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**Recherche de nouvelles substances présentes dans quelques espèces de
la famille des Astéracées**

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Liste des abréviations

CPG : Chromatographie en phase gazeuse.

CPG/SM : Chromatographie en phase gazeuse couplée à la spectrométrie de masse.

Ir : Indice de rétention.

FID : Détecteur à ionisation de flamme.

m/z : masse/charge électrique.

CC : Chromatographie liquide sur colonne ouverte.

CCM : Chromatographie sur couche mince.

RMN : Résonance magnétique nucléaire.

RMN ¹³C : Résonance magnétique nucléaire du carbone-13.

RMN ¹H : Résonance magnétique nucléaire du proton.

COSY : Correlation SpectroscopY.

HSQC : Heteronuclear Single Quantum Correlation.

HMBC : Heteronuclear multiple Bond Correlation.

J : Constante de couplage (RMN).

RMN 2D : Résonance Magnétique Nucléaire bidimensionnelle.

CDCl₃ : Chloroforme deutéré.

AA : Acide ascorbique.

DPPH : 2, 2-Diphényl-1-Picrylhydrazyle.

FRAP : Ferric Reducing Antioxidant power.

BHT : Butylhydroxytoluène.

EDTA : acide éthylènediaminetétra acétique.

ABS : Absorbance.

CI₅₀ : Concentration inhibitrice à 50% des radicaux libres.

CMI : Concentration minimale inhibitrice.

Ampho B : Amphotéricine B.

Gent : Gentamicine.

MOE : Molecular operating environment

DM : Dynamique moléculaire.

SOD : Superoxyde dismutase.

Lref : Ligand de référence.

ADMET : Absorption, Distribution, Métabolisme, Excrétion et Toxicité.

Tlemcen : Région

Introduction Générale

Depuis l'aube de l'humanité, l'homme a toujours tiré profit de son environnement végétal pour se nourrir, se parfumer, maintenir son équilibre et soigner les maladies qui nuisent à sa santé et parfois même dans ses rites religieux.

Dès leur apparition, il y a trois millions d'années, les plantes furent le principal voir l'unique recours de la médecine. Toutefois, avec leur nombre illimité, elles constituent une source qui semble inépuisable de composants à valeurs thérapeutiques, ce qui provient avant tout de sa richesse en métabolites secondaires telles que : les flavonoïdes, les alcaloïdes, les insaponifiables et les huiles essentielles [1]. En outre elles sont la source principale de découverte de nouveaux principes actifs, or environ 170000 molécules bioactives ont été identifiées telles que : la colchicine, l'artémisinine, la morphine, et la quinine [2].

En effet, les molécules issues des plantes présentent un grand avantage grâce à leurs diversités structurales chimiques et leurs propriétés biologiques. Plus de 60% des médicaments dont nous disposons sont d'origine végétale et 40% sont des médicaments de synthèse [3].

L'usage des antioxydants synthétiques dans les domaines de la pharmacie, l'agro-alimentaire et la cosmétique a procuré des solutions aux divers mécanismes pathologiques comme le cancer, l'athérosclérose, les maladies auto-immunes, Alzheimer et la maladie de Parkinson, dont le stress oxydatif est impliqué [3]. Mais suite aux doutes sur leurs effets cancérigènes et toxiques ces derniers ont été limités par des règles législatives.

La majorité des médicaments anti-inflammatoires qui sont sur le marché sont à base de produits chimiques, quoique ces derniers sont assez puissants, leur administration à longue durée engendre des effets secondaires tels que l'intolérance gastrique [4].

Par ailleurs, le phénomène de la résistance des micro-organismes ne cesse d'évoluer et de s'aggraver, en effet il est considéré par l'OMS comme un problème de santé publique. Cependant l'emploi des agents antimicrobiens qui sont des produits chimiques a entraîné l'apparition des maladies infectieuses graves qui seront dans quelques années la première cause de mortalité parmi les autres [5,6].

En outre, les plantes aromatiques et médicinales ainsi que les huiles essentielles qui de nos jours suscitent de plus en plus les chercheurs à les incorporer comme alternative aux produits chimiques de synthèse dans le traitement de quelques maladies infectieuses dans la préservation contre l'oxydation et aux antibiotiques qui devient de moins en moins actifs contre les germes, ainsi que pour le soulagement des maux avec l'avantage d'avoir des effets secondaires minimes.

Pour cela, la recherche de nouvelles substances naturelles à effet thérapeutique constitue un enjeu scientifique important.

Se trouvant dans le bassin méditerranéen avec de grandes variations climatiques du nord au sud et avec une superficie de 2.3 millions de Km², l'Algérie regorge d'une importante biodiversité floristique avec 3000 espèces dont 15% sont endémiques [7]. Cependant, malgré la tradition séculaire de l'utilisation traditionnelle des plantes, il a été démontré qu'un bon nombre de ces plantes renferment des substances naturelles actives utilisées dans divers secteurs tels que : l'agro-alimentaire, la pharmacie, l'aromathérapie et la cosmétique, leur élucidation chimique reste toujours insuffisante et leur valorisation peut avoir des retombées économiques considérables et des immenses opportunités pour le développement durable de notre pays.

Dans la perspective de promouvoir une meilleure valorisation des plantes médicinales et aromatiques de l'ouest algérien, nous nous sommes intéressés à deux espèces appartenant à la famille des *Asteraceae*, à s'avoir *Inula montana* et *Carlina hispanica* qui poussent à l'état spontané dans les environs et les monts de Tlemcen. La sélection de ces plantes a été basée sur le fait qu'elles soient utilisées en médecine traditionnelle pour le traitement de nombreuses maladies et le fait qu'elles n'ont jamais fait l'objet d'étude chimique ou biologique auparavant.

- ✓ Les principaux objectifs chimiques fixés de ce travail sont :
- L'extraction des volatils des deux plantes sélectionnées et leurs caractérisations par les techniques de la chromatographie en phase gazeuse (CPG) et la chromatographie en phase gazeuse couplée à la spectrométrie de masse (CPG/SM).
- La mise en œuvre de la RMN Carbone-13 et Proton pour la caractérisation du composé majoritaire.
- ✓ Le volet biologique complémentaire a été consacré à la mise en évidence des activités antioxydantes, antibactériennes, antifongiques et antiinflammatoires des huiles essentielles ainsi qu'à une combinaison de l'oxyde de carline avec les références synthétiques.

En vue de rendre compte de la démarche scientifique adoptée, ce manuscrit est composé de trois chapitres.

Le premier chapitre est consacré à un recueil bibliographique sur :

- Les plantes aromatiques et médicinales ainsi que les huiles essentielles, les chémotypes et les facteurs influençant leurs compositions chimiques.

- La famille des *Asteraceae*, son intérêt et sa répartition à travers le monde.
- Les deux genres de *Inula* et *Carlina*, leurs principales caractéristiques, la classification et leur répartition.
- Une description botanique des deux espèces : *Inula montana* et *Carlina hispanica*.
- Les principales techniques chromatographiques d'analyse et de caractérisation des huiles essentielles.
- Les activités biologiques des huiles essentielles : antioxydante, antibactérienne, antifongique et antiinflammatoire.

Le **second chapitre** regroupe l'ensemble des résultats de l'étude de la composition chimique des huiles essentielles de chaque espèce par CPG et CPG-SM, et par RMN et RMN à double dimension après fractionnement par chromatographie sur colonne de l'huile essentielle de *Carlina hispanica*.

Le **troisième chapitre** sera consacré à l'étude biologique qui met en évidence la valorisation des huiles essentielles ainsi que les activités biologiques des différentes combinaisons oxyde de carline-références synthétiques. Une contribution à l'étude de l'inhibition du récepteur de l'enzyme superoxyde dismutase par deux méthodes de chimie computationnelle : les analyses d'amarrage moléculaire (Docking) et les simulations de dynamique moléculaire (DM).

Enfin, la **partie expérimentale** qui traite le matériel utilisé, la démarche expérimentale et les techniques employées pour la réalisation des extractions et l'évaluation du potentiel biologique des huiles essentielle et des combinaisons de l'oxyde de carline avec les références synthétiques.

Chapitre 1 : Synthèse Bibliographique

I.1 Les plantes aromatiques et médicinales

Les plantes aromatiques et médicinales occupent une place vitale dans la vie quotidienne de l'homme avec une infinie multiplicité d'utilisation dans la préparation culinaire, la confection des parfums et pour le traitement de divers maux.

La ruée vers la phytothérapie s'explique par le fait que les plantes sont abondantes, et à la portée de tout le monde. Surtout pour les peuples des pays en voie de développement [8], et pour ceux qui vivent sous le seuil de la pauvreté constituent environ 75% de la population du monde [9]. Or il a été estimé dernièrement qu'il existe approximativement 400 000 espèces dans le monde, dont le tiers ou le quart sont exploitées pour subvenir aux besoins de santé primaire [10,11].

Les plantes aromatiques et médicinales renferment des constituants de valeurs thérapeutiques qui figurent dans plusieurs lignes permettant la sélection de la plante dont l'homme a besoin pour se soigner, et ceci par une suite d'échec et de réussite, car ces propriétés sont des connaissances empiriques qui ont été accumulées durant plusieurs millénaires. De ce fait l'histoire des PAM est liée à la progression des civilisations [12].

La découverte des secrets des remèdes n'a vu le jour qu'après les recherches faites par les chimistes et les pharmaciens qui ont pu réaliser des progrès immenses en réalisant l'isolement de quelques principes actifs tels que la morphine isolée par Serturmer en 1817, la codéine et l'asparagine par Robiquet en 1832, et aussi la papavérine isolée par Merck en 1848 [12].

L'ensemble de ces molécules naturelles isolées a servi par ailleurs comme modèle pour la synthèse et comme précurseur pour l'hémisynthèse de nouvelles drogues plus actives et plus spécifiques [12]. Cependant, l'impact massif que les médicaments synthétiques et semi-synthétiques ont engendré en raison de leur toxicité a mené à la réactualisation de l'usage des plantes dont leurs effets secondaires sont moindres voire absents [13]. Ce qui a amené les gens à se soigner par les plantes pour leurs diverses propriétés biologiques [13].

I.2 La famille des Astéracées

La famille des Astéracées connue également par « Composées » et a pour nom scientifique Astéracées a été décrite en 1972 par Paul Dietrich Giseke [14]. C'est l'une des familles les plus répandues avec une prédominance dans les régions tempérées, les plus distribuées et les plus vastes. Elle est très diversifiée dans le monde regroupant 1600 genres et

2500 espèces [15] soit environ 1/10 des angiospermes. L'Algérie détient 109 genres et 408 espèces [14], ce sont des espèces dicotylédones cosmopolites qui sont caractérisées par leur inflorescence en capitule. Par ailleurs l'organisation biologique réussie de cette famille importante et évoluée a permis à la nature de multiplier un nombre considérable de spécimens.

La place qu'occupe la famille des Astéracées dans les revues médicales est entièrement singulière au bénéfice de sa richesse en substances naturelles telles que les terpénoïdes, les flavonols, les alcaloïdes, et les flavones et qui sont d'une part la cause principale de la dermatite de contact [16], et d'autre part, la raison de l'étude taxonomique de cette famille par Hagnaur en 1964 en raison de leurs caractères phytochimiques [17]. En effet, cette famille renferme des espèces ayant différents intérêts, étant donné qu'elle fournit des espèces alimentaires : Le tournesol (*Helianthus annuus*) qui est cultivé pour son huile riche en acide gras, la laitue, comme la Laitue scariole (*Lactuca serriola*), ou la Laitue pommée (*Lactuca sativa*) ou encore l'artichaut qui est exploité même en pharmacie pour ces propriétés hépatiques ou dépuratives, ainsi que la Chamomille romaine (*Chamaemelum nobile* L.) pour les troubles digestifs. Le Semencontra (*Artemisia cina* Berge), et l'Arnica (*Arnica montana* L.) qui sont des traitements naturels pour soulager les douleurs de l'arthrite [16]. Cependant, ces dernières années beaucoup de molécules isolées de diverses Astéracées ont démontré des potentialités pharmacologiques captivantes comme l'artémisinine, qui est une lactone sesquiterpénique isolée à partir de *Artemisia annua* L ainsi que ses dérivés hémisynthétiques dans le traitement de certaines formes de paludisme. Actuellement, la silymarine et l'artémisinine sont les deux seules molécules produites industriellement.

I.3 Le genre *Inula*

Le genre *Inula* appartient à la famille des *Asteraceae*, il renferme une variété d'environ de 90 espèces représentées abondamment en Asie (Turquie, Japon, Chine...), en Europe (Italie, Grèce, France, Espagne...) et en Afrique (Algérie, Tunisie, Maroc...) [18]. Concernant l'aspect botanique, les espèces appartenant à ce genre, sont annuelles, herbacées vivaces, avec des tiges bien développées, généralement ramifiées et rarement simple. Des feuilles membraneuses, alternées, simples et généralement sessiles, contenant parallèlement des fleurs tubuleuses et des fleurs ligulées, bractées en différentes séries de fleurs à périphériques pistillées, des ligules tridentées, des anthères sagittées à la base, des chaînes munies de côtes et des aigrettes simples. Elles poussent dans les sols pierreux et salés, les prairies humides et les incultes particulièrement dans le Tell [18,19].

En médecine traditionnelle, de nombreuses espèces du genre *Inula* ont suscité un grand intérêt suite à leurs utilisations dans le traitement de quelques troubles, particulièrement la fièvre, le diabète et l'inflammation. Par ailleurs, les différentes exploitations de ces plantes en raison des attentions qui leur ont été accordé, ont abouti à l'élucidation de leurs propriétés biologiques, y compris les propriétés antioxydantes, anticancéreuses, antimicrobiennes, hépatoprotectrices et anti-inflammatoires [20-27]. En conséquence, beaucoup de chercheurs ont étudié leurs constituants chimiques, ce qui a permis l'identification de nombreux composés bioactifs. De plus, même les métabolites dotés d'activités biologiques importantes et qui sont d'un grand intérêt médicinal et scientifique tels que : les flavonoïdes, les terpénoïdes et les glycosides sont isolés à partir de ces espèces [28,29].

Inula montana

L'inule des montagnes est une espèce qui fait partie de la famille des *Asteraceae*. Elle se trouve en Europe du sud (France, Espagne, Italie, et Portugal), distribuée aussi en Afrique (Algérie, Tunisie et Maroc) [30-14].

C'est une plante vivace, à tige souterraine (rhizomateuse) ligneuse, remarquable par sa forme de rosette avec des tiges florales dressées, laineuse et blanchâtre d'une taille de 10 à 35 cm, et qui pousse dans les pentes rocailleuses arides des montagnes dépassant les 500 m de hauteur. Ces feuilles caulinaires, espacées, obtuses, entières ou à peine denticulées sur les bords, en dessous soyeux et blanchâtre, et en dessus pubescentes. Les feuilles inférieures sont atténuées en pétiole entre 5 à 12 cm de longueur. La tige florale se termine par un seul capitule à grandes ligules jaunes et linéaires de 4 à 5 cm de diamètre [14]. La floraison va du mois de mai jusqu'au juillet.

Inula montana est appelée en France par Arnica de Provence et employée traditionnellement pour soulager les ecchymoses. En Espagne l'infusion, la décoction ainsi que la macération dans l'huile d'olive ou l'alcool des parties aériennes fraîches, feuilles et fleurs ont été appliquées afin de soulager les rhumatismes et par voie orale pour les maux d'estomac [31]. En Algérie, la population locale appelle cette espèce « Boutlilis » et l'utilise en médecine traditionnelle pour ses propriétés cicatrisantes et apaisantes [14].



Figure1. Partie aérienne de *Inula montana*.

I.4 Le genre *Carlina*

Carlina est un genre de plantes herbacées appartenant à la tribu *Cardueae* et la sous-tribu *Carlininae* de la famille des *Asteraceae*, qui se compose à peu près d'une trentaine d'espèces originaire d'Europe et d'Asie, qui sont connues sous d'autres appellations « chardons carlin », « chardon Doré ou d'Argent », « artichaut sauvage cardoue », « cardonette » ou encore « caméléon blanc ». En outre, de nombreuses espèces de ce genre ont de très grandes têtes florales, ainsi que des bractées involucrelles internes voyantes [32]. Par ailleurs, les bractées extérieures semblables aux feuilles de la plante, et les bractées intérieures identiques aux ligules constituent la caractéristique la plus frappante de *Carlina*.

Certains taxons de *Carlina* sont fréquemment employés à des fins médicinales et nutritionnelles dans de nombreux pays, en vertu de leurs effets diurétiques, cholagogues, stomachiques, diaphorétiques et nettoyants [33]. De plus, leurs extraits sont aussi appliqués en usage externe afin de traiter de multiples troubles cutanés [34,35]. Cependant, il a été confirmé que les vertus thérapeutiques du genre *Carlina* se réfèrent avant tout à la composition chimique des huiles essentielles ainsi que les constituants phénoliques puisque quelques propriétés de ces dernières ont été liées aux triterpènes pentacycliques [36,37].

Carlina hispanica

La carline d'Espagne ou en corymbe est une plante très fréquentée appartenant au genre *Carlina* qui fait partie de la famille des *Asteraceae*. Elle se trouve dans la plupart des régions d'Italie, et elle pousse principalement sur le pourtour méditerranéen, dans les champs de maïs, et sur les terrains ensoleillés.

C'est une espèce vivace, caulescente à tige ramifiée en corymbe de hauteur variable (30 à 70 cm). Feuilles coriaces, pliées, à dents très épineuses. Capitules de 3 à 6 cm de diamètre, à bractées externes semblables aux feuilles. Les bractées intérieures radiantes, larges de 2 mm,

glabre, de couleur dorée, entourant le disque de fleurons jaunes. Les fruits sont des Akènes velus à aigrette. Sa période de floraison est en Juillet, Aout. *Carlina hispanica* est appelée communément « Djinza ».



Figure 2. Racine et partie aérienne de *Carlina hispanica*.

I.5 Les huiles essentielles

Depuis les temps les plus anciens, l'homme s'est toujours servi des huiles essentielles dans sa vie quotidienne pour aromatiser sa nourriture, se parfumer, parfumer son environnement et pour des fins thérapeutiques. En guise d'exemple, en période des épidémies en France, les ouvriers tanneurs et parfumeurs qui ont été en contact fréquent avec ces principes odorants des plantes aromatiques résistaient de façon quasi-absolue [38].

L'huile essentielle connue encore par essence aromatique végétale est un liquide huileux, pur et odorant extrait de la partie odoriférante de la matière végétale via diverses techniques comme l'entraînement par l'eau, par la vapeur d'eau, par incision, par distillation sèche ou par enfleurage [39,40].

La mise en évidence des pouvoirs offerts par les huiles essentielles de plusieurs espèces de différentes familles a démontré qu'elles sont dotées d'intéressantes et innombrables activités biologiques : anticancéreuses [41], anti-inflammatoires [42], antioxydantes [43,44], fongicides, insecticides, antiseptiques et bactéricides [45-49].

Actuellement, les huiles essentielles sont destinées vers de multiples secteurs car elles sont considérées comme des constituants à forte valeur ajoutée. A titre d'exemple, en agroalimentaire, elles sont employées pour une meilleure et longue conservation des aliments ainsi qu'une qualité organoleptique améliorée. En parfumerie, près de 300 huiles essentielles entrent fréquemment dans la fabrication des arômes alimentaires et de parfums. Particulièrement les essences du romarin, menthe poivrée et du citron [50]. En médecine, elles

sont énormément appliquées pour le traitement des maladies comme les infections d'origine virale et bactérienne ainsi que pour les troubles humoraux et nerveux. En médecine dentaire pour la prévention et le soin des caries ainsi que pour la désinfection de la pulpe dentaire [18]. Cependant en pharmacie, ces essences sont incorporées à la composition chimique des médicaments pour camoufler les offenses au bon goût [51]. Par contre en industrie chimique c'est plutôt les molécules extraites des huiles essentielles qui sont utilisées en hémisynthèse suite à l'intérêt qu'elles élucident du fait qu'elles sont rentables par rapport à celle préparées par synthèse chimique classique [51]. De ce fait, les huiles essentielles constituent des médicaments authentiques et leur étude persiste d'une actualité brûlante malgré leur ancienneté et les progrès de la science.

D'autres parts, cette endurance d'applicabilité des huiles essentielles repose principalement sur la richesse de leurs compositions chimiques en composés terpénoides représentée par des molécules très diversifiées au niveau structurel et en phénylpropanoïdes qui renferment des molécules odorantes comme l'eugénol, l'estragole et la vanilline. En effet ces classes de composés peuvent renfermer toutes les grandes fonctions de la chimie organique (phénols, alcools, composés carbonylés, ester, et parfois des soufrés et dérivés azotés) [49].

I.6 Chémotype

Le profil chimique des essences végétales extraites du même organe de la même variété botanique peut être dissemblable. Il s'agit donc de chémotype ou de race chimique.

En effet, le chémotype est le constituant dominant parmi les nombreux autres qui constituent l'huile essentielle et distinctif de cette dernière à d'autres de la même espèce.

Par ailleurs, l'existence de cette allotropie chimique des huiles essentielles dans la même plante est indiquée par des variations chimiques qualitatives ou quantitatives. Sans pour autant qu'il y est des différences au niveau de leur caryotype [52].

L'illustration la plus marquante de ce polymorphisme chimique est celle de l'espèce sauvage *thymus vulgaris* qu'on lui compte six chémotypes différents qui sont au niveau de la nature du monoterpène dominant de l'huile essentielle et qui peut être soit le thymol, le géraniol, le carvacrol, le γ -terpinéol, le thuyanol-4 ou le linalool [54]. En outre les espèces *Mentha spicata* et *Origanum vulgare* en sont des exemples aussi [48,54].

Toutefois, il a été constaté que cette variabilité de chémotype présente d'une part des activités variables et a permis aussi de favoriser tel ou tel essence pour des fins plus précises et

efficaces, d'autre part, des toxicités très différentes à titre d'exemple *Artemisia absinthium*, or celle qui était d'origine parisienne contenait l' α -thujone qui est une molécule fortement neurotoxique avec un pourcentage de 45%, alors que celle des régions d'alpines n'était constituée que de 3% de l' α -thujone [55].

I.7 Facteurs Influençant les huiles essentielles

Il est primordial de mettre en évidence l'étude des facteurs influençant le profil chimique des essences vu l'intérêt qu'elles présentent aux consommateurs. Or ces derniers ont la possibilité de faire énormément varier d'une façon qualitative ou quantitative l'huile essentielle extraite, ce qui peut engendrer des répercussions importantes sur sa commercialisation.

La biosynthèse est le premier facteur qui peut influencer la composition chimique des espèces, c'est pourquoi d'ailleurs, une même espèce peut avoir beaucoup de chémotypes différents [56]. Cependant, la composition et le potentiel dépendent aussi d'un autre paramètre intrinsèque qui est l'organe. Comme le cas de la sauge, l'huile essentielle des fleurs est plus riche en quelques terpènes que celle des feuilles [57]. En outre, le cycle végétatif ainsi que l'heure et la période de récolte des plantes sont également des facteurs influençants [58].

Il existe aussi des paramètres extrinsèques pouvant apporter des modifications de la composition chimiques des huiles essentielles. La température, le taux d'humidité, la pluviométrie, le climat et les conditions édaphiques, sont autant de facteurs d'ordre environnemental déterminant la composition chimique et le rendement des essences [59-63]. À titre d'exemple, chez les citrus, la teneur en huile essentielle est plus intéressante à une température élevée [62]. Cependant, il faut tenir compte aussi des métaux lourds (Plomb, zinc, cuivre, cadmium, mercure, nickel...) qui peuvent se trouver dans la composition du sol, car ces derniers peuvent jouer sur le développement des plantes et causer par la suite des problèmes de qualité finale en se retrouvant dans les essences extraites [64].

Par ailleurs, les facteurs d'origine technologique comme la durée d'extraction, le pH, la température, la pression et la dégradation enzymatique ou chimique sont à l'origine des modifications très importantes durant l'étape d'hydrodistillation [65]. Par contre, le stockage et la durée de séchage influent considérablement sur le rendement que le profil chimique [66].

I.8 Effet de synergisme et d'antagonisme

Un effet synergique est le phénomène qui survient lorsque l'effet combiné de deux substances ou produits chimiques est supérieur à la somme de leurs propres effets quand ils sont

pris individuellement. Cependant, l'effet antagoniste est le contraire de celui de la synergie, il survient quand l'interaction des combinaisons des produits est inférieure à la somme des effets de ces derniers pris séparément.

La polyvalence d'action et les propriétés biologiques des huiles essentielles s'expliquent par la diversité moléculaire qu'elles contiennent. Toutefois, les études menées sur les activités biologiques des huiles essentielles ont démontré qu'elles sont en relation avec le profil chimique et surtout avec les groupes fonctionnels des constituants majoritaires et même avec les effets synergiques entre les constituants [67].

Dans le secteur des huiles essentielles, les notions de synergie, antagonisme, additivité et indifférence sont illustrées dans divers cas de figures : soit intraspécifique par l'émulsion entre les composés de la même essence pour avoir un effet biologique ou plusieurs. Ou interspécifique résultante de la combinaison de deux essences ou plusieurs, laquelle peut également avoir un impact sur une ou plusieurs activités biologiques [68].

Le problème de la persistance des micro-organismes aux antibiotiques ne cesse de croître surtout avec l'emploi abusif des antibiotiques qui a limité leur efficacité et a engendré une augmentation rapide de la résistance de ces micro-organismes pathogènes à cette classe thérapeutique [69]. A cet égard, l'approche qui a été trouvée à cette pharmacorésistance réside à la combinaison des huiles essentielles avec des antibiotiques existants ou de combinaisons entre les huiles essentielles car ces dernières peuvent également contrôler les bactéries qui sont reconnues pour être spécifiquement résistantes aux antimicrobiens d'origine végétale comme *Pseudomonas spp* [70,71]. Par exemple, la combinaison de l'huile essentielle de *Thymus vulgaris* avec celle de l'*Origanum vulgare* a montré un meilleur résultat d'efficacité avec une concentration d'inhibition plus intéressante que les huiles essentielles séparées [72]. D'autre part, les auteurs avaient précisé que si ces combinaisons n'ont montré aucun effet de synergie, elles présentent un potentiel pour les applications industrielles en prolongeant la durée de vie nécessaire des aliments sélectionnés [72]. Une autre interaction synergique qui peut être employée comme source puissante d'agents antioxydants et antibactériens naturels sûrs et puissants dans les industries alimentaires et pharmaceutiques a été signalée sur les activités antibactériennes et antioxydantes entre le linalool de l'huile de graines de coriandre et de l'acide p-coumarique de l'huile de graines de cumin [73].

I.9 Les méthodes d'analyse

L'identification du profil chimique des huiles essentielles demeure une étape primordiale pour une bonne commercialisation et valorisation notamment pour les industriels que ces huiles essentielles constituent une matière première destinée à de nombreux secteurs d'activités.

L'analyse des huiles essentielles se fait en général à l'aide d'une technique incontournable qui est la chromatographie en phase gazeuse CPG qui sert à séparer les composants de ces mélanges complexes de volatils. D'autres parts, la quantification et le calcul d'indices de rétention des constituants s'effectuent par la combinaison de la CPG à un détecteur à ionisation de flamme FID. Toutefois, la CPG est fréquemment combinée avec une technique d'identification spectral, la Spectrométrie de masse (SM) ou parfois la Spectrométrie Infrarouge par Transformée de Fourier (IRTF) [74,75]. Au moment où l'étape d'identification se complique, elle exige le fractionnement de l'échantillon grâce à la chromatographie liquide sur colonne (CLC) ou mieux encore l'utilisation de la chromatographie flash automatisée (CFA), qui peut durer jusqu'à la purification du constituant. Le but étant d'aboutir à son élucidation structurale par les techniques spectroscopiques fréquentes : RMN¹H et ¹³C, IRTF, SM, etc.

I.9.1 Chromatographie en phase gazeuse (CPG)

La chromatographie en phase gazeuse CPG est une technique d'analyse chromatographique tant répandue en raison de ces multiples avantages tels que la polyvalence, la sensibilité, la rapidité d'optimisation de nouvelles analyses et la possibilité d'automatisation ce qui augmente davantage son intérêt [76].

Elle s'applique aux composants volatiles par chauffage sans les décomposer [77]. En effet, le perfectionnement de cette méthode lui confère la possibilité de permettre la séparation et la quantification des espèces chimiques des échantillons de l'ordre du milligramme voir du microgramme et qui peuvent comprendre jusqu'à 200 espèces chimiques qui varient à leur tour tant par leur nature que par leur volatilité [78,79].

De même que toutes les techniques chromatographiques, la CPG se base sur le principe de migration différentielle des composants [80]. Or le mélange est vaporisé dans l'injecteur à l'entrée de la colonne appelée la phase stationnaire, et transporté à travers celle-ci par la suite par le biais d'un gaz vecteur qui constitue la phase mobile. La séparation des constituants se fait de façon séquentielle, en sus les molécules sort les unes après les autres après un certain laps de temps selon l'affinité et la solubilité dans la phase stationnaire [81,82].

Pour chaque constituant, deux indices peuvent être obtenus : polaire et apolaire. Ces derniers sont calculés à partir d'une série de référence n-alcane ou parfois d'ester méthylique linéaire à température constante (indice de Kováts) ou en programmation de température (indices de rétention) [83]. Par ailleurs ces indices de rétention sont par la suite comparés avec ceux mesurés au laboratoire ou décrits dans la littérature. Toutefois, il est recommandable d'utiliser deux phases stationnaires de polarité différente, car elles ont tendance à différencier d'une analyse à l'autre.

La CPG reste un outil extra puissant pour l'analyse et le contrôle de qualité des huiles essentielles, exploitée également pour des molécules plus lourdes (stéroïdes, triterpènes,...), et qui est progressivement adoptée dans les principaux domaines de la chimie. Cependant, elle ne peut pas suffire à une identification formelle sauf si une technique spectroscopique supplémentaire est associée [64].

I.9.2 Chromatographie en phase gazeuse/ Spectrométrie de Masse (CPG/SM)

Au bénéfice de la simplicité de la combinaison de la CPG avec la SM, le progrès accompli dans le traitement des signaux en temps réel, l'établissement de banque de données de spectre de masse et le développement des algorithmes de comparaison entre les spectres des composés à identifier avec ceux listés dans la banque. Cette méthode est considérée comme une technique de référence en analyse de routine qui ne cesse de progresser et a trouvé de nombreux secteurs d'utilisation tels que celui des produits pétroliers (matières synthétiques, carburants), des produits naturels (médecine, parfumerie, cosmétique), de l'agroalimentaire (eau, aliments) et dans le secteur des huiles essentielles [84,85].

La spectroscopie de masse se repose sur la détermination des rapports masse/charge des molécules des échantillons à analyser et donc éventuellement leurs natures. Pour cela les molécules qui sont aux préalables éluées sur une colonne capillaire subissent un bombardement au moyen des électrons ou photons et fragmentées par la suite. En effet ce sont les fragments, chargés positivement qui représentent le spectre de masse de la molécule.

Cependant, les analyseurs les plus répandus pour l'analyse des volatils sont le «quadripôle» piège à ions ou « ion trap », or ils utilisent la stabilité des trajectoires pour séparer les ions selon le rapport masse sur charge m/z [86,87]. Le multiplicateur de photons donne lieu à la détection des ions positifs et dans quelques cas des ions négatifs [88]. Les signaux visualisés sous forme de pics d'intensités variables, rangés sur une échelle de masse sont enregistrés à l'aide d'un ordinateur.

L'identification des constituants est réalisée par la comparaison de leurs spectres à ceux des composants de références contenus dans des bibliothèques informatisées ou sous format papier construites au laboratoire ou commerciales [89], à condition que les indices de rétention calculés sur deux colonnes de polarité différente en CPG soient identiques dans les conditions opératoires comparables et le niveau de similitude des spectres inconnus et ceux de référence soit également suffisant [62]. Toutefois, pour l'obtention d'informations complémentaires, deux modes d'ionisation peuvent être élaborés, ionisation par impact électronique (IE) et ionisation chimique (IC) dans lequel on distingue deux modes : L'ionisation chimique positive (ICP) et l'ionisation chimique négative (ICN) [90].

I.9.3 Résonance magnétique nucléaire (RMN)

La RMN est une technique spectroscopique d'analyse de la matière non destructive, fortement puissante et riche. Elle permet de sonder la structure moléculaire à l'échelle nanométrique en faisant interagir l'aimantation naturelle des noyaux avec un champ magnétique, et permet également l'accès à des informations qui concernent le squelette des molécules ainsi que leur fonctionnalisation.

Le phénomène de la RMN s'applique dans diverses disciplines en chimie (organique, inorganique et sciences des matériaux), en biochimie (structure des molécules) et en physique. Cependant, son utilisation pour l'analyse des huiles essentielles et les mélanges complexes a été adaptée pour l'identification des constituants qui sont difficiles à séparer par la CPG, des sesquiterpènes et des stéréoisomères qui ont des indices de rétention très proches tels que les isomères du menthol, du dihydrocarvéol et leurs acétates dans les huiles essentielles de menthe verte et de menthe poivrée, et aussi pour l'étude de la variabilité chimiques des huiles essentielles [91].

I.10 Activités biologiques

I.10.1 Activité antioxydante

Les radicaux libres sont des espèces réactives de l'oxygène (E.R.O) produites par l'organisme continuellement lors des processus physiologiques. Ces E.R.O sont soit des radicaux primaires tels que : l'anion superoxyde ($O_2^{\bullet-}$), le radical hydroxyle ($\bullet OH$), le monoxyde d'azote (NO^{\bullet}), le radical peroxyde (ROO^{\bullet}) et le radical alkoxyde (RO^{\bullet}), soit des radicaux secondaires tels que : l'oxygène singulet 1O_2 , le peroxyde d'hydrogène (H_2O_2) et le nitroperoxyde ($ONOOH$), ces derniers se forment par réaction des radicaux primaires sur les composés biochimiques de la cellule [93]. Ils sont caractérisés par une instabilité et/ou un pouvoir oxydant fort, avec la capacité de réagir et altérer plusieurs biomolécules telles que

l'ADN, les protéines, les acides polyinsaturés, les lipides etc..., la dégradation des tissus et des cellules ainsi que le système immunitaire ce qui donne lieu à un phénomène connu sous le nom de stress oxydatif [92-94]. Celui-ci est sujet de nombreuses pathologies comme le cancer, la maladie d'Alzheimer et de Parkinson, le diabète, le vieillissement, et les maladies cardiovasculaire [95] [48]. Le moyen de prévenir ce stress oxydatif est de rechercher un apport supplémentaire de substances antioxydantes dans l'alimentation (α -tocophérol, vitamine C, BHT, BHA, etc.) [96].

Un antioxydant est par définition toutes substances endogènes ou exogènes, présentes en faible concentration vis-à-vis au substrat oxydable avec lesquels elles rentrent en compétition, capable de retarder significativement ou empêcher l'oxydation et la destruction de ces substrats biologiques [97]. Cependant, il a été rapporté que les huiles essentielles renferment des propriétés anti radicalaires qui constituent une alternative prometteuse aux antioxydants synthétiques qui ont des effets néfastes sur la santé humaine [98,99]. Un exemple est celui des huiles essentielles de basilic, muscade, persil, origan, cannelle, clou de girofle et le thym qui comprennent de puissantes substances antioxydantes [48]. En outre, le thymol et le carvacrol sont des composés dotés d'un pouvoir antioxydant prouvé, ce qui est en relation avec leurs structures phénoliques, en effet les constituants de ce type jouent un rôle essentiel pour la neutralisation des radicaux libres et la décomposition des peroxydes [100]. Par ailleurs, le pouvoir antioxydant des huiles essentielles est attribué également à certains alcools et aldéhydes monoterpéniques, cétones, éthers, les hydrocarbures et les composés soufrés [101,102]. À titre d'illustration le α -terpinène, le β -terpinène et le β -terpinolène présents dans l'huile essentielle de *Melaleuca alternifolia*, le menthone et l'isomenthone dans *Mentha longifolia* et *Mentha piperita* et le linalol, thymol et l'eugénol dans le gingembre, cumin et cannelle [103]. D'autres parts, la mise en évidence de l'activité antioxydante *in vivo* de l'huile essentielle d'Origan a révélé que cette dernière a démontré une activité importante en favorisant le retard de l'oxydation des lipides chez les lapins en régime excessif [104].

I.10.2 Activité antibactérienne

Les bactéries sont des organismes vivants microscopiques susceptibles de croître et de se diviser au profit de substances nutritives. En effet ce sont des cellules procaryotes (leurs ADN n'étant pas fixés dans le noyau) et ubiquistes qui se trouvent dans tous les types de biotopes rencontrés sur terre du fait de leur métabolisme très divers [105].

La résistance des micro-organismes développée aux antibiotiques pose des problèmes importants dans le monde voir graves dans certaines régions géographiques [106]. Elle est

devenue comme une évolution inéluctable et ceci depuis la fin de la seconde guerre mondiale [105]. En outre L'impact réel de ce phénomène biologique délicat se manifeste particulièrement en milieu hospitalier où les principaux types de pathogènes résistants ont augmenté durant ces dernières années.

Par conséquent, les études qui ont été menées sur l'activité antibactérienne des huiles essentielles ont révélé qu'elles peuvent constituer une alternative aux antibiotiques inefficaces. Elles ont une double action à savoir : bactéricides et bactériostatiques, ce qui leur confère un spectre d'action très étendu et aussi du fait qu'elles agissent sur un large éventail de bactéries résistantes et multirésistantes comme *Listeria innocua*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Shigella dysenteria* et *Bacillus cereus* [107], avec moins de risques et en assurant aux consommateurs plus de sécurité ce qui est avant tout au bénéfice de leur grande richesse en molécules naturelles tant en nombre qu'en variété [108-110]. Entre autres, les huiles essentielles de *Melaleuca alternifolia* et *Thymus vulgaris* qui ont inhibé la croissance de différentes souches notamment *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus hominis*, *Staphylococcus epidermidis* et *Klebsiella pneumonia* [111]. Sachant que même les combinaisons des huiles essentielles ont exercé un effet antibactérien remarquable particulièrement sur *Bacillus cereus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* et *Escherichia coli* et pas seulement les huiles individuelles [72]. A signaler aussi que c'est au fait la classe des chémotypes des huiles essentielles qui est responsable sur l'activité, à titre d'exemple la classe des phénols (carvacrol, eugénol, thymol) possède le coefficient antibactérien le plus élevé suivie par les monoterpènes (menthol, géraniol, terpinéol) ensuite les aldéhydes (néral, géraniol), etc [112].

I.10.3 Activité antifongique

En raison de la prescription des antibiotiques de façon abusive, les infections fongiques sont devenues très fréquentes dans notre société et énormément favorisées. Il s'agit de moisissures contaminantes qui sont capables de coloniser de nombreux substrats (végétal, animal, murs, papier, etc.), or elles sont connues pour leur effet d'altération des denrées alimentaires et ceci en conduisant à l'accumulation de métabolites secondaires toxiques (les mycotoxines), c'est en fait des organismes hétérotrophes que leur développement nécessite une source de composés organiques.

L'étude *in vivo* qui a été entreprise vis-à-vis d'un large éventail de pathologies fongiques agricole, animaux et humain a évoqué une sensibilité importante aux huiles essentielles ce qui

a accru l'intérêt de leurs applications industrielles et thérapeutiques, ainsi que leurs constituants actifs à savoir : les phénols et les aldéhydes monoterpéniques, les alcools monoterpéniques et les lactones..., entre autres, l'eugénol, le carvacrol et le thymol, qui ont été testés contre une large gamme de champignons: *Aspergillus* (*A. niger*, *A. flavus*, *A. fumigatus*), *Penicillium italicum*, *Penicillium chrysogenum*, *Candida* (*C. albicans*), *Penicillium digitatum*, et d'autres [113,114]. Par ailleurs, les recherches effectuées sur cette activité portent en majorité sur les huiles essentielles appartenant à la famille des *Lamiacées* comme la menthe, le thym, la lavande, le romarin, l'origan, la sauge, etc...[115]. En effet, elles constituent une alternative naturelle comme agents de protection contre les champignons phytopathogènes et les microorganismes.

I.10.4 Activité antiinflammatoire

L'inflammation est une réponse de défense immunitaire de la part des tissus de l'organisme qui se met en place lors d'une blessure locale engendrée par des germes pathogènes ou par des agents physiques ou chimiques afin de maintenir son intégrité [116-118]. Elle existe principalement en deux types : l'inflammation aiguë qui se manifeste rapidement et qui est médiée par l'activation du système immunitaire et qui dure uniquement pendant un court laps de temps. et l'inflammation chronique qui survient lorsque l'inflammation aiguë persiste et elle prédispose à plusieurs maladies chroniques comme le cancer [119]. C'est au faite un processus souhaité et bénéfique vu qu'elle permet l'élimination totale des intrus et le retour des tissus endommagés à l'homéostasie [120,121].

Depuis longtemps, les huiles essentielles ont été employées comme agents antiinflammatoires ce qui mène à dire qu'elles renferment une forte activité [122]. Cependant les études mises en évidence ont permis d'avoir une place de choix et une nouvelle option pour le traitement des inflammations et de constituer une alternative aux médicaments allopathiques classiques tels que les AINS (Anti-inflammatoires non stéroïdiens) connus pour leur mauvaise tolérance digestive et ceci à court terme. Un cas exemplaire est celui des huiles essentielles de géranium ainsi que le linalol et son acétate qui ont démontré une efficacité sur les œdèmes de pattes de souris induit par le carraghénine [123,124]. Un autre exemple, l'huile essentielle de racine de réglisse qui a signalé un fort effet antiinflammatoire dans le test de dépistage de l'inhibiteur de la lipoxycgénase [125]. Toutefois les aldéhydes monoterpéniques tels citronellal, citrals (géraniol et néral), curminal représentent les molécules principales responsables à combattre les inflammations comme l'arthrite, les allergies et les rhumatismes en empêchant la libération d'histamine.

***Chapitre 2 : Extraction Et
Caractérisation des huiles essentielles***

II.1 *Inula montana*

La recherche bibliographique menée sur l'espèce *Inula montana* indique qu'aucune étude n'a été réalisée sur l'huile essentielle de cette espèce, ceci nous a incité à étudier de près la composition chimique de l'huile essentielle des parties aériennes qui représente pour nous un champ d'investigation prometteur pour la découverte de nouvelles molécules dotées d'activités thérapeutiques.

II.1.1 Provenance du matériel végétal et rendement

Les parties aériennes de *Inula montana* ont été récoltées durant le mois de mai 2019 en période de pleine floraison à partir de sept stations différentes de la région de Tlemcen (Algérie). L'identification botanique des espèces collectées a été faite par le laboratoire d'écologie et de gestion des écosystèmes de l'université de Tlemcen. L'hydrodistillation des huiles essentielles a été réalisée au moyen d'un appareil de type Clevenger pendant 5 heures, ce qui a abouti à l'obtention d'une huile de couleur jaunâtre. Les rendements obtenus sont calculés par rapport à la masse du végétal frais.

Les informations des habitats des échantillons (lieux de récolte, altitudes et rendements) sont présentées dans le Tableau 1.

Les rendements en huile essentielle les plus élevés ont été obtenus dans les deux stations de Wardania (0,18%) (S4) et Ain fezza (0,1%) (S5) caractérisées respectivement par des altitudes de 107 m et 780 m. Les stations de Sidi wchaa (S1) et Wad tafna (S6) qui se situent respectivement à 54 m et 772 m, ont affiché le même rendement de l'ordre de 0,9%. Pour la station d'Ouchba (S3) qui se trouve à une altitude de 780 m, le rendement a été de 0,06%. Tandis que les rendements les plus bas ont été observés dans les stations de Bouzadjar (0,02%) (S2) et Oum el alou (0,01%) (S7) avec des altitudes de 26 m et 780 m respectivement (Tableau 1).

Tableau 1. Lieux de récoltes, répartitions géographique et rendements en huile essentielle des sept échantillons de *Inula montana* de la wilaya de Tlemcen.

| Echantillon | N° | Codes Voucher | Localités | Coordonnées GPS | Rendements % | Altitudes m |
|----------------------|----|---------------|-------------|------------------------|--------------|-------------|
| <i>Inula montana</i> | S1 | IM-1020-I1 | Sidi wchaa | 35°06'55"N; 1°46'37"O | 0,09 | 54 |
| | S2 | IM-1020-I2 | Bouzedjar | 35°34'42"N; 1°08'03"O | 0,02 | 26 |
| | S3 | IM-1020-I3 | Ouchba | 34°54'00"N; 1°13'20"O | 0,06 | 780 |
| | S4 | IM-1020-I4 | Werdania | 35°16'49"N; 1°30'13"O | 0,18 | 107 |
| | S5 | IM-1020-I5 | Ain fezza | 34°52'26"N; 1°12'36"O | 0,1 | 780 |
| | S6 | IM-1020-I6 | Wad tafna | 34°45'29"N ; 1°34'04"O | 0,09 | 772 |
| | S7 | IM-1020-I7 | Oum el Alou | 34°49'00"N ; 1°16'15"O | 0,01 | 1022 |



▪ Composition chimique

La composition chimique des constituants volatils des huiles essentielles obtenues des parties aériennes de *Inula montana* récoltées à partir de différentes régions de Tlemcen a été analysée par CPG et CPG/SM.

Un total de soixante-neuf composés a été identifié dans les huiles essentielles des parties aériennes représentant un pourcentage de 97.2 % à 99.6 % du mélange complexe (Tableau 2).

Tableau 2. Composition chimique des parties aériennes de l'huile essentielle de *Inula montana*.

| No | Composés | Lit /Ira ^b | Ira ^c | Irp ^d | S1 | S2 | S3 | S4 | S5 | S6 | S7 | HE Colle | Identification ^f |
|----|--------------------------|-----------------------|------------------|------------------|-----|-----|-----|-----|-----|-----|-----|----------|-----------------------------|
| 1 | Hexanal | 773 | 770 | 1055 | 0,1 | 0,2 | 0,1 | tr | Tr | Tr | 0,2 | 0,1 | IR, SM |
| 2 | Z-hex-3-en-1-ol | 834 | 831 | 1380 | 0,2 | tr | 0,3 | tr | tr | tr | 0,5 | 0,1 | IR, SM |
| 3 | β-citronelle | 938 | 940 | 1031 | tr | 0,1 | 0,1 | tr | 0,1 | tr | 0,1 | 0,05 | IR, SM |
| 4 | 6-methyl-hept-5-en-2-one | 961 | 963 | 1337 | 0,2 | tr | tr | tr | tr | tr | 0,1 | 0,05 | IR, SM |
| 5 | 1,8-Dihydro cinéole | 979 | 979 | 1180 | 0,1 | tr | 0,3 | 0,1 | 0,6 | 0,1 | 0,4 | 0,2 | IR, SM |
| 6 | Octanal | 981 | 982 | 1169 | 0,2 | 0,1 | 0,2 | 0,1 | 0,5 | 0,1 | 0,2 | 0,2 | IR, SM |
| 7 | Nonal | 1082 | 1083 | 1394 | 0,1 | 0,1 | tr | 0,4 | 0,3 | 0,2 | 0,1 | 0,2 | IR, SM |
| 8 | α-Terpinéol | 1179 | 1175 | 1688 | 0,2 | tr | 0,4 | tr | 0,1 | 0,1 | 0,1 | 0,1 | IR, SM |
| 9 | Theaspirane 1 | 1293 | 1292 | 1480 | 0,1 | tr | 0,1 | 0,1 | 0,1 | 0,1 | tr | 0,1 | IR, SM |
| 10 | Cis-3-hexenyl tiglate | 1305 | 1304 | 1641 | 0,1 | tr | 0,1 | 0,1 | 0,1 | 0,1 | 0,1 | 0,1 | IR, SM |
| 11 | Theaspirane 2 | 1308 | 1307 | 1517 | 0,1 | tr | 0,1 | 0,1 | tr | 0,1 | 0,1 | 0,1 | IR, SM |
| 12 | α-cubébène | 1348 | 1350 | 1452 | 0,1 | tr | 0,1 | 0,2 | tr | tr | 0,1 | 0,1 | IR, SM |
| 13 | α-ylangène | 1375 | 1372 | 1476 | 0,2 | 0,1 | 0,2 | 0,2 | 0,2 | 0,1 | 0,2 | 0,2 | IR, SM |
| 14 | α-Copaène | 1379 | 1379 | 1488 | 0,1 | tr | 0,2 | 0,1 | tr | tr | 0,2 | 0,1 | IR, SM |
| 15 | β-borbonène | 1383 | 1385 | 1515 | 0,2 | 0,1 | tr | 0,3 | 0,2 | 0,1 | 0,1 | 0,1 | IR, SM |
| 16 | Cyprène | 1402 | 1406 | 1525 | 0,3 | 0,3 | 0,3 | tr | tr | 0,5 | 0,7 | 0,3 | IR, SM |
| 17 | Cis-α-bergamotène | 1409 | 1411 | 1562 | 0,3 | tr | 0,2 | tr | 0,1 | tr | 0,2 | 0,1 | IR, SM |
| 18 | α-ghurjunène | 1413 | 1410 | 1524 | 0,2 | 0,4 | 0,4 | 0,9 | 0,9 | 1,1 | 1,6 | 0,8 | IR, SM |
| 19 | Aristolène | 1420 | 1416 | 1560 | 0,1 | 0,1 | 0,1 | tr | 0,1 | tr | tr | 0,05 | IR, SM |
| 20 | E-β-caryophyllène | 1424 | 1418 | 1524 | 0,3 | 0,3 | 0,9 | 0,1 | 0,1 | 0,4 | 1 | 0,4 | IR, SM |
| 21 | β-copaène | 1426 | 1431 | 1581 | 0,1 | 0,4 | 0,3 | tr | 0,2 | tr | 0,1 | 0,1 | IR, SM |
| 22 | β-ghurjunène | 1439 | 1439 | 1591 | 0,3 | 0,2 | 0,2 | 0,1 | 0,2 | 0,2 | 0,3 | 0,2 | IR, SM |
| 23 | E-β-farnésène | 1448 | 1448 | 1660 | 0,3 | 0,4 | 0,2 | 0,3 | 0,6 | 0,2 | 0,1 | 0,3 | IR, SM |
| 24 | Nomadendrène | 1452 | 1450 | 1581 | 0,2 | tr | 0,1 | tr | 0,1 | tr | 0,3 | 0,1 | IR, SM |
| 25 | α-humulène | 1456 | 1451 | 1611 | 0,4 | 0,1 | 0,1 | 0,6 | 0,1 | 0,4 | 0,4 | 0,3 | IR, SM |
| 26 | Allo-aromadendrène | 1462 | 1463 | 1476 | 0,2 | 0,1 | 0,5 | 0,3 | 0,8 | 0,4 | 0,4 | 0,3 | IR, SM |
| 27 | α-Curcumène | 1471 | 1468 | 1465 | 0,2 | 0,1 | 0,6 | 0,3 | 0,3 | 0,1 | 0,4 | 0,4 | IR, SM |
| 28 | γ-murolène | 1471 | 1471 | 1681 | 0,2 | 0,1 | 0,3 | 0,5 | 0,3 | 0,5 | 1 | 0,4 | IR, SM |
| 29 | Germacrène D | 1480 | 1480 | 1704 | 0,3 | 0,5 | 0,6 | 1,1 | 1,8 | 0,3 | 0,1 | 6,7 | IR, SM |
| 30 | Zingibrène | 1486 | 1489 | 1717 | 0,1 | 0,2 | 0,1 | 0,3 | tr | 0,2 | 0,3 | 0,2 | IR, SM |
| 31 | α-murolène | 1496 | 1994 | 1719 | 0,3 | 0,1 | 1,5 | 1,1 | 2,7 | 0,2 | 1 | 1,0 | IR, SM |
| 32 | E,E-α-farnésène | 1498 | 1500 | 1740 | 0,3 | tr | tr | 0,1 | 0,6 | 0,1 | 0,1 | 0,2 | IR, SM |
| 33 | γ-cadinène | 1507 | 1507 | 1752 | 1 | 1,4 | 2,1 | 2,1 | 1,7 | 1,2 | 1 | 1,5 | IR, SM |
| 34 | Trans-calamenène | 1512 | 1511 | 1816 | 0,3 | tr | 0,5 | 0,9 | 0,5 | 0,5 | 0,3 | 0,4 | IR, SM |

| | | | | | | | | | | | | | |
|----|--------------------------|------|------|------|------|------|------|------|------|------|------|------|-------------|
| 35 | δ-cadinene | 1516 | 1516 | 1752 | 9,7 | 9,2 | 7,7 | 8,4 | 8,9 | 5,1 | 3 | 7,4 | IR, SM |
| 36 | α-cadinene | 1535 | 1530 | 1740 | 0,3 | 0,5 | 0,7 | 0,4 | 0,7 | 0,3 | 1,3 | 1,1 | IR, SM |
| 37 | α-calacorène | 1531 | 1531 | 1895 | 1,1 | 0,2 | 0,5 | 0,4 | 0,7 | 0,2 | 1 | 0,6 | IR, SM |
| 38 | β-calacorène | 1548 | 1547 | 1936 | 0,5 | 0,3 | 0,2 | 0,4 | 0,2 | 0,2 | 0,2 | 0,3 | IR, SM |
| 39 | E-nérolidol | 1546 | 1551 | 2037 | 0,4 | 1,3 | 1,7 | 1,4 | 1,6 | 1,1 | 2,1 | 1,4 | IR, SM |
| 40 | Epi-globulol | 1558 | 1559 | 2010 | 0,6 | tr | 0,3 | 0,6 | 0,3 | 0,4 | 0,4 | 0,4 | IR, SM |
| 41 | Caryophyllene oxyde | 1576 | 1571 | 1980 | 3,2 | 1,4 | 2,1 | 3,1 | 2,1 | 2,5 | 3,5 | 2,5 | IR, SM |
| 42 | Z-nérolidol | 1518 | 1521 | 1987 | 0,3 | 0,1 | 0,2 | 0,4 | 0,5 | 0,2 | 0,3 | 0,3 | IR, SM |
| 43 | Germacrène D-4-ol | 1573 | 1566 | 2020 | 0,7 | 4,4 | 2,5 | 1,8 | 1,1 | 3,4 | 1,2 | 2,1 | IR, SM |
| 44 | Globulol | 1580 | 1576 | 2067 | 0,4 | 0,5 | 1,9 | 0,7 | 1,3 | 1,7 | 0,4 | 1,0 | IR, SM |
| 45 | Humulène époxyde | 1592 | 1601 | 2044 | 1,1 | 0,4 | 0,7 | 0,5 | 0,4 | 0,1 | 1 | 0,6 | IR, SM |
| 46 | β-oploponone | 1593 | 1590 | 2017 | 1,2 | 2,6 | 1,1 | 4,1 | 1,3 | 2,2 | 3,2 | 2,4 | IR, SM |
| | Viridiflorol | 1591 | 1591 | 2089 | 4 | 4,5 | 3,9 | 2,6 | 3,1 | 5,8 | 4,5 | 4,1 | IR, SM |
| 47 | Zingiberénol | 1598 | 1599 | 2019 | 0,5 | 0,8 | 0,6 | 0,4 | 0,3 | 0,1 | 1 | 0,5 | IR, SM |
| | 1,10-diepi-cubenol | 1608 | 1608 | 2025 | 0,4 | 1,1 | 1,3 | 1,3 | 1,2 | 1,2 | 0,8 | 1,0 | IR, SM |
| 48 | Aromadendrene | 1615 | 1617 | 2002 | 0,4 | 1,2 | 1,3 | 1,5 | 1,1 | 0,8 | 1,4 | 1,1 | IR, SM, Réf |
| 49 | époxyde | | | | | | | | | | | | |
| 50 | Eudesma-4(15)-en-6- | 1616 | 1614 | 2039 | 1,4 | 0,3 | 1,2 | 1,5 | 1,1 | 1,3 | 0,5 | 1,0 | IR, SM |
| 51 | one | | | | | | | | | | | | |
| 52 | Cadin-4-en-7-ol | 1627 | 1627 | 2096 | 1,1 | 0,5 | 0,6 | 1,6 | 1,2 | 1,6 | 1,1 | 1,4 | IR, SM |
| 53 | Trans-murolol | 1630 | 1630 | 2134 | 0,6 | 0,5 | 0,4 | 0,2 | 0,5 | 0,6 | 1,2 | 0,6 | IR, SM |
| 54 | Tau-cadinol | 1632 | 1632 | 2169 | 6,7 | 9,5 | 8,4 | 6,6 | 9,1 | 5,6 | 6,7 | 7,5 | IR, SM |
| 55 | β-eudesmol | 1638 | 1644 | 2234 | 0,5 | 0,3 | tr | tr | tr | 0,3 | 2,4 | 0,5 | IR, SM |
| 56 | α-carinol | 1644 | 1645 | 2231 | tr | tr | tr | tr | tr | tr | 0,2 | 0,02 | IR, SM |
| | α-cadinol | 1645 | 1642 | 2231 | 12,2 | 12,3 | 16,1 | 15,2 | 18,8 | 15,3 | 12,2 | 14,6 | IR, SM |
| 57 | Z,Z-farnésol | 1648 | 1653 | 2167 | 1,2 | 1 | 0,9 | 0,7 | 0,2 | 0,5 | 2 | 1,0 | IR, SM |
| 58 | α-eudesmol | 1653 | 1649 | 2220 | 1,8 | 1,8 | 1,1 | 1,6 | 1 | 1,8 | 1,1 | 1,4 | IR, SM |
| 59 | β-bisabolol | 1656 | 1653 | 2140 | 1,2 | 0,8 | 0,9 | 0,7 | 0,5 | 1,2 | 1,3 | 1,0 | IR, SM Réf |
| 60 | Ar-curcumen-15-al | 1681 | 1675 | 2164 | 3,4 | 1,5 | 1,9 | 1,6 | 1 | 3 | 3,9 | 2,2 | IR, SM |
| 61 | Eudesma-7,11-en-4-α- | 1683 | 1683 | 2300 | 0,6 | 0,3 | 0,1 | 0,5 | 0,2 | tr | 2 | 0,4 | IR, SM |
| 62 | ol | | | 2218 | 19,7 | 23,9 | 21,1 | 20,5 | 19,1 | 19,5 | | | |
| 63 | Shyobunol | 1687 | 1688 | | | | | | | | 10,5 | 16,6 | IR, SM |
| 64 | Léadol | 1696 | 1693 | 2050 | 2,1 | 0,3 | 2 | 0,7 | 1,8 | 1,1 | 4 | 1,7 | IR, SM |
| 65 | E-acétate de nérolidol | 1732 | 1738 | 2269 | 5,4 | 2,7 | 3,2 | 1,4 | 3,1 | 4,9 | 0,9 | 3,1 | IR, SM, Réf |
| 66 | β-acoradiénol | 1797 | 1792 | 2221 | 6,4 | 0,9 | 2,1 | 4,3 | 2,1 | 1,1 | 2,1 | 2,7 | IR, SM |
| 67 | Z,E-acétate de farnésyle | 1840 | 1832 | 2356 | 0,4 | 0,2 | 1,5 | 0,4 | 0,3 | 0,2 | 6,4 | 1,3 | IR, SM |
| 68 | Heptadec-8, 11,14- | 1871 | 1871 | 2330 | 1,6 | 4,1 | 1,3 | 0,4 | 0,5 | 3,7 | 0,4 | 1,6 | IR, SM |
| | trien-2-one | | | | | | | | | | | | |
| 69 | E,Z-acétate de farnésyle | 1880 | 1882 | 2450 | 1,6 | 4,1 | 1,3 | 0,4 | 0,7 | 5,4 | 1,2 | 2,1 | IR, SM |

| | | | | | | | | |
|-----------------------------------|------|------|------|------|------|------|------|-------|
| Total d'identification % | 99,2 | 98,6 | 99,6 | 98,7 | 99,3 | 99,3 | 97,2 | 98,84 |
| Pourcentages % (w/w) | 0,09 | 0,02 | 0,06 | 0,18 | 0,1 | 0,09 | 0,01 | 0,07 |
| Composés hydrocarbonés | 18 | 15,8 | 17,3 | 18,2 | 21,4 | 12,9 | 16,7 | 17,18 |
| Sesquiterpènes hydrocarbonés | 18 | 15,8 | 17,3 | 18,2 | 21,4 | 12,9 | 16,7 | 17,18 |
| Composés oxygénés | 75,3 | 75,6 | 76,5 | 72,2 | 73,3 | 80,6 | 80,5 | 76,28 |
| Monoterpènes oxygénés | 0,5 | tr | 0,9 | 0,3 | 0,8 | 0,4 | 0,6 | 0,5 |
| Sesquiterpènes oxygénés | 74,3 | 75,4 | 74 | 71,4 | 72 | 79,9 | 79,9 | 75,27 |
| Composés oxygénés non-terpéniques | 0,5 | 0,2 | 1,6 | 0,5 | 0,5 | 0,3 | 0,6 | 0,5 |

L'identification des constituants a été établie par comparaison de leurs indices de rétention (IR) et leurs spectres de masse (SM) avec ceux de la bibliothèque « Arômes » propres au Laboratoire de l'université de Corse et à l'aide des bibliothèques commerciales.

Les sesquiterpènes oxygénés (71,4-79,9 %) ont été les constituants majoritaires de la composition chimique des huiles essentielles avec 31 composés, suivis par les sesquiterpènes hydrocarbonés (12,9- 21,4 %) avec 22 composés. On révèle, par ailleurs la faible présence des deux classes de monoterpènes oxygénés (0,3-0,9 %) et de composés oxygénés non terpéniques (0,3-1,6 %) avec 8 composés dans chaque classe. (Tableau 2, Figure 3).

Les principaux composés volatils des parties aériennes ont été le shyobunol (10,5- 23,9%), le α -cadinol (12,2-18,8 %), le tau-cadinol (5,6-9,5 %), le δ -cadinène (3-9,7 %), le véridiflorol (2,6-5,8 %), l'oxide de caryophyllène (1,4-3,5 %), le Z,E- acétate de farnésyle (0,2-6,4 %) et l'ar-curcumen-15-al (1-3,9 %) (Tableau 2).

D'autre part, l'analyse de la composition chimique des huiles essentielles des parties aériennes récoltées des différents sites a montré que ces dernières sont qualitativement et quantitativement identiques avec aucune variabilité chimique.

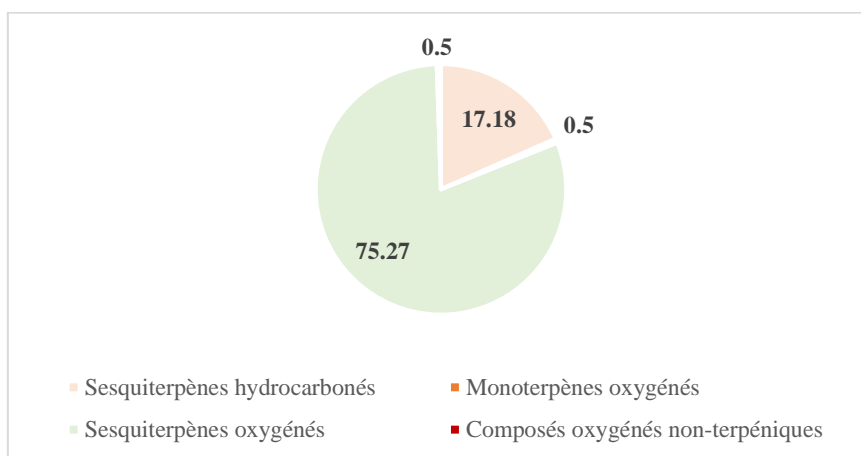


Figure 3. Variation des % des classes chimiques de la partie aérienne de l'huile essentielle de *Inula montana*.

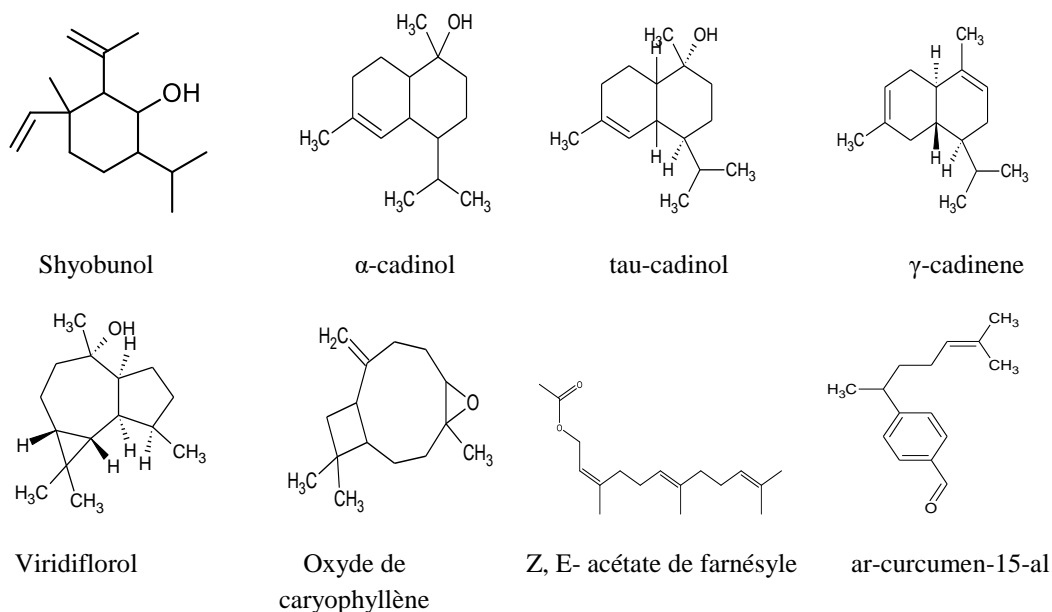


Figure 4. Structures chimiques des principaux constituants identifiés dans la partie aérienne de l'huile essentielle de *Inula montana*.

Comme nous l'avons déjà signalé précédemment, aucun travail n'a porté sur l'étude de la composition chimique de l'espèce *I. montana*. Par conséquent le genre *Inula* a fait l'objet de nombreuses études.

L'étude bibliographique réalisée sur les huiles essentielles de certaines espèces du genre *Inula* a montré qu'elles sont constituées généralement par le même composé alors qu'elles sont récoltées dans différentes régions et différents pays.

L'huile essentielle de *I. ensifolia* et *I. salicina* collectées en Bulgarie et *I. verbascifolia* collectée en Croatie se composent principalement d'acide hexadécanoïque [127,128]. De même, les huiles essentielles de *I. oculus-christi* de Turquie, *I. viscosa* d'Algérie et *I. graveolens* de France sont chemotypées par le bornylacétate [129,19].

En revanche, l'huile essentielle de *I. crithmoides* récoltée en Tunisie [130], en Espagne, Malte [131], et en Italie centrale [128] est principalement composée de p-cymène avec des pourcentages variables tandis que celle de la Grèce [130] par le β -phellandrène et celle de la Sicile (Italie) [132] par le thymol. Cependant, les huiles essentielles de *I. germanica* et *I. bifrons* [127] récoltées en Bulgarie sont caractérisées respectivement par l'acétate de cis-carvyl et le Muurola-4,10 (14) dien-1-ol, alors que l'huile de *I. graveolens* [19] de la Turquie est constituée par le 1,8-cinéole.

II.2 *Carlina hispanica*

La recherche bibliographique exhaustive menée sur l'espèce *Carlina hispanica* a montré que l'huile essentielle de la partie souterraine n'a fait l'objet d'aucune étude, ce qui nous a incité à déterminer sa composition chimique et à l'isolation de son composé majoritaire.

II.2.1 Provenance du matériel végétal et rendement

La cueillette de la partie souterraine de *Carlina hispanica* a eu lieu au mois de mai 2019 à Ain Fezza wilaya de Tlemcen (Algérie). Les racines ont été soumises à une hydrodistillation dans un appareil de type Clevenger pendant 5 heures, un rendement de 1,4 % par rapport à la masse du végétal frais a été obtenu. Du fait de la rareté de l'espèce au niveau de la région de Tlemcen la campagne d'échantillonnage a été limitée à une seule station.

II.2.2 Composition chimique de l'huile essentielle de *Carlina hispanica*

▪ Composition chimique

Après l'hydrodistillation de la partie racinaire de *Carlina hispanica*, une huile essentielle de couleur jaune-orangé a été obtenue. La composition chimique de l'huile essentielle a été analysée par CPG et CPG-SM. Ce qui a permis l'identification de sept composés constituant 98,7% de la composition totale du contenu identifié (Tableau 3).

L'huile essentielle des racines de *C. hispanica* a été caractérisée par la prédominance d'un composé oxygéné non-terpénique, le benzyle-2-furylacétylène ou l'oxyde de carline (81,1%), suivis par les sesquiterpènes hydrocarbonés de (13,1%) et un sesquiterpène oxygéné (4,5%) (Tableau 3).

Tableau 3. Composition chimique de l'huile essentielle des racines de *Carlina hispanica*.

| N | Composés | Ira ^d | Ira ^c | Lit/Ira ^b | HE | Identification |
|---------------------------------|-----------------------------|------------------|------------------|----------------------|-------------|----------------|
| 1 | Trans caryophyllène | 1424 | 1431 | 1582 | 0,1 | IR, SM |
| 2 | α -curcumène | 1471 | 1470 | 1771 | 2,3 | IR, SM |
| 3 | Trans- β -bergamotène | 1480 | 1477 | - | 4,4 | IR, SM |
| 4 | Zingébérène | 1489 | 1496 | 1717 | 4,5 | IR, SM |
| 5 | β -Sesquiphellandrene | 1516 | 1514 | 1768 | 1,8 | IR, SM |
| 6 | Benzyl-2-furylacétylène | 1580 | 2380 | - | 81,1 | IR, SM, RMN |
| | Oxyde de carline | | | | | |
| 7 | Z, Z-farnésal | 1653 | 1661 | 2172 | 4,5 | IR, SM |
| Total d'identification % | | | | | 98,7 | |
| Composé oxygéné non-terpénique | | | | | 81.1 | |
| Sesquiterpènes hydrocarbonés | | | | | 13.1 | |
| Sesquiterpène oxygéné | | | | | 4.5 | |

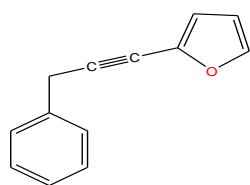
^a Ordre d'élution donné sur une colonne apolaire (Rtx-1)

^b Indices de rétention sur une colonne apolaire issus de la littérature

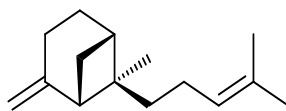
^c Indices de rétention sur une colonne apolaire Rtx-1

^d Indices de rétention sur colonne polaire Rtx-wax

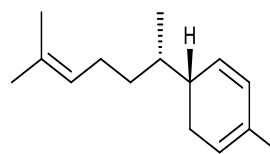
^e Ir : Indices de rétention ; SM : Spectres de Masse en impact électronique ; Réf : Composés identifiés l'aide de bibliothèques commerciales [126]



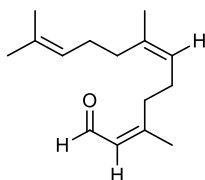
Benzyl-2-furylacétylène
(Oxyde de carline)



Trans- β -bergamotène



Zingébérène



Z, Z-farnésal

Figure 5. Structures chimiques des principaux constituants identifiés dans l'huile essentielle des racines de *Carlina hispanica*.

La recherche bibliographique effectuée sur les huiles essentielles des racines des espèces appartenant au genre *Carlina* a révélé qu'elles sont toutes chémotypées par l'oxyde de carline, en effet l'huile essentielle de *C. vulgaris* et *C. gummefera* récoltées en Algérie précisément dans la région de Tlemcen ont été caractérisées par l'oxyde de carline avec un pourcentage de 33,7% et 63,2% respectivement [133,134]. Pour l'huile essentielle de *C. acaulis* récolté en Italie, l'oxyde de carline a été détectée avec un pourcentage de 94,6% [135]. D'autre part, dans l'huile essentielle de *C. radix* elle a été signalée avec un pourcentage de 98,9% [136], Cependant, l'arcurcumene a été détecté dans les deux espèces avec un pourcentage de 0,4% et 0,2% respectivement. Enfin pour l'huile essentielle de *C. acanthifolia*, l'oxyde de carline a été caractérisée avec un pourcentage de 91,5% [136].

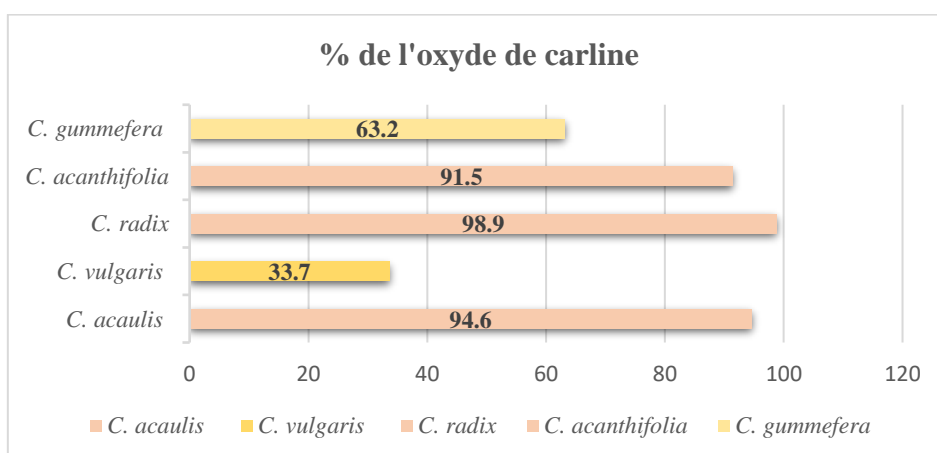


Figure 6. Pourcentage de l'oxyde de carline dans certaines plantes du genre *Carlina*.

2.2.3. Fractionnement et isolement de l'oxyde de carline

L'objectif de cette partie du travail consiste à isoler et purifier la molécule l'oxyde de carline afin d'évaluer par la suite son potentiel biologique dans l'espoir de trouver de nouvelles molécules dotées d'activités biologiques.

L'huile essentielle a été soumise à une chromatographie sur colonne sur gel de silice éluée avec de l'hexane et l'hexane/acétate d'éthyle. Le schéma de fractionnement est représenté dans la Figure 7. Les identifications qui ont été faites ont nécessité la mise en place d'autres analyses complémentaires telles que la RMN ^1H , ^{13}C et la comparaison du composé séparé avec les données décrites dans la littérature [137] nous a permis d'identifier le composé majoritaire sans ambiguïté comme étant le benzyl-2-furylacétylène connu également sous le nom de oxyde de carline.

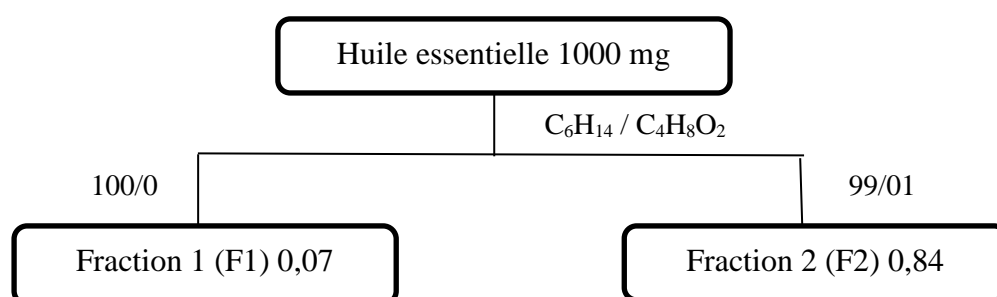


Figure 7. Schéma de fractionnement.

Tableau 4. Attribution des déplacements chimique des protons et des carbones.

| Carbones | δ ppm | Attribution | Protons | δ ppm | Attribution | HMBC |
|----------|--------------|--------------------|---------|--------------|-------------|--|
| C7 | 25,71 | 1C.CH ₂ | H7 | 3,83 | 2H.s | C7-H7 |
| C8-C9 | 91,81 | C≡C | | | | C8-H7 C9-H7 |
| C12 | 110,56 | 1C.CH.furane | H12 | 6,37 | 1H.dd | C12-H13 C12-H2.H6. |
| C11 | 114,09 | 1C.CH.furane | H11 | 6,47 | 1H.dd | C12- H7 C11-H13 C11-H2.H6 C11-H7 |
| C4 | 126,67 | 1CH.phényle | H4 | 7,33 | 6H.m | C2.C3.C4.C5. C6-H2.H3.H4. H5.H6.H13 C4-H7 |
| C3-C5 | 127,85 | 1CH.phényle | H3-H5 | 7,31 | | |
| C2-C6 | 128,48 | 1CH.phényle | H2-H6 | 7,26 | | |
| C1 | 135,79 | 1C.phényle | | | | C1-H2.H3.H4. H5.H6 C1-H7 |
| C10 | 137,24 | 1C.furane | | | | C10-H11-H12 |
| C13 | 142,81 | 1C.CH.furane | H13 | 7,42 | | C13-H11.H12 |

δ_c & δ_H : Déplacements chimique des carbones et des hydrogènes. La corrélation entre les carbones et les hydrogènes est déterminée grâce au spectre *HMBS*.

Le spectre RMN ^1H montre dans la région des champs forts un singulet intégrant 2 protons caractéristique d'un groupement éthyle (C^7H_2) avec un déplacement $\delta=3,83\text{ppm}$. On observe aussi deux doublet de dédoublets ($\text{C}^{11}\text{H}_{11}$, $\text{C}^{12}\text{H}_{12}$) à un déplacement chimique $\delta = 6,37\text{ ppm}$ et $\delta = 6,47\text{ ppm}$ attribuable aux protons furfural et 6 signaux caractéristiques de protons aromatiques $\delta=7,33-7,42\text{ ppm}$. Ce qui nous conduit vers la présence d'un cycle de type furane et d'un groupement phényle monosubstitué.

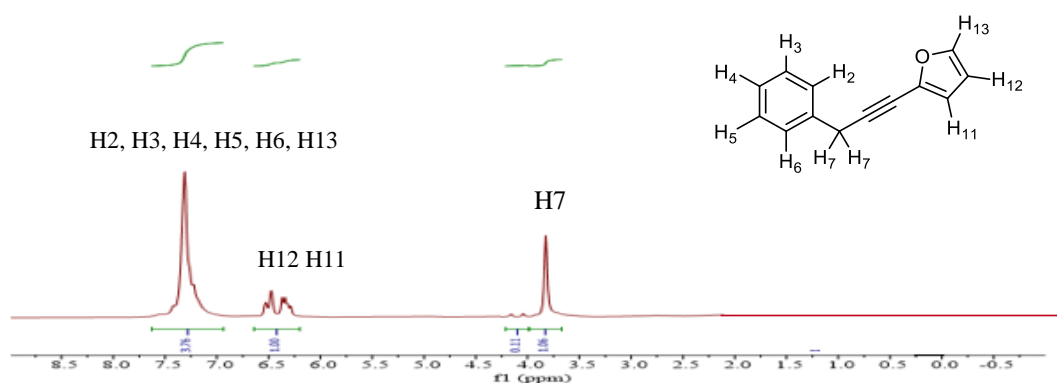


Figure 8. Spectre RMN ^1H de l'oxyde de carline.

L'examen du spectre RMN ^{13}C a permis d'observer 13 pics de résonance appartenant au composé majoritaire de l'huile essentielle dont 4 pics d'intensités faibles indiquent la présence de 4 carbones quaternaires, et 2 pics avec une très forte intensité indiquent la présence des carbones aromatiques. L'enregistrement du spectre en séquence DEPT 135, nous a emmené vers une formule brute de type $\text{C}_{13}\text{H}_{10}\text{O}$. et nous a confirmé également la présence d'un groupement éthylène CH_2 .

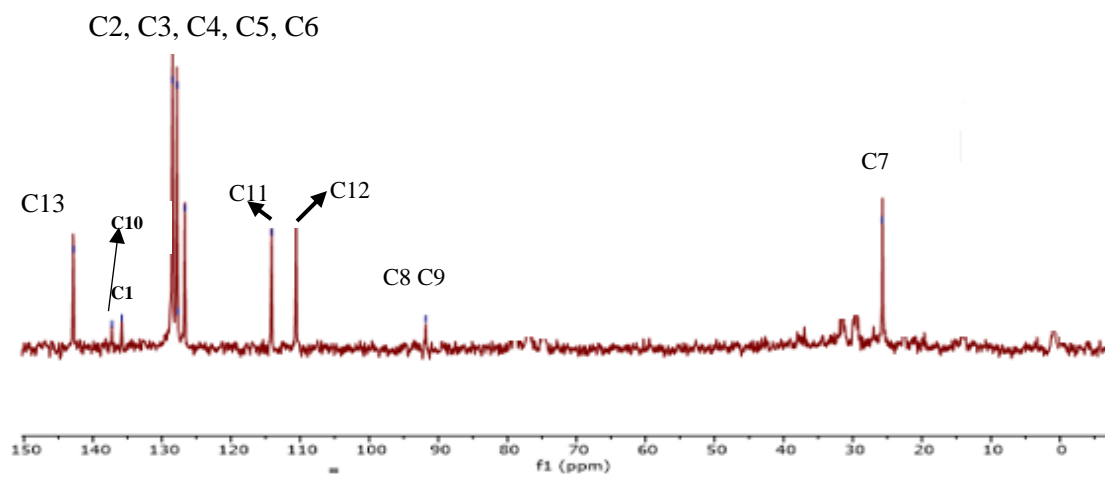


Figure 9. Spectre RMN ¹³C de l'oxyde de carline.

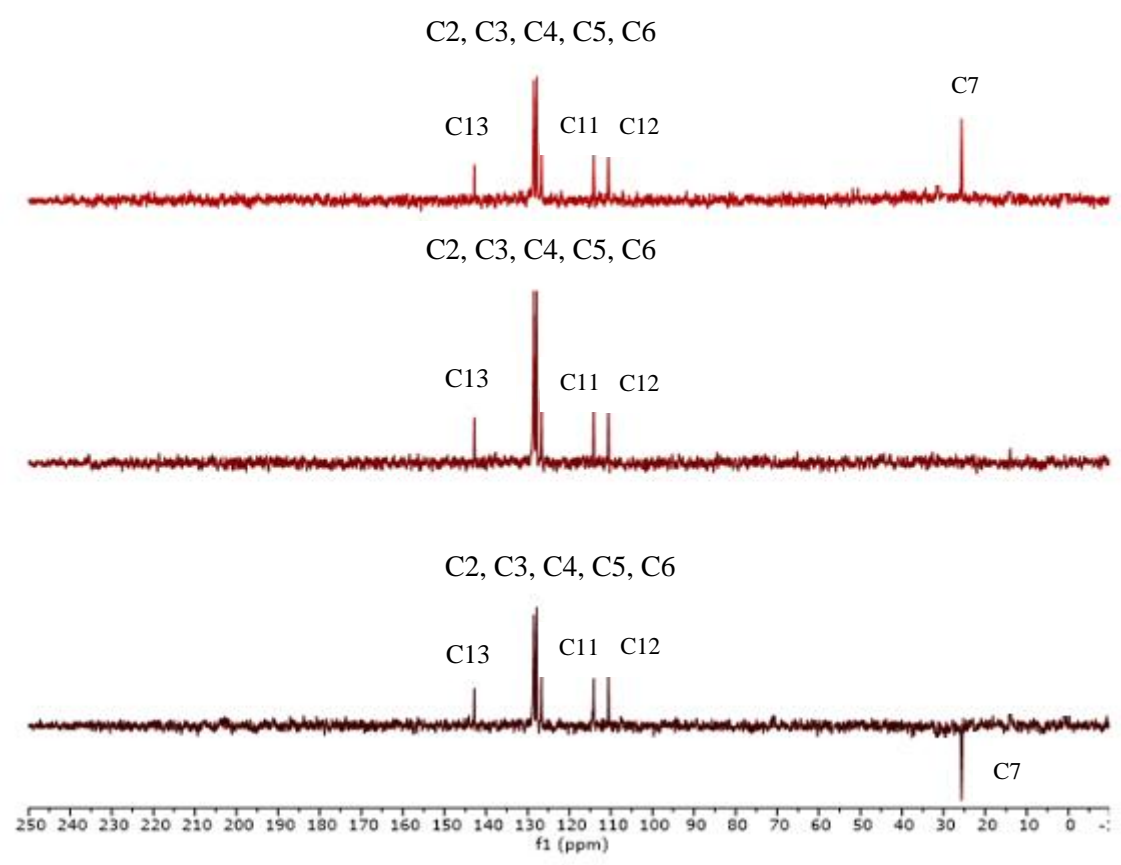


Figure 10. Spectre RMN ¹³C DEPT de l'oxyde de carline.

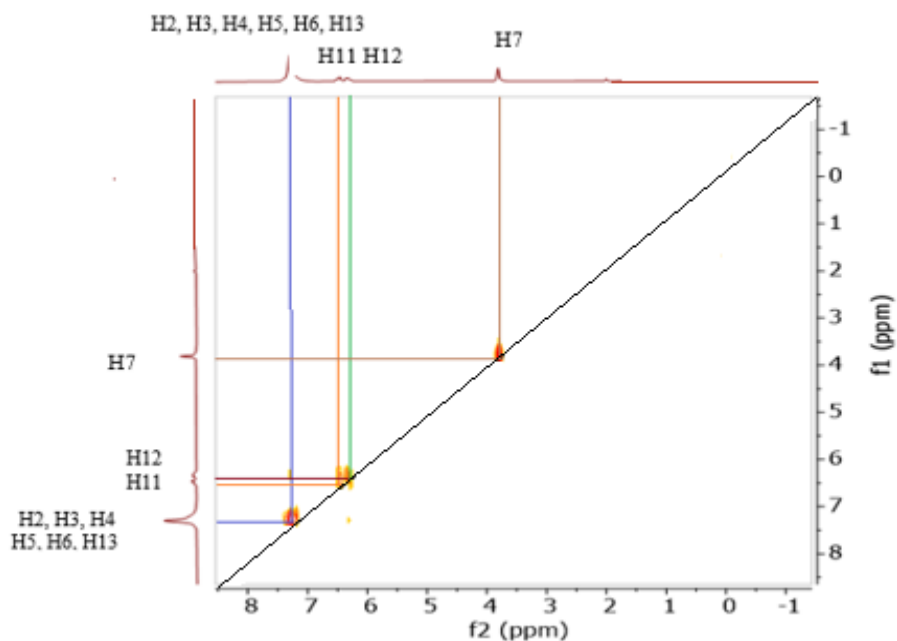


Figure 11. Spectre RMN 2D COSY de l'oxyde de carline.

L'enregistrement des séquences de corrélations proton-carbone HSQC et HMBC a confirmé la présence d'un cycle furane et un groupe benzylique associés par un système acétylénique.

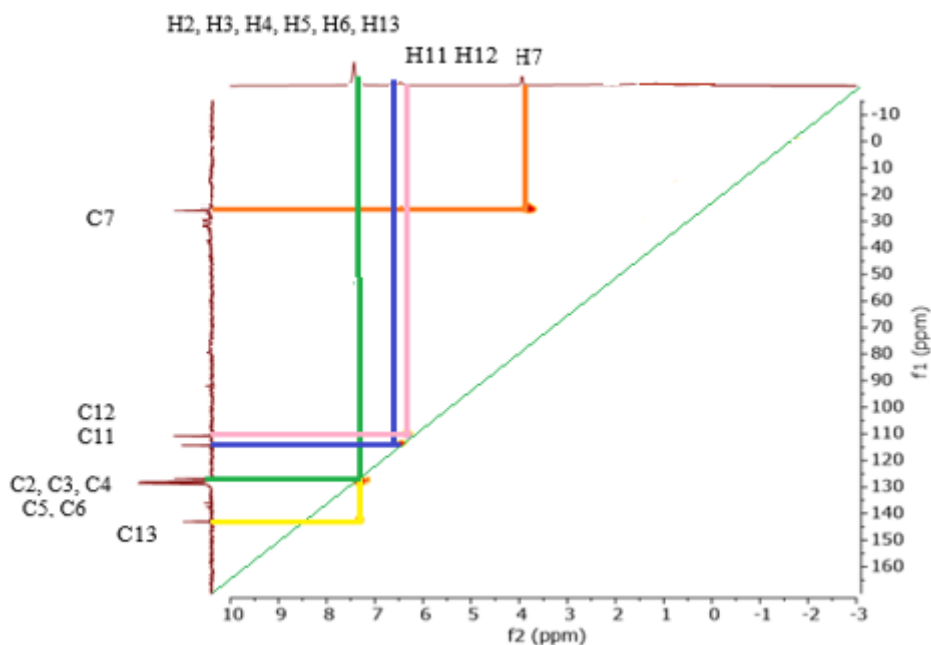


Figure 12. Spectre RMN 2D HMQC de l'oxyde de carline.

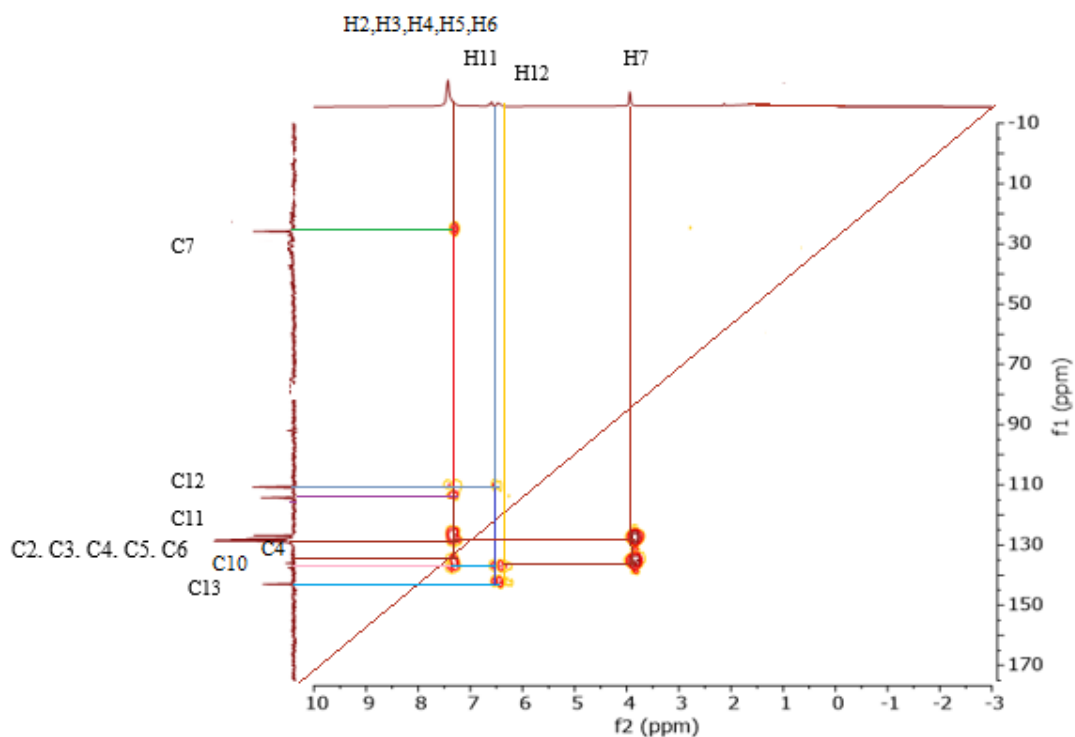


Figure 13. Spectre RMN 2D HMBC de l'oxyde de carline.

La comparaison des données RMN ^1H et ^{13}C de notre échantillon avec celles trouvées dans la littérature [140] a mené à l'identification non ambiguë du benzyle-2-furylacétylène connu également par l'oxyde de carline (Tableau 4. Figure 15).

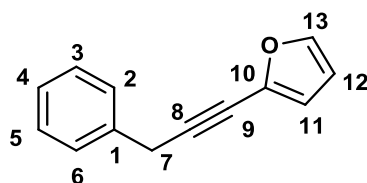


Figure 14. benzyle-2-furylacétylène ou oxyde de carline.

*Chapitre 3 : Evaluation Des Activités
Biologiques*

III.1 Evaluation des activités antioxydantes des huiles essentielles et de l'oxyde de carline

Les antioxydants sont des entités présentes à une concentration faible par rapport à celle des substrats oxydables [138], et qui sont capables de réagir avec les radicaux libres dans l'intention de retarder ou inhiber le processus d'oxydation des substrats biologiques qui se produisent sous l'influence de l'oxygène atmosphérique ou des espèces réactives de l'oxygène [139]. Cependant, les antioxydants synthétiques utilisés usuellement dans des applications alimentaires et pharmacologiques tels que le butylhydroxyanisole (BHA) et le butylhydroxytoluène (BHT) sont suspectés à long terme d'être potentiellement néfastes sur la santé humaine [15] [140,141].

Actuellement, la recherche d'alternatives aux antioxydants synthétiques par de nouveaux produits d'origine naturelle, efficace et écologique pour la conservation des produits cosmétiques et alimentaires s'oriente de plus en plus vers les huiles essentielles et plus particulièrement aux activités biologiques de leurs composants [142].

Les huiles essentielles représentent une source potentielle de molécules naturelles et bioactives en particulier les phénols, ce qui leur confèrent de bonnes propriétés biologiques et thérapeutiques puissantes notamment comme antioxydantes, anticancéreuses, antiinflammatoires, insecticides, antimicrobiennes, antivirales, et analgésiques [143-148]. D'autre part, il existe des huiles essentielles qui ne contiennent pas de composés phénoliques mais qui expriment un comportement antioxydant due à la chimie radicalaire de certains terpénoides et d'autres composants volatils [149].

Ces essences naturelles ne sont pas appliquées uniquement en monothérapie, mais plutôt en combinaison afin d'agir en synergie dans le but de rendre leurs effets meilleurs et ceci depuis plusieurs années [150].

Dans cette optique, nous avons étudié la présence d'une éventuelle activité antioxydante de l'huile essentielle de *Inula montana* en utilisant la méthode du DPPH et FRAP et DPPH, FeCl₂, β-carotène pour l'huile essentielle de *Carlina hispanica*. Dans le but de réaliser une analyse plus approfondie de la capacité antioxydante, on a évalué le pouvoir antioxydant de l'oxyde de carline (composé majoritaire) et sa combinaison avec le BHT et EDTA comme des antioxydants de référence.

Inula montana

Le pouvoir antioxydant de l'huile essentielle de *Inula montana* a été évalué à l'aide de deux méthodes : test de piégeage du radical libre DPPH et la méthode de la réduction du fer FRAP, en utilisant l'acide ascorbique comme contrôle positif.

III.1.1.1 Test de piégeage du radical libre : DPPH

La technique du piégeage du radical libre DPPH permet l'évaluation du pouvoir antioxydant des échantillons à tester. En effet, elle est basée sur la réduction du radical DPPH synthétique de couleur violette caractéristique de la solution du DPPH et qui vire vers le jaune en présence d'antioxydant possédant une capacité anti radicalaire. En outre ce virage de couleur est accompagné d'une diminution de l'absorbance (DO) à 515 nm qui s'exprime par le pourcentage de réduction.

La technique du piégeage du radical libre DPPH est présentée généralement par la valeur CI_{50} (Concentration de l'inhibiteur causant 50% d'inhibition), car elle permet le classement des échantillons entre eux. En effet plus la CI_{50} est petite, plus l'échantillon est antioxydant.

La comparaison de l'activité de balayage DPPH de l'huile essentielle de *I. montana* avec celle exprimée par l'acide ascorbique a montré une bonne activité antioxydante. En effet, l'huile essentielle a la capacité de réduire le radical DPPH• à 78,57 % avec une concentration de l'ordre de 0,09 mL/L et une CI_{50} de 0,061 mL/L légèrement supérieure à celle de l'acide ascorbique ($CI_{50}=0,051$ mL/L) (Tableau 5).

Tableau 5 : Pourcentage d'inhibition du DPPH en fonction des concentrations de l'huile essentielle de *Inula montana* et de l'acide ascorbique.

| Echantillons | | Activité antioxydante | | | | |
|-------------------|--------------------------|-----------------------|-------------|-------------|--------------|--------------|
| Huile essentielle | Concentration (mL/L) | 0,04 | 0,05 | 0,06 | 0,075 | 0,09 |
| | Pourcentage d'inhibition | 33,06 | 42,01 | 49,75 | 63,6 | 78,57 |
| | CI_{50} (mL/L) | | | | | 0,059 |
| Acide ascorbique | Concentration (mL/L) | 0,04 | 0,05 | 0,06 | 0,07 | 0,08 |
| | Pourcentage d'inhibition | 36,03 | 47,95 | 61,27 | 75,32 | 90,7 |
| | CI_{50} (mL/L) | | | | | 0,051 |

III.1.1.2 Test de la réduction du fer : FRAP

La capacité réductrice de notre échantillon a été évaluée à l'aide de la technique colorimétrique couramment employée, elle est basée sur la mesure de la capacité de

l'échantillon à tester à réduire le fer ferrique Fe^{3+} présent dans le complexe ferricyanure de potassium $K_3Fe(CN)_6$ en fer ferreux Fe^{2+} en captant un électron. La teneur en ion Fe^{2+} peut être contrôlée et mesurée grâce à la formation du bleu de perl à 700 nm. En outre une augmentation de la capacité réductrice est toujours indiquée par une augmentation de l'absorbance (DO).

La figure 15 représente les valeurs d'absorbance en fonction de différentes concentrations d'huile essentielle obtenues par la méthode FRAP. Le pouvoir antioxydant de l'huile essentielle augmente avec l'augmentation de la concentration. Cependant, à une concentration de 0,15 mL/L, l'activité antioxydante de l'huile essentielle a été supérieure à celle de l'antioxydant synthétique (acide ascorbique) (Figure 15).

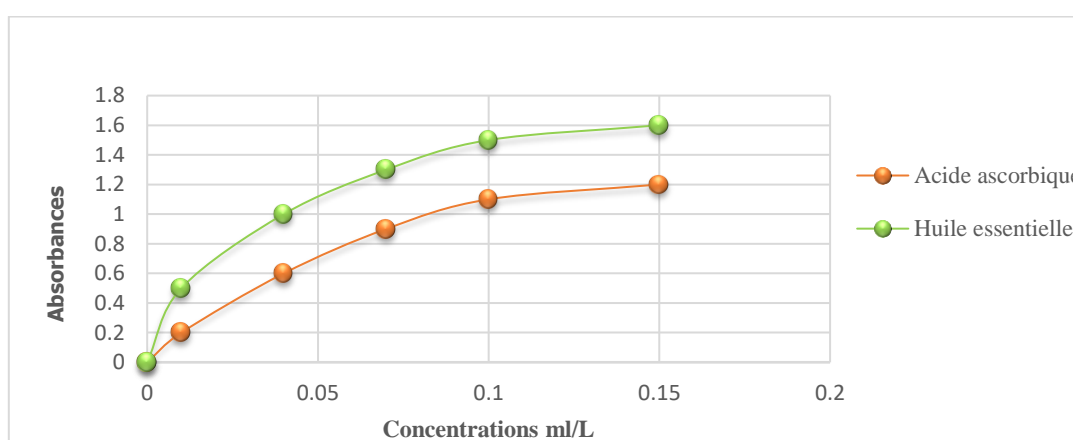


Figure 15. Pouvoir réducteur de l'huile essentielle de *I. montana* et l'acide ascorbique.

III.2 *Carlina hispanica*

L'oxyde de carline

▪ Travaux antérieurs

Les produits d'origine naturelle ont tendance à être très efficaces et ne présentent pas d'effets secondaires. Les composés acétyléniques peuvent se montrer très actifs contre les pathogènes et peuvent être avantageux sur la santé. Parmi cette classe de composés, l'oxyde de carline un composé obtenu à partir des racines des huiles essentielles ou des extraits aux solvants de certaines espèces appartenant à la famille des *Astéracées* tel que le genre *Carlina*.

L'étude des travaux préliminaires des activités biologiques des huiles essentielles riches en oxyde de carline ont montré des effets prometteurs. Ce qui a permis de tirer l'hypothèse que l'origine de ces activités est principalement due au constituant majoritaire. A titre d'exemple, les espèces *C. gummifera*, *C. acaulis*, *C. acanthifolia* et *C. radix*, leurs huiles essentielles ont

montré plusieurs potentiels biologiques comme antioxydant, antimicrobien, antiinflammatoire, gastro protectrice et insecticide [159-163].

III.2.1.1 Test de piégeage du radical libre : DPPH

Le pouvoir antioxydant de l'huile essentielle, l'oxyde de carline et de l'antioxydant standard est reporté dans le Tableau 6.

Tableau 6 : Pourcentage d'inhibition du DPPH en fonction des concentrations de l'huile essentielle, l'oxyde de carline et de la combinaison.

| Echantillons | | Activité antioxydante | | | | | |
|---|--------------------------|-----------------------|----------|----------|----------|-----------|-----------|
| Huile essentielle | Concentration (mg/mL) | 0,5 | 1 | 3 | 5 | 10 | 15 |
| | Pourcentage d'inhibition | 37,44 | 40,3 | 42,35 | 45,02 | 49,5 | 52,59 |
| | CI ₅₀ (mg/mL) | | | | | | |
| Oxyde de carline | Concentration (mg/mL) | 0,5 | 1 | 3 | 5 | 10 | 15 |
| | Pourcentage d'inhibition | 37,27 | 40,1 | 43,61 | 45,98 | 52,6 | 61,28 |
| | CI ₅₀ (mg/mL) | | 1 | | | | |
| BHT | Concentration (mg/mL) | 0,5 | 1 | 3 | 5 | 10 | 15 |
| | Pourcentage d'inhibition | 28,5 | 30,4 | 35,9 | 40,8 | 64,7 | 75,6 |
| | CI ₅₀ (mg/mL) | | | | | | |
| Combinaison Oxyde de carline-BHT | Concentration (mg/mL) | 0,5 | 1 | 3 | 5 | 10 | 15 |
| | Pourcentage d'inhibition | 38,8 | 42,13 | 55,04 | 63,5 | 78,71 | 92,3 |
| | CI ₅₀ (mg/mL) | | | | | | |

La comparaison de l'activité de balayage DPPH de l'huile essentielle de *C. hispanica* et de l'oxyde de carline avec celui exprimé par le BHT a montré une bonne activité antioxydante par rapport au composé synthétique BHT pour les concentrations inférieure à 10 mg/mL. Les activités de piégeage des radicaux libres obtenues ont été d'environ de 61,28% pour l'oxyde de carline et 52,59% pour l'huile essentielle, tandis que la plus forte activité a été obtenue par le mélange de l'oxyde de carline et du BHT (92,3%) à une concentration de 15 mg/mL (Tableau 6). La concentration inhibitrice (CI₅₀) fournissant une inhibition de 50% de DPPH dans la solution d'essai a été calculée (voir Tableau 6).

La combinaison de l'oxyde de carline avec le BHT a indiqué un effet synergique très prometteur dans la trempe de radical DPPH•, avec une CI₅₀ de 2,5 mg/mL, soit environ 3 fois plus élevé que l'oxyde de carline seule (CI₅₀=7,81 mg/mL) et l'antioxydant synthétique utilisé

comme référence ($CI_{50} = 6,93$ mg/mL), et 5 fois plus élevée que celle de l'huile essentielle ($CI_{50} = 11,32$ mg/mL ; Tableau 6).

III.2.1.2 Test de blanchissement du β -carotène

Dans la méthode de blanchissement du β -carotène, nous avons utilisé de l'acide linoléique qui est un acide gras polyinsaturé, où les radicaux peroxydes formés par l'oxydation de ce dernier suite à l'abstraction de ses atomes d'hydrogènes à partir des groupements méthylènes dans une émulsion, oxyde le β -carotène qui est hautement insaturé et entraîne aussi la perte de sa couleur orange suivie par spectrophotométrie à 470 nm.

La capacité antioxydante des échantillons a été déterminée en mesurant les absorbances dans le temps dans des milieux réactionnels. En absence des échantillons testés l'absorbance diminue tandis qu'en leur présence ce phénomène est ralenti.

Tableau 7 : Activité antioxydante de l'huile essentielle, l'oxyde de carline et de la combinaison par la méthode de dosage de blanchiment du β -carotène /acide linoléique à différentes concentrations.

| Echantillons | | Activité antioxydante | | | | | | |
|---|--|-----------------------|----------|----------|----------|------------|-----------|--------------|
| Huile essentielle | Concentration (mg/mL) | 0,5 | 1 | 3 | 5 | 5,5 | 10 | 15 |
| | Test de blanchiment du β -carotene [%] | 4,17 | 5,25 | 15,06 | 22,18 | 26,13 | 43,42 | 56,06 |
| | CI_{50} (mg/mL) | | | | | | | 12,59 |
| Oxyde de carline | Concentration (mg/mL) | 0,5 | 1 | 3 | 5 | 5,5 | 10 | 15 |
| | Test de blanchiment du β -carotene [%] | 28,13 | 31,53 | 39,16 | 50,3 | 55,75 | 75,49 | 88,54 |
| | CI_{50} (mg/mL) | | | | | | | 5,08 |
| BHT | Concentration (mg/mL) | 0,5 | 1 | 3 | 5 | 5,5 | 10 | 15 |
| | Test de blanchiment du β -carotene [%] | 30,62 | 32,93 | 44,51 | 51,7 | 56,72 | 71,35 | 83,49 |
| | CI_{50} (mg/mL) | | | | | | | 4,87 |
| Combinaison Oxyde de carline-BHT | Concentration (mg/mL) | 0,5 | 1 | 3 | 5 | 5,5 | 10 | 15 |
| | Test de blanchiment du β -carotene [%] | 39,0 | 45,8 | 53,34 | 57,25 | 63,98 | 85,49 | 98,45 |
| | CI_{50} (mg/mL) | | | | | | | 2,45 |

Les résultats obtenus ont montré que l'huile essentielle et l'oxyde de carline sont dotées d'une très bonne activité vis-à-vis les radicaux peroxydes générés par l'oxydation de l'acide linoléique (Tableau 7).

L'activité d'élimination des radicaux libres la plus intéressante a été révélée par la combinaison de l'oxyde de carline et le BHT qui a un effet synergique avec une CI_{50} de l'ordre de 2,45 mg/mL soit environ 5 fois plus forte que celle de l'huile essentielle avec une CI_{50} de

12,59 mg/mL, suivie par l'antioxydant synthétique BHT (CI_{50} = 4,87 mg/mL) et de l'oxyde de carline (CI_{50} = 5,08 mg/mL).

III.2.1.3 Test de chélation du fer ferreux

La chélation du fer ferreux a été évaluée en utilisant la ferrozine qui forme un complexe avec le fer résiduel dans le milieu réactionnel et forme un chromophore rouge (Fe (II)-Ferrozine) ayant un maximum d'absorption à 562 nm.

Tableau 8 : Activité antioxydante de l'huile essentielle, oxyde de carline et de la combinaison oxyde de carline-EDTA par le test de chélation du fer ferreux.

| Echantillons | | Activité antioxydante | | | | | | |
|--|-----------------------|-----------------------|----------|----------|----------|------------|-----------|--------------|
| Huile essentielle | Concentration (mg/mL) | 0,5 | 1 | 3 | 5 | 5,5 | 10 | 15 |
| | Test de chélation [%] | 3,08 | 4,11 | 14,04 | 20,29 | 25,31 | 41,62 | 55,06 |
| | CI_{50} (mg/mL) | | | | | | | 12,95 |
| Oxyde de carline | Concentration (mg/mL) | 0,5 | 1 | 3 | 5 | 5,5 | 10 | 15 |
| | Test de chélation [%] | 9,09 | 13,37 | 20,82 | 33,22 | 34,82 | 53,00 | 69,46 |
| | CI_{50} (mg/mL) | | | | | | | 9,7 |
| EDTA | Concentration (mg/mL) | 0,5 | 1 | 3 | 5 | 5,5 | 10 | 15 |
| | Test de chélation [%] | 39,79 | 59,00 | 60,9 | 73,25 | 75,49 | 94,68 | - |
| | CI_{50} (mg/mL) | | | | | | | 0,76 |
| Combinaison Oxyde de carline-EDTA | Concentration (mg/mL) | 0,5 | 1 | 3 | 5 | 5,5 | 10 | 15 |
| | Test de chélation [%] | 23,57 | 32,76 | 55,24 | 64,56 | 65,25 | 98,62 | - |
| | CI_{50} (mg/mL) | | | | | | | 3,28 |

Les résultats obtenus révèlent que l'huile essentielle ainsi que l'oxyde de carline possèdent une activité chélatrice intéressante vis-à-vis à celle de l'ion ferreux.

Une chélation de 55,06% du fer ferreux est obtenue avec une concentration de 15 mg/mL de l'huile essentielle. À la même concentration, l'oxyde de carline exerce une chélation de 69,46%. L'EDTA donne une chélation maximale de 94,68% à une concentration de 10 mg/mL. Cependant, la combinaison de l'oxyde de carline et l'EDTA à une concentration de 10 mg/L a donné une chélation maximale de 98,62% ce qui montre que le mélange présente un effet synergique très intéressant.

La demi-concentration maximale inhibitrice (CI_{50}) la plus puissante a été obtenue par la combinaison de l'oxyde de carline et l'EDTA (CI_{50} = 3,28 mg/mL) qui est 4 fois plus élevée que celle de l'huile essentielle (CI_{50} = 12,95 mg/mL) et 3 fois plus élevée que l'oxyde de carline seule (CI_{50} = 9,7 mg/mL).

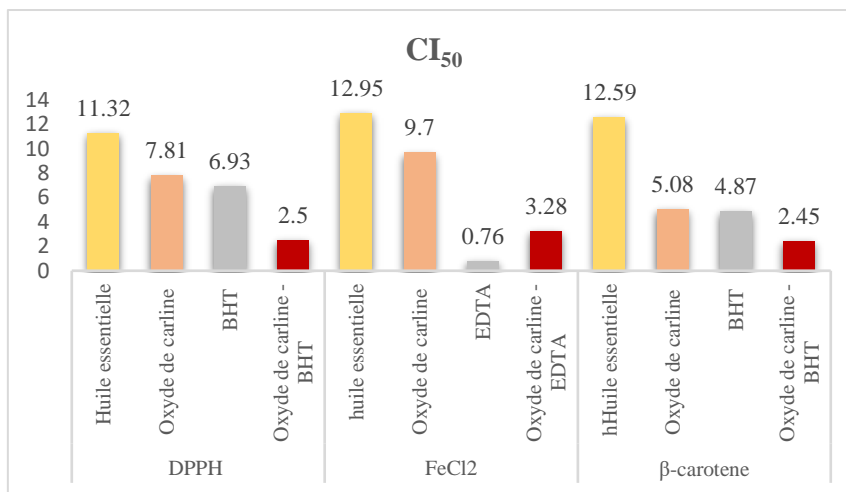


Figure 16. CI₅₀ déterminées par les tests DPPH, FeCl₂ et β-carotène.

L'histogramme (Figure 16) montre la corrélation entre les résultats obtenus par les trois méthodes utilisées pour l'évaluation de l'activité antioxydante.

D'après la figure 16, on peut en conclure que la plus grande activité antioxydante obtenue par les trois méthodes a été attribuée à la combinaison de l'oxyde de carline avec les références synthétiques avec des CI₅₀ respectivement de 2.5 mg/mL et 2.45 mg/mL par la méthode de DPPH, FeCl₂ et β-carotène, meilleure que les antioxydants de synthèse.

Cependant, le pouvoir antioxydant le plus faible obtenu par les trois méthodes a été observé pour l'huile essentielle avec les CI₅₀ les plus élevées, largement supérieures aux antioxydants synthétiques.

Dans la présente étude, nous avons constaté que l'ensemble des échantillons testés a montré une activité antioxydante prometteuse avec des CI₅₀ proches à celles des antioxydants synthétiques comme l'acide ascorbique, BHT et EDTA, avec de bonne cohérence des résultats entre les différents tests effectués.

Le pouvoir antioxydant de l'huile essentielle de la partie aérienne de *Inula montana* peut être lié à son profil chimique, notamment aux sesquiterpènes oxygénés et hydrocarbonés présents en pourcentage élevé tels que le shyobunol, le α-cadinol, le tau-cadinol, le δ-cadinène, le viridiflorol, l'oxyde de caryophyllène, le Z, E- acétate de farnésyle et l'ar-curcumen-15-al, car il a été noté que les monoterpènes et les sesquiterpènes comprennent des propriétés antioxydantes [164]. Cependant, il a été signalé aussi que l'activité antioxydante ne résulte pas purement des composés majoritaires mais aussi d'autres composés minoritaires ayant la possibilité d'agir de manière synergique ou antagoniste afin de créer un système efficace contre les radicaux libres [165].

De même, l'huile essentielle de la partie souterraine de *Carlina hispanica* a révélé une bonne activité antioxydante, ce qui peut s'expliquer par le pourcentage élevé de l'oxyde de carline, qui a déjà été suspecté d'être responsable de la propriété antioxydante que possède l'huile essentielle de *Carlina acanthifolia* [166]. Par ailleurs, d'autres études menées sur les différentes propriétés biologiques de l'oxyde de carline ont montré qu'elle est dotée de plusieurs activités : antitrypanosmiques, antiulcéreuse, antimicrobienne, antiinflammatoire et antioxydante [167-171]. Sur cette base nous avons décidé d'évaluer la capacité antioxydante de cette molécule ainsi de sa combinaison avec les antioxydants synthétiques qui a révélé un pouvoir antioxydant plus fort avec un effet synergique important. Selon des travaux antérieurs réalisés sur les effets synergiques des huiles essentielles de *A. verticulata*, *S. argentea* et *S. candidissima*, il a été observé que le mélange de ses dernières a donné un meilleur pouvoir antioxydant que celui des huiles individuelles [172].

Les résultats obtenus ouvrent des perspectives de l'utilisation de ces huiles essentielles ainsi que de leurs combinaisons comme agents antioxydants dans différentes applications, tout en diminuant la dose minimale efficace, ce qui peut aider par la suite à minimiser les effets secondaires indésirables des antioxydants synthétiques en les rendant plus efficaces.

III.3 Activité antiinflammatoire de l'huile essentielle de *Carlina hispanica* et de l'oxyde de carline

L'inflammation est un moyen de défense naturelle de l'organisme supérieur à une agression extérieure telles que les blessures et les infections dans l'intention d'éliminer l'agent pathogène, réparer les lésions et pour maintenir son intégrité [172-174]. Son rôle essentiel repose sur la mobilisation du système immunitaire.

Cependant, les médicaments antiinflammatoires commercialisés (stéroïdiens, non stéroïdiens et opioïdes) sont contraignants et insatisfaisants bien qu'ils soient assez puissants à cause d'effets indésirables qu'ils entraînent comme les accidents cardiovasculaires, les intolérances gastriques, les convulsions et les actions immunosuppressives [175-177].

De ce point de vue, il est impérieux de substituer ces traitements chimiques par des agents thérapeutiques antiinflammatoires de source naturelle, efficace, avec moins d'effets secondaires et une sécurité prouvée tels que certains constituants des huiles essentielles possédant un effet similaire voir plus efficace.

Les huiles essentielles constituent une nouvelle option dans la médication des maladies inflammatoires inclusivement l'arthrite, les rhumatismes et les allergies [178]. L'activité antiinflammatoire de l'huile essentielle de *Melaleuca alternifolia* ainsi que son composé

principal l' α -terpinéol sont parmi les études qui ont été mise en évidence pour l'évaluation de l'activité antiinflammatoire [179-182].

À notre connaissance, aucune étude sur l'évaluation de la capacité antiinflammatoire *in vitro* par le test d'inhibition de la dénaturation des protéines de l'espèce *Carlina hispanica* étudiée n'a été rapportée dans la littérature. Cette partie d'étude met en évidence l'évaluation *in vitro* de l'huile essentielle de *C. hispanica*, l'oxyde de carline et de la combinaison oxyde de carline-diclofénac de sodium.

Effet sur la dénaturation protéique

Les résultats de l'effet inhibiteur de la dénaturation de l'albumine en fonction des différentes concentrations des échantillons et comparés à ceux enregistrés par le diclofénac de sodium, considéré dans ce test comme un médicament de référence, sont résumés dans la Figure 17.

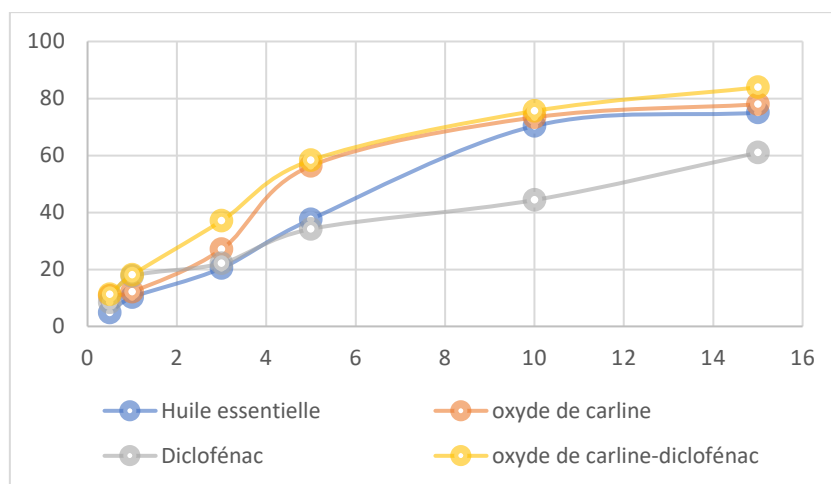


Figure 17. Pourcentage d'inhibition de la dénaturation de l'albumine d'œuf par l'huile essentielle de *C. hispanica*, oxyde de carline et de la combinaison oxyde de carline-diclofénac de sodium.

L'ajout de concentration croissante de l'huile essentielle de *C. hispanica*, oxyde de carline et du mélange oxyde de carline-diclofénac de sodium à la solution d'albumine d'œuf empêche la précipitation de cette protéine. Or, à une concentration de 15 mg/mL, les pourcentages d'inhibition ont été de 75,02% pour l'huile essentielle et 77,95% pour l'oxyde de carline. Cependant à la même concentration, le mélange oxyde de carline-diclofénac de sodium a représenté un effet synergique important avec pourcentage plus élevé 83,90%.

Les composés volatils des huiles essentielles sont mis à profit depuis fort longtemps par les anciennes civilisations en raison de leurs effets bénéfiques afin de soigner les pathologies

courantes, L'évaluation du pouvoir antiinflammatoire de l'huile essentielle, l'oxyde de carline et du mélange oxyde de carline-diclofénac de sodium élucidé dans cette étude a affiché des résultats intéressants.

En majeure partie, les propriétés thérapeutiques des huiles essentielles sont référées à leurs structures chimiques à titre d'exemple, l'effet antiinflammatoire est attribué aux structures terpénoides tels que le trans-cinnamaldehyde, (-) aromadendrene, l'oxyde de caryophyllene, le T-cadinol, et le α -cadinol qui ont montré une excellente activité antiinflammatoire [124].

L'activité antiinflammatoire de l'huile essentielle de *C. hispanica* est attribuée à sa teneur en oxyde de carline, une structure acétylénique, qui a affiché un pouvoir antiinflammatoire plus élevé que celui de l'huile essentielle [166]. D'autre part, le mélange de l'oxyde de carline-diclofénac de sodium a affiché l'activité la plus prometteuse avec un effet synergique intéressant.

III.4 Activité antimicrobienne des huiles essentielles et de l'oxyde de carline

Les bactéries et les fongicides résistent de plus en plus aux antibiotiques et ne s'arrêtent pas d'évoluer et de développer des résistances multiples. Ils ne cessent également de défier le secteur de la santé dans les pays développés et en voie de développement [167-169].

À cause de leur toxicité aigüe, les effets cancérogènes et les risques sur l'environnement, l'emploi des agents antimicrobiens qui sont à l'essor de la chimie est limité. Par ailleurs, l'utilisation non adéquate des antibiotiques afin de lutter contre les micro-organismes pathogènes délimite leur efficacité et entraîne une augmentation rapide de leur résistance [170].

A cet égard et en réponse à la demande accrue des consommateurs de denrées naturelles, la recherche de nouvelles molécules bioactives pour minimiser ou éliminer les affections est d'actualité [171].

L'utilisation des huiles essentielles constitue une alternative très prometteuse aux antibiotiques qui sont attribuables avant tout à leurs compositions chimiques, plus précisément à la nature des constituants volatils majoritaires ou minoritaires [172]. Cependant, leurs actions exercent un large spectre sur les bactéries à gramme positif et à grammes négatifs [173], la raison pour laquelle elles sont considérées comme une source prometteuse de nouveaux médicaments naturels [174-175].

Dans ce contexte et en dépit des résultats préliminaires de l'activité antioxydante des huiles essentielles des deux espèces, l'oxyde de carline et des combinaisons oxyde de carline-

références, nous nous sommes intéressés à tester l'activité antimicrobienne en utilisant deux méthodes : la méthode de diffusion sur gélose et la méthode des microplaques à 96 puits.

III.4.1 *Inula montana*

Le pouvoir antimicrobien de l'huile essentielle de *I. montana* a été évalué contre six micro-organismes : cinq bactéries [deux à Gram positif et trois à Gram négatif] et une levure. L'activité a été testée qualitativement (méthode de diffusion sur disque en papier) et quantitativement (méthode de CMI). Les résultats sont mentionnés respectivement dans les Tableaux 8 et 9.

Les résultats obtenus montrent que l'huile essentielle évaluée a contribué à l'inhibition des souches testés à des degrés différents. En présence de l'huile essentielle, *Klebsiella pneumoniae* a été la souche la plus sensible avec un diamètre d'inhibition de 16 mm, suivie par *Listeria monocytogenes*, *Pseudomonas aeruginosa* et *Staphylococcus aureus* avec des diamètres d'inhibition de 15,23 et 28 mm respectivement.

Cependant, l'effet de l'huile essentielle a été moins prononcé contre *Escherichia coli* (13,6 mm) qui reste la souche la plus résistante et contre la souche fongique *C. albicans* ATCC 10231 (12,04 mm).

Les sensibilités des souches bactériennes et fongiques aux antibiotiques de références ont été également testées, en effet les diamètres d'inhibition ont été compris entre 12 et 32 mm pour la gentamicine et 30 mm pour l'amphotéricine B (Tableau 9).

Tableau 9 : Zones d'inhibition de l'huile essentielle de *I. montana*.

| A. Souches bactérienne à gram positif | | | | | |
|---|-----------------------|---|--|--|-----------------------|
| <i>Staphylococcus aureus</i> ATCC 25923 | | | <i>Listeria monocytogenes</i> ATCC 19115 | | |
| Zones d'inhibition (mm) | | | | | |
| HE (10 µl/disc) | Gent (10 µl /disc) | HE (10 µl/disc) | Gent (10 µl /disc) | HE (10 µl/disc) | Gent (10 µl /disc) |
| 28±0,5 | 32 ± 0,6 | 15 ± 0,6 | | 14 ± 0,577 | |
| B. Souches bactérienne à gram négatif | | | | | |
| <i>Escherichia coli</i> ATCC 25922 | | <i>Pseudomonas aeruginosa</i> ATCC 27853 | | <i>Klebsiella pneumoniae</i> ATCC 70603 | |
| HE (10 µl/disc) | Gent (10 µl /disc) | HE (10 µl/disc) | Gent (10 µl /disc) | HE (10 µl/disc) | Gent (10 µl /disc) |
| 13,6 ± 0,7 | 20 ± 0,5 | 23 ± 1,7 | 24 ± 1,5 | 16 ± 1,5 | 12 ± 0,70 |
| Levure | | | | | |
| <i>Candida albicans</i> ATCC 10231 | | | | | |
| HE (10 µl /disc) | | | Ampho B (0.2mg/ disc) | | |
| 12 ± 0,4 | | | 30 ± 1,2 | | |

La concentration minimale inhibitrice (CMI) a été obtenue en utilisant la méthode de la microplaque (96 puits). Les valeurs des CMI ont été comprises entre 0,75 µg/mL et 2,5 µg/mL pour les bactéries. Les CMI obtenues pour *Listeria monocytogenes* (1,25 µg/ mL) et *Klebsiella pneumoniae* 2,5µg/ mL ont été plus importantes que la CMI obtenue par la gentamicine (8 µg/mL) ce qui montre le bon pouvoir antibactérien de l'huile essentielle de *I. montana*.

Pour la levure *Candida albicans ATCC 10231* qui est une souche multi résistante, la valeur de la CMI a été plus élevée (> 5 µg/ mL).

Tableau 10 : Concentration minimale inhibitrice de l'huile essentielle de *I. montana*.

| A. Souches bactérienne à gram positif | | | | | |
|---|--|--|--|---|--|
| <i>Staphylococcus aureus ATCC 25923</i> | | | <i>Listeria monocytogenes ATCC 19115</i> | | |
| Concentration minimale inhibitrice | | | | | |
| HE (10 µl/disc) | | Gent (10 µl /disc) | | HE (10 µl/disc) | |
| | | | | Gent (10 µl /disc) | |
| 0,75±00 | | 0,5±0,000 | | 1,25±00 | |
| | | | | 8±0,000 | |
| B. Souches bactérienne à gram négatif | | | | | |
| <i>Escherichia coli ATCC 25922</i> | | <i>Pseudomonas aeruginosa ATCC 27853</i> | | <i>Klebsiella pneumoniae ATCC 70603</i> | |
| HE (10 µl/disc) | | Gent (10 µl /disc) | | HE (10 µl/disc) | |
| | | | | Gent (10 µl /disc) | |
| 1,25±00 | | 0,5±0,000 | | 2,5±00 | |
| | | | | 8±0,000 | |
| Levure | | | | | |
| <i>Candida albicans ATCC 10231</i> | | | | | |
| HE (10 µl /disc) | | | Ampho B (0.2mg/disc) | | |
| > 5 | | | 8 | | |

III.4.2 *Carlina hispanica*

Le pouvoir antimicrobien de l'huile essentielle de *C. hispanica*, oxyde de carline et la combinaison oxyde de carline-référence sur six souches de référence a été réalisé par la technique de diffusion sur disque, nommée aussi aromatoگرامme qui est en fait un procédé qualitatif qui permet de tester la résistance ou la sensibilité des microorganismes vis-à-vis les substances réputées antimicrobiennes. Concernant la concentration minimale inhibitrice, elle a été déterminée quantitativement par la méthode de microdilution en milieu liquide en utilisant des microplaques à 96 puits.

Pour les souches à gram négatif, aucun effet n'a été obtenu contre *Pseudomonas aeruginosa* que ce soit par l'huile essentielle, l'oxyde de carline ou encore la combinaison oxyde de carline-gentamicine, pour *Escherichia coli*, l'huile essentielle a montré une faible efficacité avec une zone d'inhibition de 10,5 mm, alors que l'oxyde de carline ainsi que la combinaison n'ont montré aucune efficacité sur cette souche.

En revanche, *Enterococcus faecalis* est faiblement sensible vis-à-vis de l'huile essentielle et l'oxyde de carline avec la même zone d'inhibition de l'ordre de 7.5 mm. Par ailleurs, l'huile

essentielle et l'oxyde de carline possèdent une certaine sensibilité avec respectivement des diamètres d'inhibition de 11,5 et 9 mm contre la souche *Staphylococcus aureus*. D'autre part, un effet inhibiteur synergique a été observé par le mélange oxyde de carline -gentamicine avec des zones d'inhibition de 10 mm contre *Enterococcus faecalis* et 22 mm pour *Staphylococcus aureus* (Tableau 11).

Concernant les souches fongiques, les résultats obtenus ont été plus importants par rapport à ceux obtenus contre les souches bactériennes. Les diamètres d'inhibition de l'huile essentielle, l'oxyde de carline et la combinaison oxyde de carline-amphotéricine contre la souche *Candida albicans ATCC 10231* ont été respectivement de 19,5, 13,5 et 22 mm et de 16,66, 11,66 et 18 mm contre *Candida albicans IPP444* (Tableau 11).

Tableau 11 : Zones d'inhibition de l'huile essentielle de *C. hispanica* et de l'oxyde de carline.

| A. Souches bactérienne à gram positif | | | | | | | |
|---|-----------------------------|-------------------------------------|--------------|--|-----------------------------|-------------------------------------|--------------|
| <i>Staphylococcus aureus ATCC 25923</i> | | | | <i>Enterococcus.faecalis TCC49452</i> | | | |
| Zones d'inhibition (mm) | | | | | | | |
| HE(10 µl/disc) | Oxyde de carline (10µl/dis) | Combinaison Oxyde de carline - Gent | Gent (10µg) | HE (10µl/disc) | Oxyde de carline (10µl/dis) | Combinaison Oxyde de carline - Gent | Gent (10µg) |
| 11,5±1,5 | 9±1 | 22±0,5 | 33 | 7,5±0,5 | 7,5±0 | 10±0,5 | 13 |
| B. Souches bactérienne à gram ngatif | | | | | | | |
| <i>Escherichia coli ATCC 25922</i> | | | | <i>Pseudomonas aeruginosa ATCC 27853</i> | | | |
| HE (10 µl/disc) | Oxyde de carline (10µl/dis) | Combinaison Oxyde de carline - Gent | Gent (10µg) | HE (10µl/disc) | Oxyde de carline (10µl/dis) | Combinaison Oxyde de carline - Gent | Gent (10µg) |
| 10,5±0,5 | - | - | 23±0,0 | - | - | - | 25±00 |
| Levures | | | | | | | |
| <i>Candida albicans ATCC 10231</i> | | | | <i>Candida albicans IPP444</i> | | | |
| HE (10 µl/disc) | Oxyde de carline (10µl/dis) | Combinaison Oxyde de carline - Gent | Am B (0.2mg) | HE (10µl/disc) | Oxyde de carline (10µl/dis) | Combinaison Oxyde de carline - Gent | Am B (0.2mg) |
| 19,5±0,5 | 13,5±0,5 | 22±0,5 | 30±0,0 | 16,66±1,2 | 11,66±1,2 | 18±0,2 | 30±0,0 |

La CMI est la plus faible concentration de l'extrait testé susceptible d'inhiber la croissance microbienne. Dans cette étude la méthode de micro dilution en milieu liquide a été appliquée afin de déterminer la CMI de nos échantillons.

Les résultats obtenus indiquent qu'une faible CMI de l'huile essentielle a été observée contre *Staphylococcus aureus* avec 1,562 µg/mL. Pour les autres bactéries aucune CMI n'a été affichée ce qui est le cas aussi pour l'oxyde de carline et la combinaison de l'oxyde de carline -gentamicine.

Cependant, nous avons obtenu des CMI intéressantes contre les souches fongiques. Pour l'huile essentielle, les CMI ont été de 0,39 µg/mL pour *Candida albicans ATCC 10231* et

Candida albicans IPP444. Quant à l'oxyde de carline, nous avons obtenu des CMI de 3,125 µg/mL pour *Candida albicans* ATCC 10231 et 1,562 µg/mL pour *Candida albicans* IPP444.

Par ailleurs, la combinaison oxyde de carline-amphotéricine B s'est révélée la plus prometteuse avec un effet synergique et des CMI de 1,25 et 0,56 µg/mL contre *Candida albicans* ATCC 10231 et *Candida albicans* IPP444 respectivement.

Table 12 : Concentration minimale inhibitrice de l'huile essentielle de *C. hispanica* et de l'oxyde de carline.

| A. Gram-Positive Bacterial Strains | | | | | | | |
|---|-------------------------------------|---|--|--------------------------------|------------------------------------|--|-----------------|
| <i>Staphylococcus aureus</i> ATCC 25923 | | | | | | | |
| Concentration minimale inhibitrice | | | | | | | |
| HE (10 µl/disc) | Oxyde de carline (10 µl/disc) | | Combinaison Oxyde de carline - Gent | | Gent (10µg) | | |
| 1.562±0.00 | - | | - | | 0.5± 0.000 | | |
| Levures | | | | | | | |
| <i>Candida albicans</i> ATCC 10231 | | | | <i>Candida albicans</i> IPP444 | | | |
| HE (10 µl/disc) | Oxyde de carline (10 µl/disc) | Combinaison Oxyde de carline - Gent | Am B (0.2mg) | HE (10 µl/disc) | Oxyde de carline(10 µl/disc) | Combinaison Oxyde de carline- Gent | Am B (0.2mg) |
| 0.39±0.00 | 3.125± 0.00 | 1.25±0.00 | 8±0.00 | 0.39±0.00 | 1.562±0.00 | 0.56±0.00 | 4±0.00 |

Le pouvoir antimicrobien des huiles essentielles est déterminé principalement par le type et les caractéristiques de leurs constituants actifs, plus précisément de leurs propriétés hydrophobes qui leur permet de pénétrer dans la double couche phospholipidique de la membrane des cellules bactériennes [177]. Comme il dépend aussi du type des microorganismes ciblés [178].

Plusieurs études réalisées sur le pouvoir antimicrobien des huiles essentielles ont montré qu'il résulte de la présence des terpénoïdes oxygénés, et que les molécules oxygénées sont généralement plus actives que les molécules hydrocarbonées [12]. Il a été révélé aussi que la classe des terpènes est connue pour être active contre un large éventail de microorganismes [179]. Ce qui explique et affirme le pouvoir antimicrobien très prometteur de l'huile essentielle de *Inula montana* riche en sesquiterpènes oxygénés (74.3%).

Les résultats obtenus via la méthode de micro dilution en milieu liquide ont indiqué que l'huile essentielle de *Carlina hispanica* a été plus intéressante que ceux de l'oxyde de carline seul. Une étude de l'activité antimicrobienne a prouvé que cette activité peut avoir lieu suite à un effet synergique entre les constituants de l'huile essentielle et non pas exclusivement des principaux constituants [180].

Cependant, la combinaison de l'oxyde de carline avec les antibiotiques de références s'avère avoir les meilleurs effets inhibiteurs avec un effet synergique, témoigné par les faibles valeurs des CMI. Une étude récente réalisé sur l'effet antimicrobien de la combinaison de l'huile essentielle du *Thymus vulgaris* et le céfotaxime s'est révélée efficace contre *E. coli* [181].

Ces activités prometteuses ouvrent des perspectives comme alternatives aux conservateurs synthétiques qui présentent des effets néfastes sur la santé humaine. À cet effet, l'utilisation de l'huile essentielle de *Carlina hispanica* et la combinaison de l'oxyde de carline avec les antibiotiques de référence comme conservateur alimentaire naturel et comme fongicide dans diverses applications présente une bonne alternative très intéressante. Cependant, d'autres travaux sont nécessaires pour valider ces résultats.

III.5 Etude in silico de l'activité antioxydante

III.5.1 Approche théorique

L'accumulation des radicaux libres dans l'organisme développe des maladies chroniques et dégénératives tels que le cancer, les maladies auto-immunes, la polyarthrite rhumatoïde, les maladies cardiovasculaires et neurodégénératives. Cependant, les antioxydants synthétiques couramment utilisés dans les applications pharmacologiques et alimentaires sont des composés phénoliques tels que le butylhydroxytoluène (BHT) et le butylhydroxyanisole (BHA) dont leur utilisation a été limitée par des règles législatives suite aux des doutes sur leurs effets cancérigènes et toxiques [182].

En conséquence, la recherche et l'identification de sources alternatives naturelles et sûres d'antioxydants est une stratégie essentielle pour les utiliser par la suite comme conservateurs dans différents domaines.

Les plantes médicinales sont dotées de beaucoup de propriétés thérapeutiques. Outre cela, elles sont à l'origine d'une autre richesse dues à leurs huiles essentielles qui constituent une source potentielle de molécules naturelles et bioactives.

En outre, les techniques de modélisation et de simulation sont devenues une méthode de recherche importante pour l'étude des systèmes opérationnels et organisationnels, particulièrement en chimie. Elles sont souvent nécessaires lorsque l'expérience réelle est trop difficile, dangereuse et/ou coûteuse. La chimie numérique subventionne une meilleure compréhension de l'action des plantes médicinales contre les pathologies et offre une formation de haut niveau, centrées sur l'étude du vivant au niveau moléculaire. De plus, elle représente un

pont entre la théorie et l'expérience, et permet de représenter, interpréter et prédire les structures et les fonctions biomoléculaires [183,184].

Ce travail visait donc à étudier pour la première fois, les molécules de l'huile essentielle de la partie aérienne de *Inula montana* comme nouveaux agents antioxydants, en s'intéressant à l'interaction avec les modèles de liaison et à l'activité antioxydante de l'ADNct et la cible de la superoxyde dismutase qui est un antioxydant important, active dans les cellules endothéliales, le cytoplasme et la matrice intermembranaire mitochondriale [185].

▪ Méthodes théoriques et computationnelles

L'énergie minimisée des ligands (constituants) de l'huile essentielle de la partie aérienne de *Inula montana* ainsi que leur toxicité obtenue par le logiciel MOE, sont rapportées dans le Tableau 13.

Tableau 13. Propriétés et minimisation d'énergie des meilleurs ligands (composés) de l'huile essentielle de la partie aérienne de *Inula montana*.

| Ligands | Composés | Toxicité | LogP | LogS | Hdon+Hacc | Flexibilité |
|---------|--------------------------|----------|------|-------|--------------|-------------|
| L32 | E,E- α -farnésène | No | 5,20 | -6,01 | don:0; acc:0 | 2 out 2 |
| L27 | α -curcumène | No | 4,84 | -5,22 | don:0; acc:0 | 4 out 4 |
| L30 | Zingébérène | No | 4,89 | -4,87 | don:0; acc:0 | 4 out 4 |
| L34 | trans-calamenene | No | 4,69 | -5,80 | don:0; acc:0 | 1 out 1 |
| L42 | Z-Nérolidol | No | 4,40 | -3,93 | don:1; acc:1 | 7 out 7 |
| L56 | α -Carinol | No | 1,23 | -2,09 | don:5; acc:7 | 9 out 9 |
| L65 | E- acétate de nérolidol | No | 4,97 | -4,55 | don:0; acc:1 | 9 out 9 |
| L66 | β -acoradiénol | No | 3,70 | -4,64 | don:1; acc:1 | 2 out 2 |
| L67 | Z,E-acétate de farnésyle | No | 4,97 | -4,87 | don:0; acc:1 | 9 out 9 |
| L69 | E,E-acétate de farnésyle | No | 4,97 | -4,87 | don:0; acc:1 | 9 out 9 |

LogP : Coefficient de partage octanol/eau ; Log S : Coefficient de solubilité ; Hdon et Hacc : nombre de donneurs et d'accepteurs possibles de liaisons hydrogène, (la capacité de liaison hydrogène d'un soluté médicamenteux est reconnue comme un déterminant crucial de la perméabilité. Un potentiel élevé de liaison hydrogène est souvent lié à une faible perméabilité et absorption) ; flexibilité : nombre de liaisons rotatives.

L'inhibition du récepteur de la superoxyde dismutase a été étudiée théoriquement par deux méthodes de chimie computationnelle : les analyses d'amarrage moléculaire et les simulations de dynamique moléculaire (DM). Dans cette étude, une combinaison de trois approches théoriques a été utilisée pour explorer les inhibiteurs potentiels parmi les composés des huiles essentielles des parties aériennes de *Inula montana* contre l'enzyme (SOD). La

structure complète du récepteur avec sa cavité enzymatique et les résidus du site actif a été obtenue par la méthode de diffraction des rayons X (Figure 18).

D'après le Tableau 13 et le Tableau 13.a (annexes), nous remarquons que tous les ligands répondent à la règle de Lipinski. (Poids moléculaire < 500, pas plus de cinq donneurs de liaisons hydrogène, pas plus de dix accepteurs de liaisons hydrogène et une valeur de coefficient de partage ($\log P$) < 5) sauf le ligand L32 E,E- α -farnésène plus au moins élevé $\log P = 5.20$. a été conçue dans l'objectif d'aider à la conception de nouveaux médicaments, à leur formulation en phase *in vitro* et aussi au développement de médicaments biodisponibles par voie orale [186].

Selon les résultats obtenus, nous constatons que les composés E,E-acétate de farnésyle, Z,E-acétate de farnésyle, E-acétate de Nérolidol et E,E- α -farnésène ont les plus grandes valeurs de $\log P$ et les plus petites valeurs de $\log S$, ce qui veut dire une bonne solubilité dans l'eau et donc une meilleure perméabilité à travers la membrane cellulaire.

De même, les ligands L69, L67 et L65 ont une valeur élevée d'angle de torsion 9 out 9 vis-à-vis aux autres constituants, ce qui montre que ces composés sont plus flexibles.

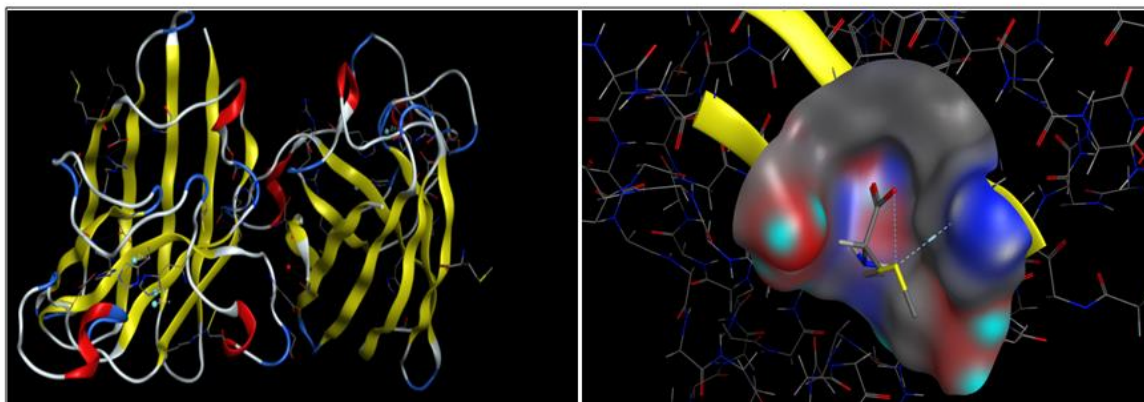


Figure 18 : Cavité enzymatique

Figure 19 : Enzyme dimère

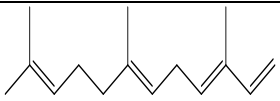
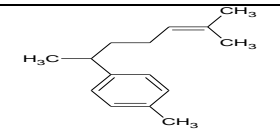
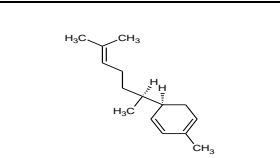
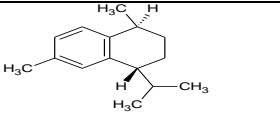
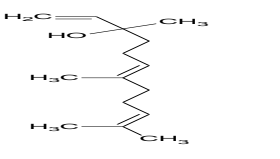
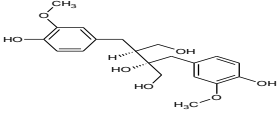
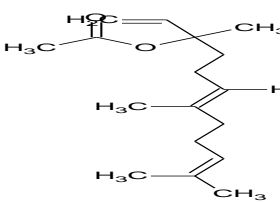
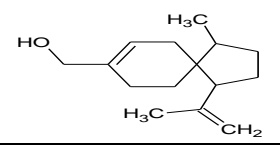
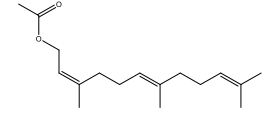
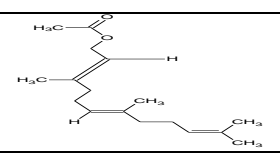
III.6 Affinité de liaison des composés avec la cible de la Superoxyde dismutase

III.6.1 Évaluation du docking moléculaire

III.6.1.1 Interaction avec la superoxyde dismutase

Les résultats du bilan énergétique des meilleurs complexes formés avec la cible de la SOD sont donnés dans le Tableau 14. Les énergies des autres complexes sont mentionnées dans le Tableau 14.a en annexe.

Tableau 14. Résultat du bilan énergétique des meilleurs complexes (enzyme-ligands)
Kcal/mol.

| Ligands | Structure chimique | Énergie de liaison (Kcal/mole) | Rmsd - refine | Energy-Conf | Energy-Place | Energy-Refine | RMSD |
|---------------------------|---|--------------------------------|---------------|-------------|--------------|---------------|-------|
| E,E- α -farnésène |  | -3,915 | 1,511 | -1,997 | -5,738 | -3,758 | 1,154 |
| α -curcumène |  | -4,093 | 1,503 | 35,082 | -0,102 | -12,536 | 0,214 |
| Zingébérène |  | -4,032 | 2,021 | 36,990 | 25,109 | -13,224 | 0,198 |
| Trans- -calamenene |  | -4,100 | 2,831 | 44,135 | -33,581 | -9,968 | 0,434 |
| Z-Nérolidol |  | -4,000 | 3,403 | -10,987 | -1,967 | -10,604 | 0,116 |
| α -Carinol |  | -4,150 | 3,192 | 3,192 | 75,237 | -17,291 | 0,984 |
| E- acétate de Nérolidol |  | -4,226 | 5,681 | 33,046 | -2,144 | -10,626 | 1,101 |
| β -Acoradienol |  | -4,011 | 1,236 | 38,401 | -12,797 | -12,301 | 0,476 |
| Z,E- acétate de farnésyle |  | -4,175 | 2,665 | 23,760 | -14,341 | -10,152 | 1,007 |
| E,E- acétate de farnésyle |  | -4,401 | 3,665 | -22,011 | 1,348 | -11,109 | 0,539 |

bleau 15 : Résultats des liaisons entre les atomes des inhibiteurs et les résidus du site actif de la cible.

| N° | Composés | S-score (kcal/mol) | Liaisons entre atomes des composés et résidus du cite actif | | | | | |
|-----------------------------------|---------------------------|-----------------------|---|-----------------------------------|------------------------------------|--|------------------------------|------------------------------|
| | | | Atome de composés | Atomes récepteurs impliqués | Résidus récepteurs impliqués | Type d'interactions | Distances (Å) | Energies (kcal/mol) |
| Superoxide Dismutase (SOD) | | | | | | | | |
| L27 | α -curcumène | -4,093 | 6-ring | O | HOH 2043 | pi-H | 4,24 | -0,7 |
| L54 | Tau-cadinol | -4,087 | O1 1 | OG | SER 98 | H-acceptor | 2,91 | -1,6 |
| L56 | α -carinol | -4,150 | 6-ring | CD | LYS 30 | pi-H | 4,57 | -0,6 |
| L66 | β -acoradiénol | -4,011 | O1 1 O1 1 | O O | HOH 2051 HOH 2042 | H-donor H-acceptor | 2,52 2,43 | 0,7 5,1 |
| L2 | Z-Hex-3-en-1-ol | -3,212 | O1 1 | 5-ring | TRP 32 | H-pi | 3,81 | -0,7 |
| L6 | Octanal | -3,300 | O1 1 | ND2 | ASN 19 | H-acceptor | 3,15 | -1,3 |
| L20 | E- β -Caryophyllène | -3,154 | O1 1 | NE1 | TRP 32 | H-acceptor | 3,50 | -0,5 |
| L39 | E-nérolidol | -3,752 | O1 1 | O | HOH 2130 | H-donor | 2,61 | -0,6 |
| L40 | Epi-globulol | -3,438 | O1 1 | O | HOH 2128 | H-acceptor | 2,73 | -1,1 |
| L46 | β -oplopénone | -3,354 | O1 1 | OG | SER 98 | H-acceptor | 2,48 | 2,9 |
| L47 | Viridiflorol | -3,677 | O1 1 | O | HOH 2126 | H-acceptor | 2,53 | -0,1 |
| L53 | Trans-murolol | -3,655 | O1 1 | OG | SER 98 | H-acceptor | 2,90 | -1,6 |
| L55 | β -eudesmol | -3,578 | O1 1 | O | HOH 2043 | H-acceptor | 2,97 | -0,6 |
| L59 | α -eudesmol | -3,571 | O1 1 | O | HOH 2043 | H-acceptor | 2,97 | -0,6 |
| L63 | shyobunol | -3,433 | O1 1 | NZ | LYS 75 | H-acceptor | 3,34 | -1,5 |
| L65 | E-acétate de nérolidol | -4,226 | H2 C14 C18 C19 | O2 O O O | HOH2043 LYS30 LYS75 LYS75 | WaterH-bond Alkyl Interaction Alkyl Interaction Alkyl Interaction | 2,10 4,83 2,87 4,87 | -0,9 -1,2 -0,5 -1,1 |
| L67 | Z,E- acétate de farnésyle | -4,175 | C15 | O | LYS30 | Alkyl Interaction | 4,83 | -1,2 |
| L69 | E,E-acétate de farnésyle | -4,401 | H1 | O2 | HOH2043 | Water H-bond | 2,21 | -0,6 |

S-score : énergie de liaison. H=Liaison hydrogène conventionnelle, C= Liaison hydrogène carbone, Aa= Alkyl-alkyle, Ap=Alkyl-Pi, Aps=Amide-Pi empilés, Ppt=Pi-pi en forme de T, X=Halogène

D'après les résultats des Tableaux 13 et 14, parmi les meilleurs composés étudiés, le E, E-acétate de farnésyle (Ligand 69) (Figure 18), a été prédit comme étant le liant le plus fort des récepteurs de la superoxyde dismutase qui forme un complexe avec une stabilité importante et l'énergie la plus faible (-4,401 Kcal/mol). Le deuxième meilleur ligand a été le E-acétate de nérolidol (Ligand 65) avec une énergie de -4,226 Kcal/mol qui interagit avec l'accepteur HO 2043 H à une distance d'interaction forte de 2,10Å et une énergie de liaison de -0,9 Kcal/mol (Figure 19). Les autres ligands qui interagissent avec la superoxyde dismutase sont: le ligand L67 Z,E-acétate de farnésyle avec une énergie de -4.175 Kcal/mol interagit avec un acide aminé Lys30 alkyl à une forte distance de 4.83 Å et une énergie de liaison de -1.2 Kcal/mol, le ligand L27 α -curcumène interagit avec HOH 2043 H-pi à une forte distance de 4,24 Å et une énergie

de liaison de - 0,7 Kcal/mol, le ligand Tau-cadinol L54 interagit avec un acide aminé accepteur SER 98 H-accepteur à une forte distance de 2,91 Å et une énergie de liaison de -1,6 kcal/mol de même, le ligand L66 β -acoradiénol interagit avec HOH 2051 et HOH 2042, (H-accepteur, H-donneur) à une forte, faible distance respectivement de 2,52. 2,43 Å et une énergie de liaison de 0,7, 5,1 Kcal/mol, le ligand Z-Hex-3-en-1-ol L2 interagit avec TRP 32 H-pi à une distance de 3,81 Å de liaison faible et une énergie de -0,7 Kcal/mol, le β-oplopénone L46 interagit avec un acide aminé SER 98 32 H accepteur à une distance de liaison de 2,48 Å et d'énergie de 2,9 Kcal/Mol. Le E-β-caryophyllène (L20), Trans-murolol (L53) et le shyobunol (L63) interagissent avec un acide aminé TRP32, SER98 et LYS75 H-accepteur à une distance de 3,50, 2,90 et 3,34 Å c'est une interaction forte d'énergie de -0,5, -1,6 et -1,5 Kcal/mol. Une interaction avec le E-acétate de nérolidol (L39), l'épi-globulol (L40), le Viridiflorol (L47), le β-eudesmol (L55) et α-eudesmol (L59) avec HOH 2130, HOH 2128, HOH 2126 et HOH 2043 respectivement. H-donneur et H-accepteur à une distance de 2,61,2,73,2,53 et 2,97 Å interaction forte, faible, moyenne et énergie de liaison de -0,6,-1,1, -0,1 et -0,6 Kcal/mol respectivement. On note que les interactions entre les résidus du site actif de 2wz5 et le ligand E, E- acétate de farnésyle forme le complexe le plus stable et que le ligand E-acétate de nérolidol peut inhiber la superoxyde dismutase.

L'affichage du diagramme d'interaction 3D de la cavité du site de liaison du superoxyde dismutase du E, E- acétate de farnésyle avec poche (a) et sans poche (b) sont donnée dans la Figure 19.

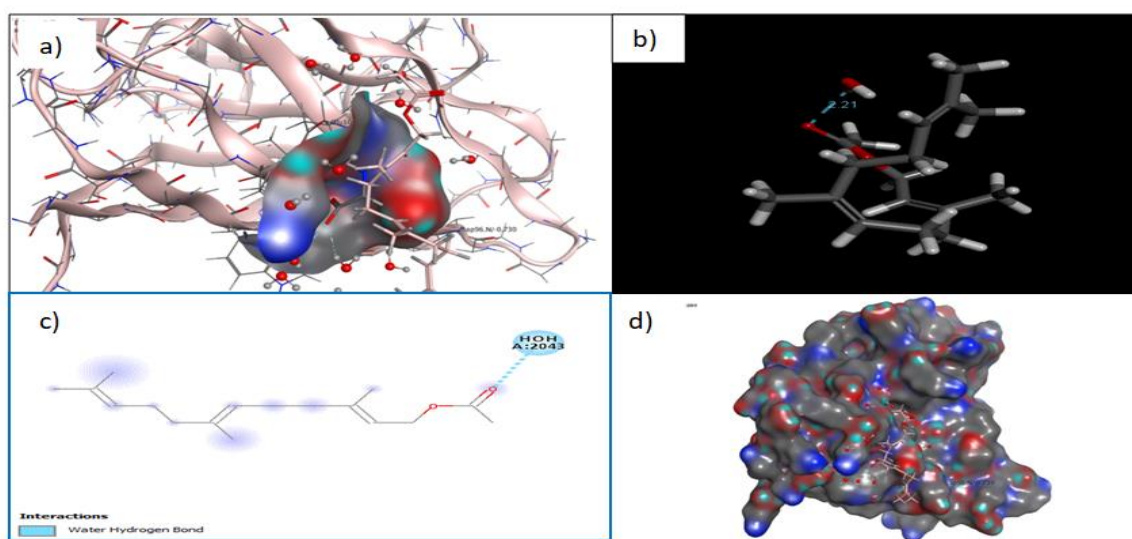


Figure 20: Affichage du diagramme d'interaction 3D de la cavité du site de la liaison de la superoxyde dismutase du E,E-acétate de farnésyle avec poche (a) et sans poche (b) ; Mode de liaison du E,E-acétate de farnésyle sous forme de diagramme 2D (c) ; les motifs de liaison du E,E-acétate de farnésyle dans le site actif de la superoxyde dismutase, dans lesquels le E,E-acétate de farnésyle a disparu dans le modèle de bâton avec du rose tandis que la surface de la SOD est représentée en bleu rouge vert (d).

L'affichage du diagramme d'interaction 3D de la cavité du site de liaison de la superoxyde dismutase du E-nérolidol acétate avec poche (a) et sans poche (b) est illustré dans la **Figure 20**.

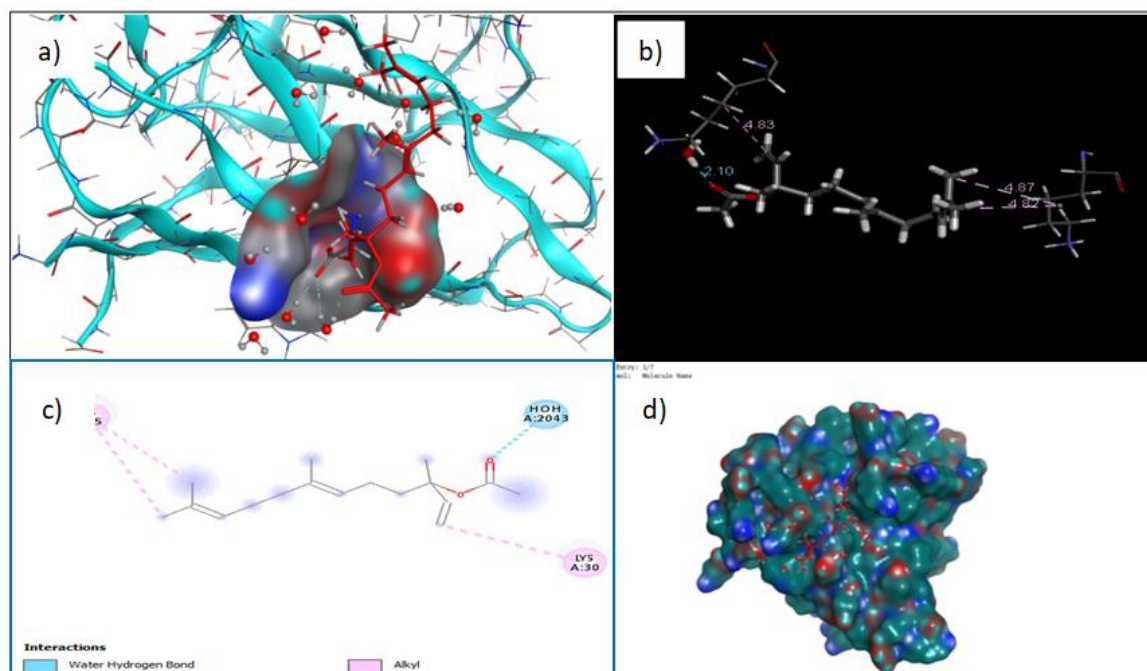


Figure 21 : Affichage du diagramme d'interaction 3D de la cavité du site de liaison de la Superoxyde Dismutase du E-acétate de nérolidol avec poche (a) et sans poche (b) ; Mode de liaison du E-acétate de nérolidol sous forme de diagramme 2D (c); Les motifs de liaison du E-acétate de nérolidol dans le site actif de la superoxyde dismutase, dans lesquels le E-acétate de nérolidol a disparu dans le modèle du bâton avec le rose tandis que la surface des SOD est montrée en bleu rouge vert (d).

Les affinités de liaison et les interactions sans liaison sont répertoriées dans le Tableau 14. Les ajouts d'halogène augmentent non seulement les propriétés physico-chimiques mais également l'affinité et la spécialité de liaison [187]. L'incorporation du groupe $-CF_3$ a augmenté les propriétés inhibitrices et médicinales [188,189]. Le trifluorure de carbone du groupe a de grandes applications importantes dans le domaine des colorants agronomiques, des pigments, des produits pharmaceutiques, des polymères et de la science des matériaux lorsqu'il est incorporé à différentes molécules organiques en raison de ses fortes caractéristiques électro-négatives et hydrophobes, qui peuvent être utilisées dans la conception de médicaments pour améliorer la fonctionnalité sélective [190, 191].

Dans notre étude, tous les composés présentent plusieurs liaisons hydrogène significatives. Certaines études récentes ont rapporté que la liaison halogène similaire à la liaison hydrogène joue un rôle crucial pour le système biologique et chimique [192,193]. L'affinité de liaison de L69, L65, L67, L56, L27 et L54 a considérablement augmenté à -4,401, -4,226, -4,175, -4,150, -4,093 et -4,087 kcal/mol respectivement du E,E-acétate de farnésyle, E

-acétate de nérolidol, Z,E- acétate de farnésyle, α -carinol α -curcumène et Tau-cadinol. Une liaison hydrogène améliorée est observée dans L69 et L65 non seulement contribue à augmenter l'affinité de liaison, mais améliore également la spécificité de liaison [194,195]. Cette observation a permis de confirmer que (les sesquiterpènes oxygénés) et les composés (L67, L56 et L27) se lient au site de liaison souhaité de la protéine réceptrice après l'amarrage moléculaire.

III.6.1.2 Interaction avec la séquence d'ADN

Les radicaux libres, en particulier OH, peuvent attaquer l'ADN. Ils réagissent avec les nucléotides. Ils peuvent entraîner, par exemple, des modifications de l'azote bases, fragmentation de l'ADN, ruptures de brins ou pontage de bases. Par conséquent, ils seraient nécessaires la cause de maladies comme le cancer, les maladies neurodégénératives et la pathogenèse d'infections virales. L'accumulation avec l'âge de radicaux et donc les dommages qu'ils génèrent seraient l'un des facteurs expliquant le vieillissement cellulaire. Une étude d'amarrage moléculaire bio-informatique a été réalisée sur un duplex d'ADN de séquence d(CGCGAATTCGCG)₂ dodécamère (PDBID:1BNA) et est pré

sentée dans le Tableau 16.

Tableau 16 : Les énergies d'amarrage des inhibiteurs d'ADN.

| | Composés | DE*(kcal/mol) DNAct | DE*(kcal/mol) Enzyme | ETOR (kT) | VDW (kT) | EIE (kT) |
|-----------------------|--------------------------|------------------------|-------------------------|--------------|-------------|----------|
| Ligand control | Acid ascorbic | -4.383 | -3.329 | 345.417 | 553.157 | -1639.53 |
| Lref | L-methionine | - | -3.388 | 331.046 | 412.988 | -1941.78 |
| L69 | E,E-acétate de farnésyle | -6.855 | -4.401 | 331.636 | 412.865 | -1941.94 |
| L67 | E,Z-acétate de farnésyle | -6.336 | -4.175 | 331.503 | 412.724 | -1941.62 |
| L56 | α -carinol | -6.276 | -4.150 | 330.732 | 413.030 | -1942.17 |
| L65 | E- acétate de nérolidol | -6.087 | -4.226 | 345.417 | 553.157 | -1639.53 |
| L32 | E,E- α -farnésène | -6.084 | -3.915 | 331.636 | 412.864 | -1941.94 |
| L27 | α -curcumene | -5.727 | -4.093 | 330.612 | 413.093 | -1942.17 |
| L42 | Z-nérolidol | -5.569 | -3.980 | 330.570 | 412.976 | -1942.21 |
| L54 | Tau-cadinol | -4.708 | -4.087 | 330.567 | 412.975 | -1942.22 |
| L30 | Zingébèrene | -5.176 | -4.032 | 330.174 | 413.373 | -1942.50 |
| L66 | β -acoradiénol | -4.742 | -4.011 | 330.719 | 412.932 | -1942.20 |

* DE : énergie Docking (Liaison); ETOR : Énergie de torsion ; VDW : Van der Waals ; EIE : Énergie d'interaction électrostatique.

Nos résultats ont démontré que le: E,E- α -farnésène (Ligand 23), le α -carinol (Ligand 56), le E- acétate de nérolidol (Ligand 65), le E,E-acétate de farnésyle (Ligand 69) et le E,Z- acétate de farnésyle ont été les meilleurs composés interagissant (voir Tableau 15). Les énergies du docking calculées pour ces composés ont été respectivement -6.084, -6.276, -6.087, -6.855 et -6.336 kcal/mol. À l'exception du Tau-cadinol et du β -Acoradiénol, autres composés sont situés dans le petit sillon de l'ADNc. Les résultats du docking moléculaire ont montré que le Tau-

cadinol et le β -acoradiénol ont existé dans la région du sillon principal. L'énergie calculée du docking de ce composé a été respectivement de -4.708 et -4.742 kcal/mol voir Tableau 16).

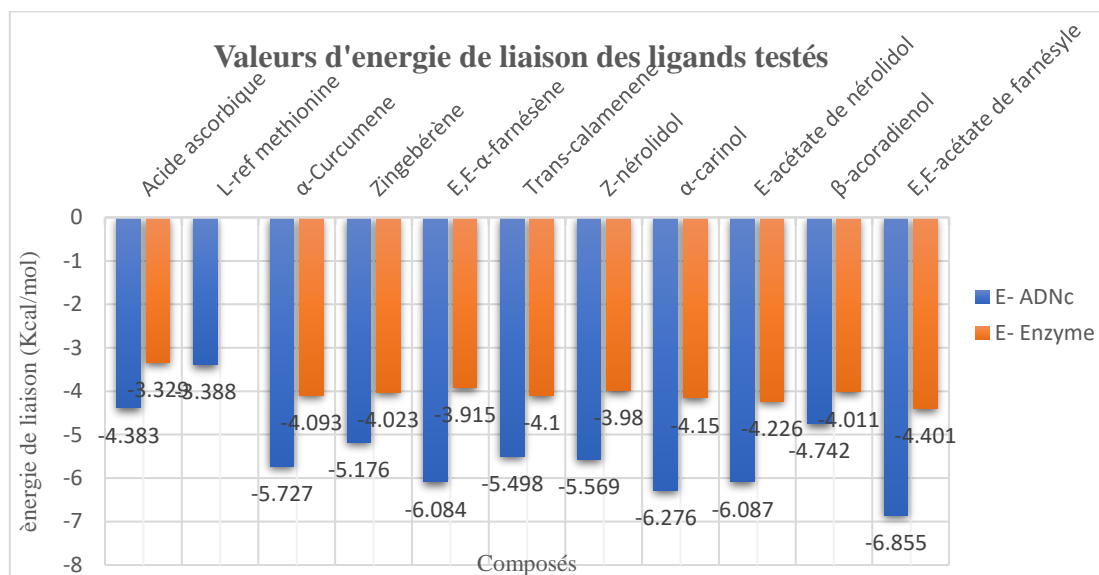


Figure 22 : Valeurs d'énergie de liaison des ligands testés ; Lref : ligand de référence, acide ascorbique : ligand témoin.

L'énergie de liaison est le paramètre principal qui est généré à la suite du docking moléculaire. Cela nous donne l'idée de la force et l'affinité de l'interaction entre le ligand et le récepteur.

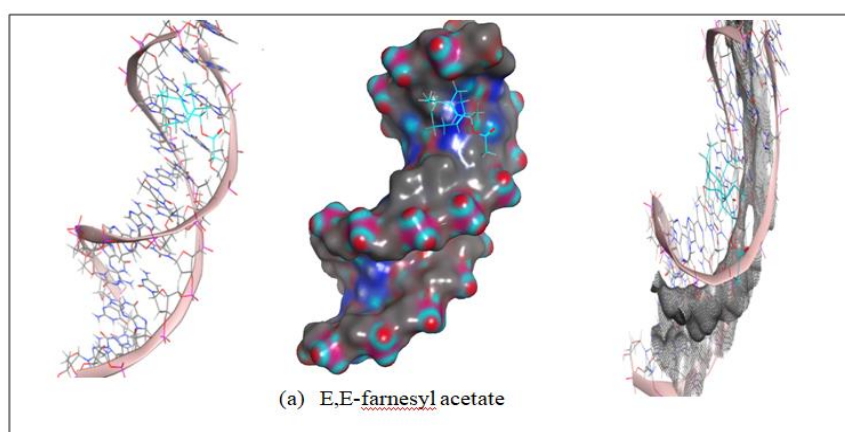
Plus l'énergie de liaison est grande, plus l'interaction est faible et vice versa. Ainsi, lors de toute étude du docking, nous entendons chercher le ligand qui affiche le moins d'énergie de liaison, donc la meilleure affinité parmi les molécules testées [214]. Parmi les candidats aux tests de cette étude, le E,E-acétate de farnésyle et le E-acétate de nérolidol ont affiché l'énergie de liaison la plus faible de -6.865 et -6.087 kcal/mol pour la séquence d'ADN et -4.733 et -4.226 kcal/mol pour l'enzyme. L'énergie de liaison du Lref (-3.388 kcal/mol) pour l'enzyme et le ligand contrôle ; l'acide ascorbique (-4.383 kcal/mol pour la séquence d'ADN et -3.329 kcal/mol pour l'enzyme). En effet, ils ont été beaucoup plus élevés que le E, E-acétate de farnésyle et le E-acétate de nérolidol qui ont présenté une liaison bien meilleure (Figure 20).

L'énergie de liaison relative la plus négative pour le E,E-acétate de farnésyle a indiqué une plus grande tendance à la liaison de ce composé avec l'ADN. Ainsi, les résultats obtenus à partir du docking moléculaire se sont avérés cohérents avec les études de la simulation de dynamique moléculaire, ce qui peut valider nos résultats de simulation.

Les études du docking et de dynamique suggèrent que le composé E,E-acétate de farnésyle a une forte affinité de liaison avec l'ADN que le composé E-acétate de nérolidol.

Cependant, Le groupe acétate supplémentaire du composé E,E-acétate de farnésyle crée des interactions hydrophobes spécifiques avec les bases nucléotides de l'ADN.

De plus, les études de docking ont également suggéré qu'une grande partie de l'énergie de liaison provient d'interactions hydrophobes. De ces résultats, il convient de noter que plusieurs facteurs, notamment la variabilité des structures des ligands et la capacité à construire des liaisons covalentes et/ou non covalentes, pourraient affecter leur affinité de liaison au petit sillon d'ADN. Il existe même d'autres facteurs tels que la répartition des charges électrostatiques avec l'ADN et aussi les paires de bases A : T [215]. Des études ont suggéré que les ligands de liaison au petit sillon portent une charge cationique, complétant le potentiel des régions A : T. Nos résultats sont tout à fait cohérents avec les rapports précédents et les composés étudiés ont interagi avec les paires de bases A : T. Le E,E-acétate de farnésyle et le E-acétate de nérolidol ont présenté une liaison bien meilleure. (Voir Figure.22).



E. E-acétate de farnésyle

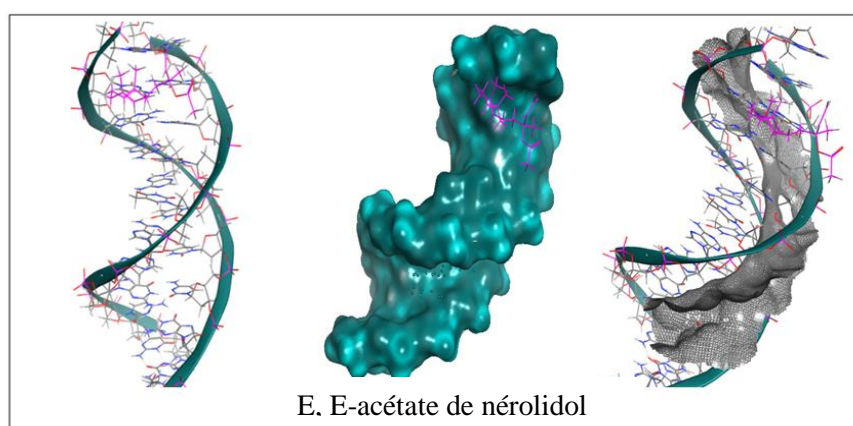


Figure 23. Modèle du docking moléculaire du site de la plus favorable liaison des composés (a) L69 et (b) L65 avec le duplex d'ADN dodécamère de séquence d(CGCGAATTCGCG)₂ (PDB ID : 1BNA).

Les résultats de l'amarrage moléculaire ont révélé que le Trans-calamenene (Ligand 34), le α -carinol (Ligand 56), le Zingébérène (Ligand 30), le E-acétate de nérolidol (Ligand 65), le Z,E-acétate de farnésyle (Ligand 67) et le E,E-acétate de farnésyle (Ligand 69) ont été les meilleurs composés interagissant avec les résidus de liaison suspectés au site actif de la superoxyde dismutase (Figure 20). Les énergies du docking calculées pour ces molécules ont été respectivement de : -4,100, -4,150, -4,032, -4,226, -4,175 et -4,401 kcal/mol. Par ailleurs, les interactions les plus faibles avec ce récepteur ont été données par le Z-nérolidol et le E,E-acétate de farnésène avec des énergie de : -3,980 et -3,915 kcal/mol, de manière respective. Les représentations 3D des meilleures interactions de pose sont illustrées dans la Figure 23.

Les paramètres du docking comprennent l'énergie de liaison libre, l'énergie intermoléculaire et les valeurs d'énergie libre de torsion. Nous avons observé que le E,E- α -farnésène (Ligand 32), le α -curcumène (Ligand 27), le α -carinol (Ligand 56) et le E-acétate de nérolidol (Ligand 65), le E,E- acétate de farnésyle (Ligand 69) et le Z,E- acétate de farnésyle (L67) ont montré une affinité de liaison pour interagir avec l'ADNc, avec des énergies du docking de : -6.084, -5.727, -6.276, -6.087, -6.855, -6.336 Kcal/mol.

De ces résultats donnés, nous pouvons conclure que la meilleure inhibition se produit par deux molécules le E- acétate de nérolidol (Ligand 65) et le E,E- acétate de farnésyle (Ligand 69).

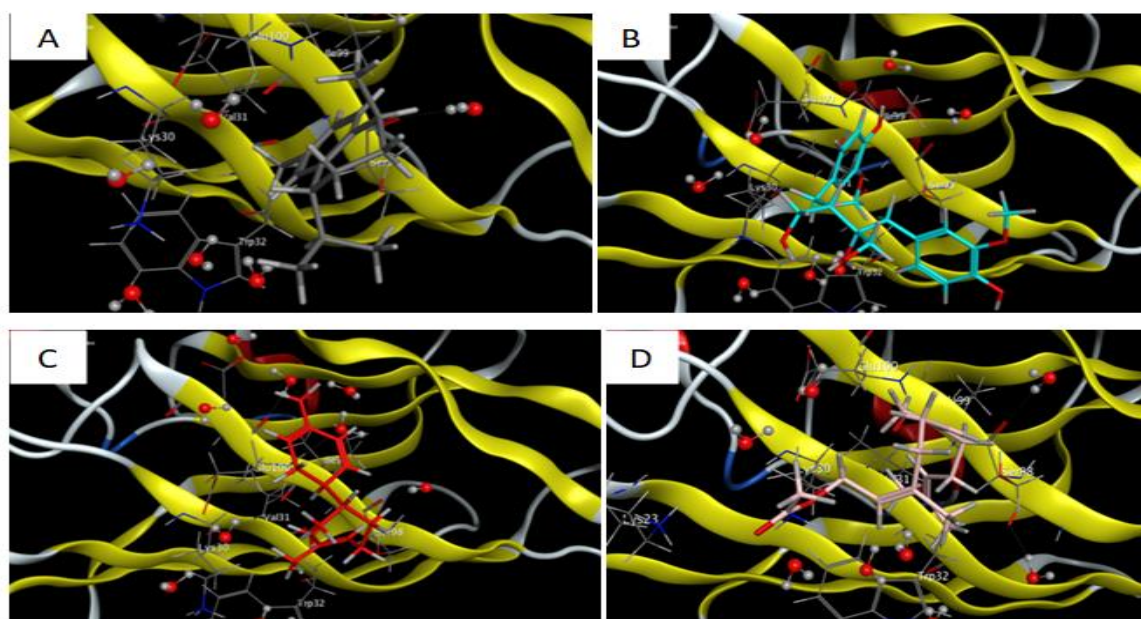


Figure 24. Représentations 3D des meilleures interactions de pose entre les ligands et le récepteur. Les protéines sont représentées dans le modèle de ruban solide et les ligands sont représentés dans le modèle de bâton. A. le trans-calamenene B. le α -carinol, C. le zingebrene. D. Z,E- acétate de farnésyle. Avec la SOD.

III.7 Interaction avec les systèmes antioxydants enzymatiques endogène

- **Le cas de la superoxyde dismutase (SOD)**

Les antioxydants peuvent être classés comme molécules liposolubles ou hydrosolubles. Selon leurs caractéristiques physico-chimiques, ils auront une localisation cellulaire préférentielle : les membranes cellulaires pour les substances liposolubles et le cytosol et/ou le milieu extracellulaire pour les substances hydrosolubles. Ils seront particulièrement efficaces sur les radicaux libres présents dans chaque type de support, respectivement (Figure 24).

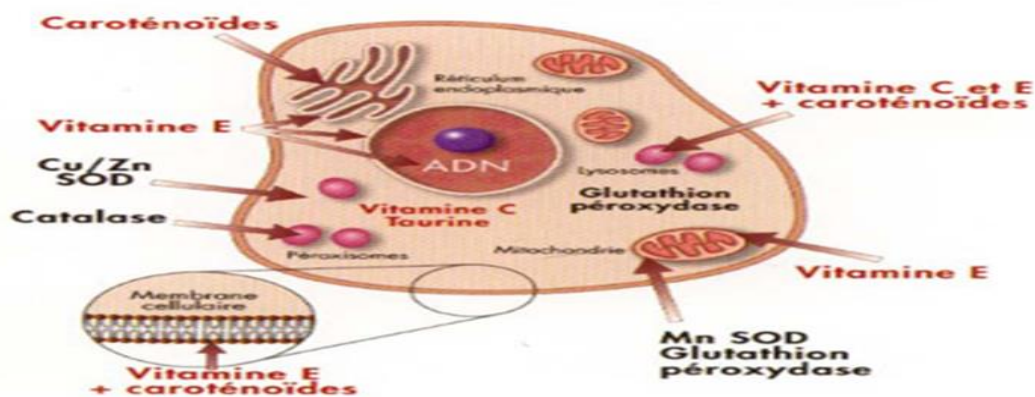


Figure 25 : Sites d'action des nutriments antioxydants (en rouge) et des enzymes antioxydantes (en noir) (OPARA, 2002).

Les défenses exogènes (Superoxyde dismutase, Catalase, Glutathion peroxydase) semblent les plus faciles à supporter puisqu'elles pourraient être renforcées par la prise de compléments alimentaires. En effet la superoxyde dismutase : Convertit les radicaux libres en substances moins nocives, tels que le peroxyde d'hydrogène, La catalase : Élimine le peroxyde d'hydrogène du corps et la glutathion peroxydase : Élimine le peroxyde d'hydrogène.

III.8 Évaluation de la dynamique moléculaire

- **Propriétés thermodynamiques**

L'évolution des propriétés thermodynamiques du meilleur ligand E,E-acétate de farnésyle a été étudié par l'approche de simulation DM dans l'ensemble NVT et NPT (Tableau 16). Nous avons effectué des minimisations énergétiques du meilleur complexe après un amarrage de 600ps. Puis réalisé des simulations jusqu'à (cycles de production DM) 100ns en trois étapes sous contraintes (voir Tableau 17).

Etape d'amorçage sp1 0-20ns : Chauffage (thermalisation) de 0 à 300 K par exemple.

Etape d'équilibrage sp2 20ns -40ns : Simulation normale mais le système n'est pas en équilibre.
40ns-60ns relaxation (adaptation) de l'eau à la surface de la protéine

Phase de production sp3 60ns-100ns : Nous avons enregistré les trajectoires

Enfin, l'analyse des résultats et la validation par contribution au score (énergie) obtenu par amarrage.

Tableau 17 : Propriétés thermodynamiques calculées en unités de bobines

| Stage | Method | H | U | EKT | P | V |
|-----------------|-----------|-----------|-----------|----------|----------|-------------|
| SP ₁ | PEROX1NVT | -165.222 | -1121.029 | 2397.107 | -259.305 | 21731.382 |
| | PEROX2NPT | 36.306 | -2679.731 | 1027.389 | -57.269 | 417.362 |
| | PEROX1NVT | 1.584 | -2233.072 | 1930.936 | -184.288 | 21731.382 |
| | PEROX2NPT | 1.320 | -2874.760 | 1199.694 | -58.531 | 218.488 |
| | PEROX1NVT | -1.367 | -2258.180 | 1976.864 | 296.146 | 21731.382 |
| | PEROX2NPT | -0.943 | -2929.258 | 1269.987 | -52.646 | 501.078 |
| SP ₂ | PEROX1NVT | -2273.905 | 1975.170 | 1297.141 | 286.447 | 21057.382 |
| | PEROX2NPT | 0.835 | -2909.660 | 1252.199 | -57.083 | 601...500 |
| | PEROX1NVT | 2.523 | 2356.218 | 2016.582 | 114.555 | 21757...382 |
| | PEROX2NPT | 92.991 | -2923.494 | 1358.883 | -49.508 | 129...876 |
| | PEROX1NVT | 3.171 | -2332.877 | 2332.877 | 148.306 | 21761.382 |
| | PEROX2NPT | -1903.034 | -5548.395 | 1990.654 | -58.157 | 700.555 |
| SP ₃ | PEROX1NVT | 1986.618 | -2372.214 | 1986.618 | -125.724 | 21731.382 |
| | PEROX2NPT | -1993.422 | -5682.353 | 2034.241 | -59.258 | 680.882 |
| | PEROX1NVT | 2.226 | -2339.252 | 1965.961 | -136.010 | 21731.382 |
| | PEROX2NPT | -3045.507 | -2253.922 | 2253.925 | -57.256 | 351.001 |
| | | | | | | |
| | PEROX1NVT | 1.678 | -2429.067 | 1953.394 | -132.638 | 21731.382 |
| PEROX2NPT | -5154.32 | -5839.098 | 3174.523 | -53.748 | 143.483 | |

Pression $P = P^* \epsilon / \sigma^3$. Energie de configuration $U = U^* N\epsilon$ Energie cinétique de translation $EKT = EKT^* N\epsilon$ et Enthalpie $H = H^* N\epsilon$.

Les résultats représentés dans le tableau 16 ont révélé que les énergies cinétiques de translation et l'énergie interne pour le E,E-acétate de farnésyle (Ligand 69) dans l'ensemble NVT et NPT.

Dans les unités NVT, les énergies cinétiques de translation et l'énergie interne du E,E-acétate de farnésyle sont faibles par rapport comparé à l'ensemble NPT. Et la fluctuation de pression pour le récepteur complexe est importante. Par conséquent, le E,E-acétate de farnésyle (Ligand 69) devrait être le système le plus interactif. Ces résultats sont en accord total avec les résultats de prédiction d'amarrage (voir les Tableaux 12-13). Nous pouvons montré l'analyse détaillée des résultats de simulation DM du composé E,E-acétate de farnésyle (Ligand 69) avec la cible de l'enzyme superoxyde dismutase (SOD) (Figure 25 et 26).

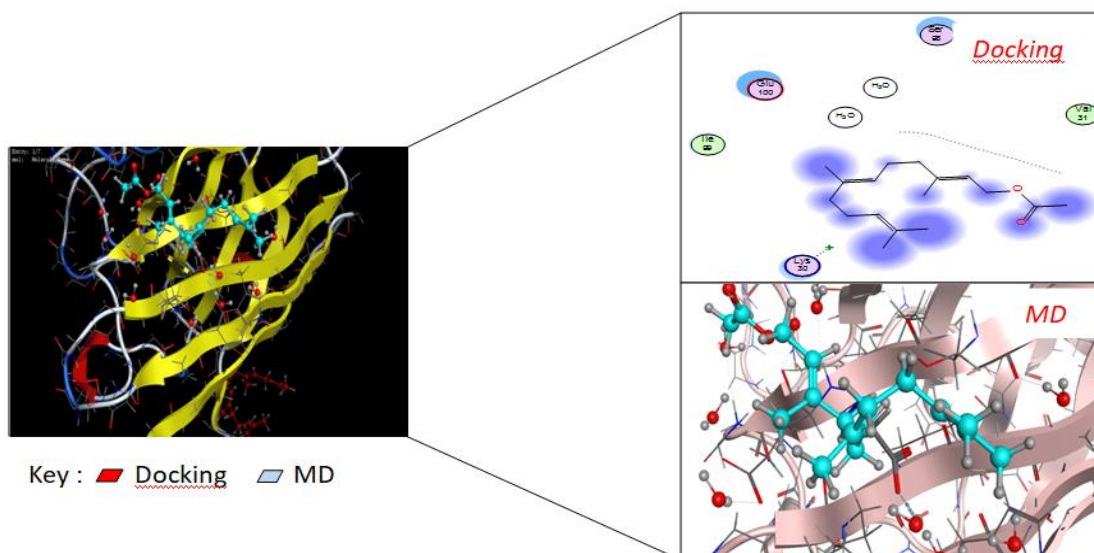


Figure 26 : le docking du composé – 69, E,E-acétate de farnésyle sans eau dans le site de liaison de la SOD; il existe également une nette différence entre la pose finale du ligand lors du docking après une simulation de dynamique moléculaire (DM) dans NVT.

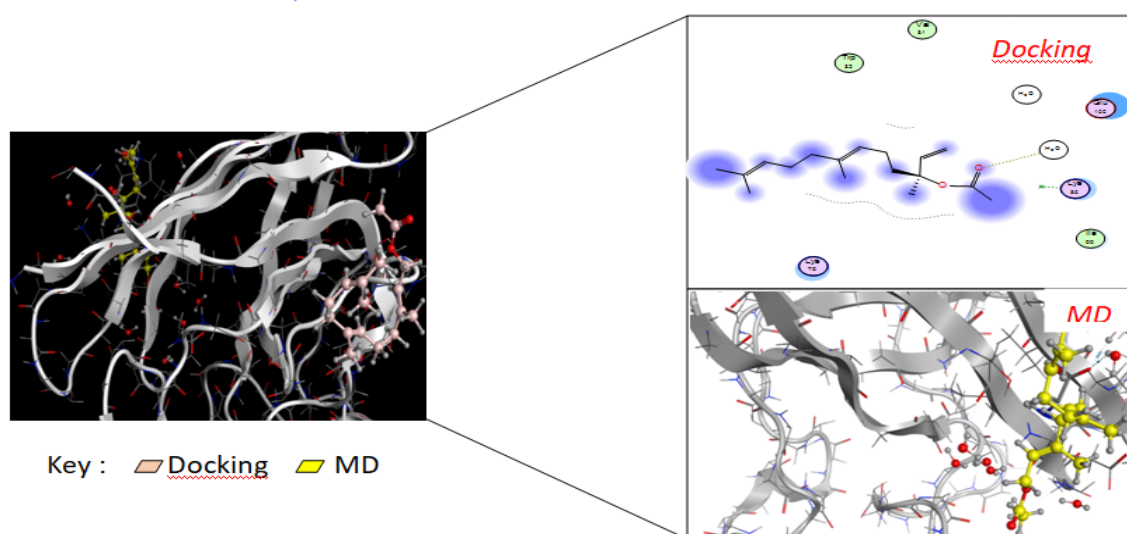


Figure 27. docking du composé – 69 ; E,E- acétate de farnésyle sans eau dans le site de liaison de la SOD; il existe également une nette différence entre la pose finale du ligand lors du docking après une simulation de dynamique moléculaire (DM) dans NPT.

Les propriétés dynamiques structurales sont simulées par la DM, nous avons étudié la dynamique structurale d'évolution de la meilleure molécule (ligand L69) (voir Tableau 16) par IMODS. L'analyse en mode normal (AMN) du complexe préparé E,E-acétate de farnésyle-superoxyde dismutase (SOD) a été illustrée dans (Figure 27).

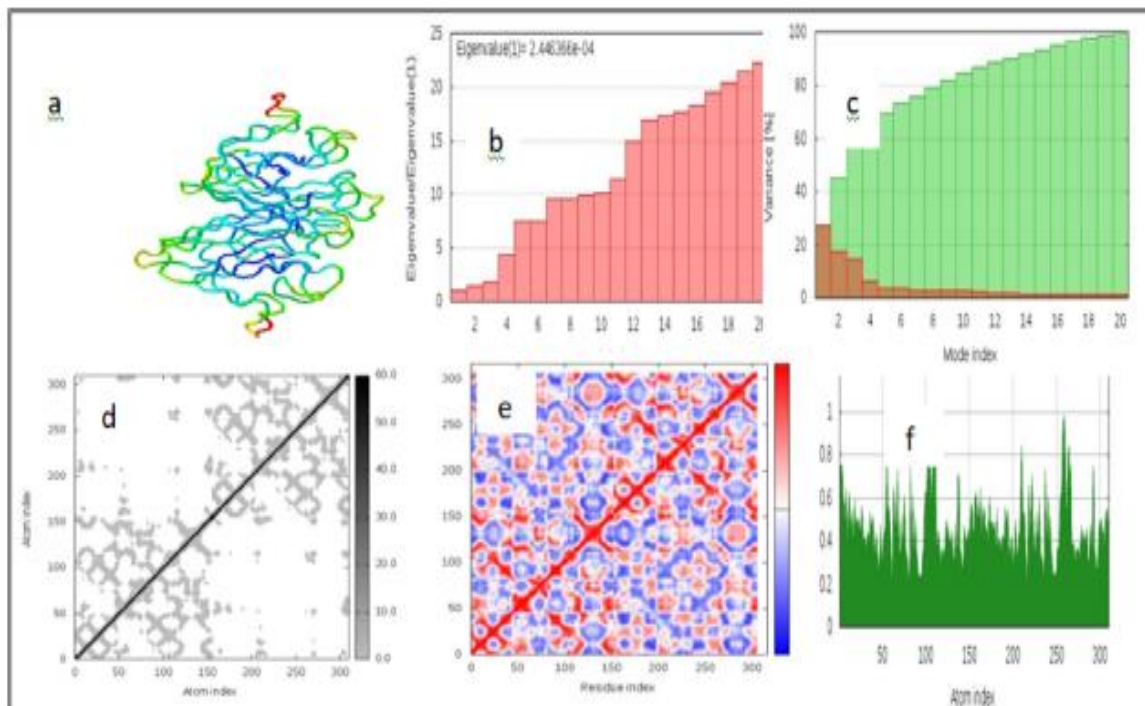


Figure 28. Résultats de la simulation de la dynamique moléculaire du complexe E,E-acétate de farnésyle - Superoxyde Dismutase (SOD). (a) mobilité AMN, (b) valeur propre, (c) variance (la couleur rouge indique les variances individuelles et la couleur verte indique les variances cumulées), (d) réseau élastique (les régions gris foncé indiquent les régions plus rigides) du complexe, (e) carte de covariance (mouvements corrélés (rouge), non corrélés (blanc) ou anti-corrélés (bleu)) et (f) déformabilité.

À partir de l'étude DM du complexe préparé E,E-acétate de farnésyle-superoxyde dismutase (SOD), il a été clair que le complexe enzyme-ligand préparé avait une valeur propre assez élevée de $2,446366e-04$, la valeur propre est illustrée dans (Figure 27b). Cependant, la carte des variances a montré un degré élevé de variances cumulatives par rapport aux variances individuelles (Figure 27c). La carte de covariance et de réseau élastique a également produit des résultats tout à fait satisfaisants (Figure 27d et Figure 27e) respectivement.

Les courbes de déformabilité du complexe E,E-acétate de farnésyle-superoxyde dismutase (SOD) illustrent les pics des graphiques qui correspondent aux régions de la protéine avec déformabilité (Figure 27f). La molécule (ligand sélectionné) peut être utilisée comme agent potentiel pour épuiser les radicaux libres.

III.9 Évaluation in silico des propriétés ADMET

Les résultats des paramètres d'évaluation pharmacocinétique et de toxicité des deux meilleurs composés sont dans le Tableau 18.

Les composés L69 et L65 présentent une disponibilité orale prometteuse, en raison de la perméabilité cellulaire optimale de Caco-2 et de et de l'intestinal humain >90% respectivement. Par conséquent, ils présentent une biodisponibilité orale prometteuse.

Les volumes de distribution (VDs) des deux meilleurs ligands L65 et L69 ont été respectivement de 0,273 et 0,275, ce qui suggère que les médicaments seront bien distribués dans le plasma puisque les valeurs de VDss sont inférieures à -0,15. Ainsi, VDss décrit l'étendue de la distribution du médicament et la fraction non liée décrit la portion de médicament libre dans le plasma qui peut s'extravaser. De plus Le Ligand 69 et le Ligand 65 ne sont pas des substrats du transporteur de cations organiques rénal 2 (OCT2) et ne sont pas toxiques (test d'AMES). La pénétration à travers la barrière hémato-encéphalique (BHE) s'est avérée aussi bonne puisque les valeurs ont été supérieur à 0,3 : 0,570 pour L65 et 0,621 pour L69. Par conséquent, les composés L69 et L65 peuvent être des agents antioxydants avec plus de potentiels intéressants.

Tableau 18 : Paramètres d'évaluation pharmacocinétiques et de toxicité des deux meilleurs composés.

| | | L 65 | L 69 |
|---------------------|--|---------|---------|
| Absorption | intestinale Humain | 93.999 | 93.643 |
| | perméabilité cutanée | -1.93 | -1.896 |
| | perméabilité Caco-2 | 1.64 | 1.632 |
| | Superficie | 117.785 | 117.785 |
| Distribution | VDss (humain) | 0.273 | 0.275 |
| | Fraction non liée (humain) | 0.176 | 0.157 |
| | perméabilité BHE | 0.57 | 0.621 |
| | perméabilité SNC | -2.133 | -1.973 |
| Excrétion | clairance totale | 1.752 | 1.749 |
| | Transporteur rénal de cations organiques | No | No |
| Toxicité | Toxicité aiguë orale chez le rat (DL50) | 1.631 | 1.633 |
| | toxicité AMES | No | No |
| | Toxicité de Tetrahymena Pyriformis | 2.251 | 2.344 |

III.1 Interaction des composés majoritaires de quelques espèces du genre *Inula* avec la superoxyde dismutase

La validation de nos résultats, pour l'huile essentielle des parties aériennes de *I. montana*, l'antioxydant synthétique acide ascorbique et l'inhibiteur co-cristallisé de la méthionine et certaines espèces du genre *Inula* est mentionnée dans le Tableau 19.

Tableau 19 : Bilan énergétique des complexes formés avec la Superoxyde Dismutase (SOD) sous puissant antioxydant clinique, quelques espèces du genre *Inula* et nos résultats pour les huiles essentielles de *I. montana*.

| Payé | Score | Espèces | Composés majoritaires des huiles essentielles |
|------------------------------|---------------|---|---|
| Bulgarie [133] | -3.738 | <i>I. germanica</i> | cis- acétate de carvyl 20.7% |
| | -3.265 | <i>I. bifrons</i> | Muurola-4,10 (14) dien-1-ol 8.6% |
| | -3.866 | <i>I. ensifolia</i> | Acide hexanoïque 8.1% |
| | | <i>I. salicina</i> | Acide hexanoïque 8.3% |
| Turquie [135] | -3.135 | <i>I. graveolens</i> | 1,8-cinéole 22.4% |
| | | <i>I. oculus-christi</i> | acétate de bornyle 21.3% |
| Algérie (Oran) [19] | -3.601 | <i>I. viscosa</i> | acétate de bornyle 39.81% |
| France [19] | | <i>I. graveolens</i> | acétate de bornyle 54% |
| Italie (Sicile) [133] | -3.674 | | Thymol 11.8% |
| Tunisie [131] | -3.450 | | p-cymene 27.3% |
| Italie [134] | | <i>I. crithmoides</i> | p-cymene 30.1% |
| Espagne | | | p-cymene 53.8% |
| Malte [137] | | | p-cymene 26.2% |
| Grèce | -3.142 | | β-Phellandrène 30.7% |
| Croatie (île Badija) [134] | -4.051 | <i>I. verbascifolia</i> | Acide hexanoïque 10.4% |
| Clinique | | | |
| Acide ascorbique | -3.329 | | |
| Ligand de référence | | | |
| Méthionine | -3.388 | | |
| Nos résultats | | | |
| E,E-acétate de farnésyle L69 | -4.401 | | |
| E- acétate de nérolidol L65 | -4.226 | Huile essentielle de <i>Inula Montana</i> | |

D'après les résultats d'amarrage moléculaire des composés majoritaires des huiles essentielles de quelques espèces du genre *Inula* avec la superoxyde dismutase, nous constatons que tous les composés forment des complexes avec une stabilité importante et des énergies

faibles, mais le résultat le plus prometteur reste celui du E,E-acétate de farnésyle. Ce qui met ces composés en perspectives pour d'autres investigations biologiques.

L'inhibition du récepteur de l'enzyme superoxyde dismutase a été étudiée théoriquement par deux méthodes de chimie computationnelle : les analyses du docking moléculaire, les simulations DM.

Les résultats d'amarrage moléculaire ont montré que parmi tous les composés le E,E-acétate de farnésyle **L69** et le E-acétate de nérolidol **L65** ont une puissante affinité de liaison avec la superoxyde dismutase et l'ADNc, et donc les meilleurs inhibiteurs. Cependant le composé E,E-acétate de farnésyle a montré une meilleure activité de piégeage des antioxydants que les autres composés, ce qui peut être attribué d'après la simulation d'amarrage moléculaire et les simulations de dynamique moléculaire (DM) à l'allongement de l'alkyle au niveau de la chaîne d'acétate.

Nos résultats d'amarrage moléculaire avec la superoxyde dismutase (SOD) et l'ADNc coïncident avec les résultats cliniques. En comparant nos résultats avec les inhibiteurs : l'acide ascorbique et la méthionine, nos ligands naturels E,E-acétate de farnésyle **L69** et E-acétate de nérolidol **L65** ont mieux stabilisé les systèmes (Tableau 13).

Par ailleurs, l'évaluation *in silico* des propriétés ADMET ainsi que le tracé BOILED-Egg ont validé que les composés L69 et L65 traversent la barrière cérébrale et présentent une lipophilie élevée et un coefficient élevé de perméabilité cutanée dans les intestins avec une bonne biodisponibilité.

Le E,E- acétate de farnésyle peut être un candidat potentiel pour de meilleures performances. En effet, il est proposé comme médicament naturel actif par voie orale et il peut également être un bon candidat pour d'autres investigations biologiques et pharmacologique. Ainsi, le E,E-acétate de farnésyle et le E-acétate de nérolidol peuvent être étudiés plus afin d'évaluer davantage leur efficacité dans la réduction du stress oxydatif et en tant qu'antioxydants éventuels à utiliser dans l'industrie pharmaceutique.

Conclusion générale

L'Algérie occupe une position stratégique dans le monde et se caractérise par une biodiversité des reliefs et des étages bioclimatiques, ce qui lui confère l'opportunité d'avoir une très grande biodiversité végétale originale. De ce fait, l'étude de cette richesse inestimable constitue une piste de recherche prometteuse en vue de la valoriser par la suite.

Ce projet de thèse de doctorat a porté sur l'étude de la composition chimique et des activités biologiques des huiles essentielles de deux espèces médicinales spontanées de la région de Tlemcen, il s'agit de *Inula montana* et *Carlina hispanica*. Le choix de ces dernières a été porté sur le fait qu'elles sont utilisées dans la médecine traditionnelle dans le traitement de plusieurs pathologies, et qui n'ont pas fait l'objet d'aucune étude auparavant.

Ce travail avait pour objectif la contribution à l'amélioration des connaissances des ressources naturelles de la biomasse végétale de l'Ouest Algérien en fournissant des informations scientifiques objectives dans l'espoir de trouver de nouvelles substances bioactives destinées à des applications économiquement durables dans divers secteurs.

L'investigation chimique des huiles essentielles a été réalisée avec l'aide de la mise en œuvre d'une procédure basée sur la complémentarité des techniques d'analyses incluant la CPG, la CPG/SM, ainsi que la chromatographie liquide et la RMN mono et bidimensionnelle.

L'extraction des huiles essentielles a été menée par hydrodistillation dans un appareil de type Clevenger. Le rendement des huiles essentielles de *Inula montana* en provenance des 7 stations a été entre 0.01% et 0.18%. Soixante-neuf composés ont été identifiés représentant un pourcentage de 97.2%-99.6% du mélange complexe. Le profil chromatographique a montré que les sesquiterpènes oxygénés (74.3%) constituent la fraction majoritaire avec 31 composés, suivis de 22 composés sesquiterpéniques hydrocarbonés. Par ailleurs, la classe des monoterpènes oxygénés et des composés oxygénés non terpéniques ont été détectées avec un pourcentage de 0,5% et 8 composés dans chaque classe. Les principaux constituants volatils des parties aériennes ont été le shyobunol, α -cadinol, tau-cadinol et le δ -cadinène. De plus, l'analyse nous a montré que le profil chimique des huiles essentielles des différentes stations est le même et qu'aucune variabilité chimique n'a été observé. L'huile essentielle de la partie souterraine de *Carlina hispanica* a été obtenue avec un rendement de 1.4%. La composition chimique a révélé la prédominance d'un composé oxygéné non-terpénique, le benzyle-2-furylacétylène ou l'oxyde de carline (81,1%), suivis par les sesquiterpènes hydrocarbonés (de 13,1%) et un sesquiterpène oxygéné (4,5%).

Le volet appliqué de cette thèse a été consacré aux tests biologiques qui apportent une dimension supplémentaire à la valorisation des huiles essentielles des espèces sélectionnées. Pour *Inula montana*, nous avons testé le pouvoir de piégeage des radicaux *in vitro* de l'huile essentielle à l'aide de deux méthodes DPPH et FRAP. L'huile essentielle de *Inula montana* s'est avérée être dotée d'une bonne activité. Qui peut être liée à son profil chimique notamment à la présence des pourcentages élevés de sesquiterpènes oxygénés et hydrocarbonés

En ce qui concerne l'huile essentielle des racines de *Carlina hispanica* ainsi qu'à sa composante majoritaire l'oxyde de carline, l'évaluation de l'activité antioxydante a été faite via les tests DPPH, FeCl₂ et β-carotène et a démontré une activité prometteuse. Ce qui a été confirmé par la cohérence des résultats entre les différents tests. Toutefois, les combinaisons oxyde de carline-EDTA et oxyde de carline-BHT ont affiché le pouvoir antioxydant le plus intéressant avec un effet synergique, avec des CI₅₀ inférieures même à celles des références.

Pour l'activité antimicrobienne, elle a été évaluée à l'aide de la méthode de diffusion sur disque en papier et les concentrations minimales inhibitrices par la méthode des microplaques à 96 puits les résultats obtenus ont indiqué que l'huile essentielle de *Inula montana* a présenté des effets prometteurs, en particulier contre la souche *Klebsiella pneumoniae* ATCC 70603 et *Listeria monocytogenes* ATCC 19115.

D'autre part, l'oxyde de carline et l'huile essentielle de *Carlina hispanica* ainsi que les combinaisons oxyde de carline-références se sont montrées moins actives vis-à-vis les bactéries et les levures et carrément inactives contre *Pseudomonas aeruginosa* ATCC 27853. Cependant, les résultats obtenus via la technique de diffusion sur disque en papier, les CMI de l'activité antifongique se sont révélées intéressantes. Les CMI de l'huile essentielle ont été meilleures que celle de l'oxyde de carline, d'autre part, les valeurs des CMI ont témoigné aussi que la combinaison de l'oxyde de carline avec les antibiotiques de références se sont montrées meilleures avec un effet synergique remarquable.

De même, nous avons testé l'effet anti-inflammatoire de l'oxyde de carline, l'huile essentielle de *Carlina hispanica* et la combinaison oxyde de carline-diclofénac de sodium via la méthode de l'inhibition de la dénaturation protéique. Les résultats obtenus démontrent que l'effet le plus prometteur a été obtenu par la combinaison de l'oxyde de carline-diclofénac de sodium. À une concentration de 15 mg/mL, le pourcentage d'inhibition a été de 83,90%, suivi par celui de l'oxyde de carline 77,95% et enfin par celui de l'huile essentielle 75,02%.

L'inhibition du récepteur de l'enzyme superoxyde dismutase a été théoriquement étudiée par deux méthodes de chimie computationnelle : les analyses d'amarrage moléculaire, les

simulations DM, les propriétés ADME et les connaissances pharmacologiques. Les résultats ont révélé que les inhibiteurs naturel le E,E-acétate de farnésyle L69 et le E- acétate de nérolidol L65 de l'huile essentielle des parties aériennes de *I. montana* ont une affinité pour interagir avec l'ADN et la superoxyde dismutase. Bien que ces deux composés L65 et L69 aient une puissante affinité de liaison avec la superoxyde dismutase dans la simulation d'amarrage. Le composé E,E-acétate de farnésyle a montré une meilleure activité antioxydante que les autres composés. La simulation d'amarrage moléculaire et les simulations de dynamique moléculaire (DM) ont indiqué que l'activité supérieure du composé E,E-acétate de farnésyle parmi d'autres composés pourrait être attribuée à l'allongement alkyle de la chaîne acétate. De plus, l'évaluation *in silico* des propriétés ADMET et le graphique BOILED-Egg valident que les composés 65 et 69 traversent la barrière cérébrale et ont une lipophilie élevée et un coefficient de perméabilité cutanée élevé dans les intestins avec une bonne biodisponibilité. Le E,E- acétate de farnésyle (sesquiterpènes oxygénés) a l'affinité de liaison la plus élevée parmi tous les inhibiteurs, il est proposé comme médicament naturel actif par voie orale et il peut également être un bon candidat pour d'autres investigations biologiques et pharmacologiques. Les résultats obtenus dans cette étude révèlent que L65 et L69 ont une puissante capacité antioxydante dans au moins deux récepteurs (superoxyde dismutase et la séquence d'ADN) via la génération de ROS. Ainsi, le E,E- acétate de farnésyle et le E- acétate de nérolidol nécessitent d'autres analyses afin d'évaluer davantage leur efficacité dans la réduction du stress oxydatif et comme antioxydants possible à utiliser dans l'industrie pharmaceutique.

Au vu des résultats intéressants obtenus, nous pouvons conclure que les huiles essentielles de *Carlina hispanica*, *Inula montana*, et la molécule « oxyde de carline » présentent des activités biologiques intéressantes. Ce qui ouvre des perspectives et des pistes de recherche au niveau de la connaissance scientifique en les explorant par des études *in vivo*, pour les utiliser dans la prolongation de la durée des aliments et pour les incorporer dans le secteur pharmaceutique en tant qu'agents antiinflammatoire et antimicrobiens.

Par conséquent, d'autres études pourront être envisagées :

- Une étude expérimentale complémentaire *in vivo* du pouvoir antioxydant de l'huile essentielle de *Inula montana* pour vérifier les résultats théoriques obtenus *in silico*.
- Le test de l'activité antiinflammatoire *in silico*, *in vitro* et/ou *in vivo*.
- La recherche du composé responsable de l'activité antimicrobienne de l'huile essentielle de *Inula montana*.

- L'étude *in silico* et *in vivo* des activités antioxydante, antiinflammatoire et antifongique de l'huile essentielle de *Carlina hispanica* et l'oxyde de carline.
- La poursuite de notre investigation sur les extraits des espèces étudiées et le test de d'autres activités biologiques.



Partie expérimentale

1. Matériel végétal

La matière végétale de *Carlina hispanica* et *Inula montana* ont été identifiées conjointement avec le Professeur BNABADJI Noury du laboratoire de botanique d'Ecologie et gestion des écosystèmes de l'Université de Tlemcen et le Docteur BABALI Brahim du laboratoire d'Ecologie et gestion d'écosystèmes naturels, Département de biologie et environnement, Faculté des sciences, Université de Tlemcen.

Inula montana

Le matériel végétal (parties aériennes) de *Inula montana* a été récolté à partir de 7 stations en mai 2019 de la région de Tlemcen au stade de pleine floraison, répartie entre différentes zones. Les coordonnées GPS des différentes stations sont les suivantes :

Sidi ouchaa (S1) [35°06'55"N, 1°46'37"O], Bouzedjar (S2) [35°34'42"N, 1°08'03"O], Ouchba (S3) [34°54'00"N, 1°13'20"O], Werdania (S4) [35°16'49"N, 1°30'13"O], Ain fezza (S5) [34°52'26"N, 1°12'36"O], Wed tafna (S6) [34°45'29"N, 1°34'04"O], Oum el Alou (S7) [34°49'00"N ; 1°16'15"O].

Carlina hispanica

La partie souterraine (racines) de *Carlina hispanica* a été récoltée le 1 mai 2019 à partir de la région de Ain fezza [34°51'44.98"N, 1°12'2.59"O] située à environ 8 km au Nord-Ouest de Tlemcen-Algérie.

2. Extraction des huiles essentielles

Pour chaque prélèvement végétal, les parties aériennes et les parties racinaires (500-1000g) ont été hydrodistillées pendant 5h à l'aide d'un appareil de type Clevenger dans le respect du procédé validé par la pharmacopée européenne [198]. Afin d'optimiser les conditions d'extraction, les rendements en huile essentielle ont été calculés par rapport à la masse du matériel sèche. La teneur en eau de chaque espèce a été déterminée en fonction de la perte de masse après 7 jours de séchage à température ambiante. Les huiles essentielles ont été conservées dans des flacons opaques, bien scellés, à 5°C pour des utilisations ultérieures.



Figure 30. Dispositif de l'extraction des huiles essentielles de type Clevenger.

2.1. Rendement

Selon la norme AFNOR (1986), le rendement en huile essentielle est défini par le rapport entre le poids de l'huile extraite obtenue et le poids du matériel végétal utilisé. Il est exprimé en pourcentage (%), et calculé selon la formule suivante :

$$\text{Rdt}_{(\text{HE})} = \frac{\text{M}_{\text{HE}}}{\text{M}_{\text{MV}}} \times 100$$

Où :

Rdt (HE) : Rendement en huile essentielle (%).

M HE : Masse de l'huile essentielle.

MMV : Masse de la matière végétale.

3. Fractionnement, purification et identification

La séparation de l'oxyde de carline a été faite sur une colonne chromatographique remplie par un mélange de gel de silice-hexane constituant la phase stationnaire. 1g de l'échantillon a été introduit. L'élution a été effectuée en utilisant de l'hexane et hexane-acétate d'éthyle comme éluant en augmentant la polarité avec des gradients de 100% allant à 99/1% [199].



Figure 31. Fractionnement de l'huile essentielle.

4. Méthodes d'analyse des huiles essentielles

4.1. Analyse par CPG/FID

Les analyses par chromatographie en phase gazeuse ont été réalisées à l'aide d'un chromatographe Perkin Elmer Clarus 600 (Walton, MA, Etat Unis), équipé d'une double ionisation de flamme, et de deux colonnes capillaires en silice (60 mx 0,22 mm D.I., épaisseur du film de phase stationnaire 0,25 μm), Rtx-1 (polydiméthylsiloxane) et Rtx-Wax (polyéthylèneglycol). La température du four a été programmée avec une augmentation progressive de 60 °C à 230 °C à 2°C/min puis maintenue isotherme pendant 35min à 230°C. Pour les injecteurs et les détecteurs, les températures ont été maintenues à 280°C. La pression en tête de colonne a été de 25 psi avec un débit de 1 mL/min. L'échantillon a été injecté avec un volume de 0.2 μL , en utilisant de l'hydrogène comme gaz vecteur (0,7 ml.min⁻¹). Les indices de rétention polaire et apolaire (IR) des composants ont été déterminés à partir du logiciel Perkin-Elmer.

4.2. Condition CPG-SM

L'huile essentielle a été analysée par chromatographie en phase gazeuse couplée à la spectrométrie de masse à l'aide d'un chromatographe Perkin Elmer Turbo Mass. Doté d'un injecteur automatique et de deux colonnes (60 mx 0,22 mm D.I., épaisseur du film de phase stationnaire 0,25 μm), polaire (Rtx-Wax) et apolaire (Rtx-1). L'énergie d'ionisation est de 70eV, La température de la source d'ion est de 150°C, la gamme de masse avec laquelle les spectres de masse ont été acquis est de 35-350 Da. L'injection de l'échantillon a été faite par un mode split avec un rapport de division de 1/80 avec un volume de 0, 2 μL .

2.6. Identification et quantification des composés

L'identification des composants a été réalisée par (i) comparaison des indices de rétention calculés respectivement sur colonnes polaires et apolaires avec celles des composés standards (bibliothèque laboratoire "Arôme") ou ceux rapportés dans la littérature. (ii) correspondance informatique des spectres de masse (impact électronique) avec ceux de composés standards (bibliothèque laboratoire) ou ceux présents dans les banques informatisées.

La quantification des constituants a été effectuée à l'aide d'un détecteur à ionisation de flamme par standardisation interne des aires des pics en utilisant les facteurs de réponse calculés par rapport au tridécano (0,7g/100g) utilisé comme étalon interne d'après la méthode décrite par Bicchi et al [200] et adaptée au sein du laboratoire de Corse [201].

4.3. Résonance magnétique nucléaire

Les analyses ont été faites sur un appareil Magritek Spinsolve 60MHz, opérant à 60MHz pour le proton et 15,08MHz pour le carbone-13. L'appareil est calibré selon le pic du D₂O à 4,71ppm, considéré comme référence interne.

Sauf autrement spécifier, les spectres ont été enregistrés dans le chloroforme deutéré. Les données des spectres sont traitées en utilisant le logiciel MestReNova-14.1.0-24037_x645.

Activités biologiques

5.1. Activité antioxydante

5.1.1. Piégeage du radical libre DPPH (2,2-diphényle-1-picrylhydrazyl)

Le pouvoir antioxydant de l'huile essentielle de *Inula montana*, *Carlina hispanica* et de l'oxyde de carline a été testé par la méthode DPPH (2,2-Diphenyl Picryl-Hydrazyl) [144].

- Pour *Inula montana*, la réaction a été réalisée dans un volume total de 100 µL de différentes concentrations comprises entre [0,05-0,09 mL/L]. Ajoutée par la suite à 25 µL de la solution méthanolique de DPPH (0,5 mM).
- Pour *Carlina hispanica*, 20 mg/mL d'huile essentielle et une solution mère d'oxyde de carline ont été préparées dans une série de concentrations différentes comprises entre [0,5-15 mg/mL]. 1000 µl de chaque concentration ont ensuite été mélangées avec 1000 µl de la solution éthanolique de DPPH (0,5 mM).

L'ensemble des échantillons a été incubé pendant 30 minutes à l'obscurité et à température ambiante. Les absorbances ont été testées à l'aide d'un spectrophotomètre à 517 nm. Les mêmes concentrations ont été préparées pour l'acide ascorbique et le BHT qui servent

de témoin positif pour *Inula montana*, *Carlina hispanica* et l'oxyde de carline respectivement. L'activité antioxydante est estimée selon l'équation suivante :

$$\% \text{ inhibition} = [(Abs_{\text{control}} - Abs_{\text{test}}) / Abs_{\text{control}}] \times 100$$

Où :

- **Abs_{control}** : Absorbance à 517 nm du contrôle.
- **Abs_{test}** : Absorbance à 517 nm des échantillons.

Les CI₅₀ ont été calculées graphiquement par la formule de régression linéaire des pourcentages d'inhibition en fonction de différentes concentrations de l'échantillon testé.

5.1.2. Réduction du pouvoir antioxydant ferrique (FRAP)

Le pouvoir réducteur du fer (Fe³⁺) a été évalué en utilisant la technique d'Oyaizu [202]. Les différentes concentrations de l'huile essentielle (0,5 à 50 mL/L) ont été mélangées avec 2,5 mL de la solution tampon phosphate (0,2 M, pH 6,6) et 2,5 mL d'une solution de ferricyanure de potassium K₃Fe (CN)₆ à 1%. Ensuite le tout a été incubé pendant 20 mn à 50°C. Une fois l'incubation a été achevée, 2,5 mL de l'acide trichloracétique (10%) a été ajouté afin de stopper la réaction. Le tout a été centrifugé à 3000 tours pendant 10mn. A la fin, 2,5 mL du surnageant a été mélangé avec 2,5 mL d'eau distillée. Les absorbances ont été mesurées à 700 nm utilisant un spectrophotomètre à double faisceau visible. Le BHT a été utilisé comme contrôle positif. Les analyses ont été réalisées en triplicata.

5.1.3. Test de blanchissement du β-carotène

Le pouvoir antioxydant de l'huile essentielle et de l'oxyde de carline a été déterminé en mesurant l'inhibition de la dégradation oxydative du β-carotène (décoloration) par les produits d'oxydation de l'acide linoléique selon la méthode décrite par Bougateg et al [203]. L'émulsion β-carotène /acide linoléique a été préparée en dissolvant 2 mg de β-carotène dans 10 mL de chloroforme, 25 μL d'acide linoléique et 200 mg de Tween 40 ont été mélangés, le chloroforme a été complètement évaporé à 40 °C au rota-vapeur, puis 100 mL d'eau distillée saturée en oxygène ont été ajoutés, l'émulsion résultante a été agitée vigoureusement. Un volume de 2,5 mL de l'émulsion précédente a été transféré dans des tubes à essai contenant 1 mL d'extrait dans de l'éthanol à différentes concentrations. Chauffé ensuite à 50 °C dans un bain-marie.

L'absorbance a été mesurée pour tous les échantillons à 470 nm, à t = 0 min et après un temps de 120 min, à l'aide d'un spectrophotomètre contre un blanc constitué d'une émulsion sans β-carotène. Le pourcentage d'inhibition a été calculé selon la formule suivante :

$$\% \text{inhibition} = 100 \times [1 - (A_E(0) - A_C(120)) / (A_C(0) - A_C(120))]$$

- **A_E (120)** : Absorbance à 470 nm des échantillons à t = 120 min.
- **A_C (0)** : Absorbance à 470 nm du contrôle à t = 0 min.
- **A_C (120)** : Absorbance at 470 nm du contrôle à t = 120 min

5.1.4. Test de Chélation du fer ferreux

L'effet chélateur des ions ferreux de l'huile essentielle et de l'oxyde de carline par le système de test ferrozine Fe²⁺ a été testé par la méthode de Chua et al [204].

Les solutions des échantillons et de l'antioxydant de référence EDTA (500 µl) ont été initialement mélangées avec 100 µL de FeCl₂ (0,6 mM dans l'eau distillée) et 900 µl d'éthanol. Après 5 min, 100 µl de Ferrozine (5 mM dans l'éthanol) sont ajoutés au milieu réactionnel. Le mélange a été bien agité puis laissé à réagir pendant 10 minutes à température ambiante. Le contrôle négatif contient tous les réactifs à l'exception de l'échantillon à tester qui est remplacé par un volume égal d'éthanol. L'absorbance de la réaction a été mesurée à 562 nm et le rapport d'inhibition de la formation du complexe ferrozine Fe²⁺ a été calculé comme suit :

$$\% \text{Inhibition} = [(A_c - A_s) / A_c] \times 100$$

- **A_c** : Absorbance at 562 nm du contrôle.
- **A_s** : Absorbance at 562 nm des échantillons.

5.2. Activité antiinflammatoire

L'activité antiinflammatoire *in vitro* a été évaluée par la méthode de dénaturation des protéines. Le diclofénac, un puissant antiinflammatoire est utilisé comme référence. Le mélange réactionnel est constitué de 2mL des différentes dilutions des huiles essentielles ou du control (eau distillée) et 2, 8 mL du tampon phosphate salin (PBS, pH 6,4) mélangé avec 0,2 mL d'albumine d'œuf (frais), ensuite le mélange est incubé à 37° C pendant 15 minutes. La dénaturation de l'albumine a été induite en bain marie à 70°C pendant 5 min. Après refroidissement, on mesure l'absorbance à 660 nm [205]. Le pourcentage d'inhibition de la dénaturation est calculé selon la formule suivante :

$$\% \text{inhibition} = [(A_{\text{abs control}} - A_{\text{abs test}}) / A_{\text{abs control}}] \times 100 \quad [206]$$

- **A_{abs control}** : Absorbance à 660 nm du contrôle.
- **A_{abs test}** : Absorbance à 660 nm des échantillons.

5.3. Activité antimicrobienne

5.3.1. Microorganismes et conditions de croissance

Dans cette étude, les souches cibles ont été cultivées dans du Muller-Hinton Agar à une température de 37°C pendant 24 h, en revanche la levure a été cultivée dans du Sabouraud Agar (Merck) à une température de 37°C durant 48h.

Ces souches ont été constituées de :

✚ **Pour *Inula Montana* :**

- Deux bactéries à gram positif : - *Staphylococcus aureus* (ATCC 25923).
- *Listeria monocytogenes* (ATCC 19115).
- Trois bactéries à gram négatif : - *Escherichia coli* (ATCC 25922).
- *Pseudomonas aeruginosa* (ATCC 27853).
- *Klebsiella pneumoniae* (ATCC 70603).
- Une levure : - *Candida albicans* (ATCC 10231).

✚ **Pour *Carlina hispanica* :**

- Deux bactéries à gram positif : - *Enterococcus faecalis* (ATCC 29212).
- *Staphylococcus aureus* SAMS (ATCC 25923).
- Deux bactéries à gram négatif : - *Escherichia coli* (ATCC 25922).
- *Pseudomonas aeruginosa* (ATCC 27853)
- Deux levures : - *C. albicans* IPP444.
- *C. albicans* (ATCC10231).

Les suspensions microbiennes ont été réalisées dans de l'eau physiologique stérile (0,85%) et ajustée à une concentration finale correspondant à 10^7 – 10^8 CFU/mL pour les bactéries et à 1 à 5×10^6 CFU/mL pour les levures. Les huiles essentielles ainsi que la molécule ont été dissoutes dans du DMSO afin de faciliter leurs diffusions sur la gélose.

5.3.2. Technique de diffusion sur gélose

La méthode des disques permet de déterminer le pouvoir antimicrobien de ces composés vis-à-vis des microorganismes testés en utilisant le milieu Muller-Hinton Agar (MHA, Oxoid) pour les bactéries et le milieu Sabouraud Dextrose Agar (SDA, Merck) pour la levure. Cette technique est réalisée selon les recommandations de Clinical and Laboratory Standards Institute [207]. Les disques de papier filtre stériles de 05 mm (Whatman, no.2) ont été imprégnés de 10 µL de l'huile essentielle de *Carlina hispanica* ainsi que la molécule et de 15 µL de l'huile essentielle de *Inula montana*, ensuite placés sur la surface de la gélose appropriée (MHA ou SDA) préalablementensemencée par écouvillonnage, les boîtes sont ensuite incubées pendant 37°C durant 24 à 48h. La gentamicine (10µg) et l'amphotéricine B (0,2 mg), un

antibiotique et un antifongique commerciaux ont été utilisés comme contrôle positif pour les bactéries et les levures respectivement. Les diamètres de la zone d'inhibition (en mm) ont été mesurés. Ce test a été fait en duplicata, la moyenne et l'écart type ont été calculés avec le logiciel PAST VERSION 3.22.

5.3.3. Méthode de micro dilution sur milieu liquide

Dans cette technique, des microplaques à fond rond (96 puits) sont utilisées pour déterminer la concentration minimale inhibitrice, qui est la plus faible concentration de l'agent antimicrobien qui inhibe la croissance des microorganismes selon les recommandations de Clinical and Laboratory Standards Institute [208].

Dans chaque ligne de la microplaque, 100 µL du bouillon Mueller Hinton ou 100 µL du bouillon du Sabouraud Dextrose est déposé. Ensuite, 100 µL de chaque échantillon est introduite dans le 1er puits. Après avoir bien mélangé le contenu du 1er puits, 100 µL est prélevé, puis déposé dans le 2ème puits, et ainsi de suite jusqu'au 10ème puits où 100 µL restantes a été éliminé. Par conséquent, une dilution de ½ entre chaque puits sera obtenue. Les deux derniers puits représentent des témoins négatifs : le puits n°11 contient le milieu de culture et l'inoculum et le puits n°12 contient uniquement le bouillon.

Enfin, 100 µL de l'inoculum bactérien (5×10^5 UFC/mL) ou levurien (1 à 5×10^4 cellules/mL) a été ajouté dans chaque puits. Les microplaques ont été scellées et incubées à $37 \pm 2^\circ\text{C}$ pendant 24h. La concentration minimale inhibitrice (CMI) a été déterminée comme étant la concentration la plus faible pour laquelle aucune croissance ne s'est produite dans le puits.



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Annexes

Table 13a: Propriétés et minimisation d'énergie des ligands de l'huile essentielle de la partie aérienne de *Inula montana*.

| Molécules | Energies (Kcal/mol) | LogP | LogS | Toxicité |
|-----------------------------|---------------------|------|-------|----------|
| Hexanal | -2.10017e+000 | 1.77 | -1.38 | No |
| Z-hex-3-en-1-ol | 6.05208e+000 | 1.94 | -0.93 | No |
| β -Citronellene | 1.09326e+001 | 3.55 | -3.92 | No |
| 6-methyl-hept-5-en-2-one | 6.02568e+000 | 2.32 | -1.48 | No |
| 1,8-Dihydro cineole | -2.50281e+000 | 2.55 | -2.41 | No |
| Octanal | -2.50281e+000 | 2.55 | -2.41 | No |
| Nonal | 4.80498e+000 | 1.91 | -2.53 | No |
| α -terpineol | 3.87834e+001 | 4.27 | -5.91 | No |
| theaspirane 1 | 4.34100e+001 | 4.27 | -5.91 | No |
| cis-3-hexenyl tiglate | 1.21711e+001 | 2.85 | -2.38 | No |
| α -Cubebene | 3.87834e+001 | 4.27 | -5.91 | No |
| α -ylangene | 4.34100e+001 | 4.27 | -5.91 | No |
| α -Copaene | 4.34100e+001 | 4.27 | -5.91 | No |
| β -Bourbonene | 4.34100e+001 | 4.27 | -6.41 | No |
| Cyprene | 4.88527e+001 | 4.27 | -6.41 | No |
| cis- α -Bergamotene | 3.91656e+001 | 4.73 | -5.29 | Yes |
| α -gurjunene | 5.36129e+001 | 4.27 | -6.41 | No |
| Aristolene | 2.95612e+001 | 4.58 | -5.17 | No |
| (E)- β -Caryophyllene | 3.91656e+001 | 4.73 | -5.29 | No |
| β -Copaene | 4.34100e+001 | 4.27 | -5.91 | No |
| β -ghurjunene | 2.59488e+001 | 4.89 | -4.87 | No |
| Aromadendrene | 4.88527e+001 | 4.27 | -6.41 | No |
| α -Humulene | 4.28781e+001 | 5.04 | -3.92 | No |
| allo-Aromadendrene | 5.36129e+001 | 4.27 | -6.41 | No |
| γ -Muurolene | 2.95612e+001 | 4.58 | -5.17 | No |
| Germacrene-D | 3.17611e+001 | 4.89 | -4.74 | No |
| α -Murolene | 2.95612e+001 | 4.58 | -5.17 | No |
| Delta-cadinene | 2.61312e+001 | 4.73 | -4.80 | No |
| α -cadinene | 3.90002e+001 | 3.78 | -3.23 | No |
| α -calacorene | 3.54879e+001 | 3.78 | -3.54 | No |
| β -calacorene | 4.47669e+001 | 3.92 | -4.36 | No |
| E-nerolidol | 3.54879e+001 | 3.78 | -3.54 | No |

| | | | | |
|---------------------------------|--------------|------|-------|-----|
| epi-globulol | 8.54552e+001 | 1.23 | -2.09 | No |
| caryophyllene oxyde | 8.54552e+001 | 4.40 | -4.25 | No |
| Globulol | 5.49887e+001 | 3.47 | -4.79 | No |
| Humulene epoxyde II | 4.77798e+001 | 4.25 | -3.09 | Yes |
| β -oplopenone | 5.30337e+001 | 3.80 | -3.98 | No |
| viridiflorol | 4.03789e+001 | 3.70 | -4.64 | No |
| Zingiberenol | 2.38235e+001 | 4.25 | -3.09 | Yes |
| 1,10-diepi-cubenol | 8.27697e+000 | 5.38 | -5.86 | No |
| Aromadendreneepoxyde II | 4.99269e+001 | 3.48 | -4.96 | Yes |
| eudesma-4(15)-en-6-one | 3.57237e+001 | 3.69 | -2.29 | No |
| Cadin-4-en-7-ol | 4.09548e+001 | 3.78 | -3.23 | No |
| T-Muurolol | 3.54879e+001 | 3.78 | -3.54 | No |
| β -eudesmol | 4.47669e+001 | 3.92 | -4.36 | No |
| α -Cadinol | 3.54879e+001 | 3.78 | -3.54 | No |
| Z-Z-farnesol | 1.60145e+001 | 4.40 | -4.25 | No |
| α -eudesmol | 2.18401e+001 | 5.20 | -6.01 | No |
| β -bisabolol | 2.76289e+001 | 4.58 | -4.67 | No |
| Curcumen-15-al | 2.19599e+001 | 4.35 | -4.75 | No |
| Eudesm-7(11)-en-4- α -ol | 4.93225e+001 | 4.06 | -3.67 | No |
| Shyobunol | 5.30337e+001 | 3.80 | -3.98 | No |
| Ledol | 5.91711e+001 | 3.47 | -4.79 | No |
| 8,11,14-Heptadecatrien-2-one | 8.27697e+000 | 5.38 | -5.86 | No |

Table 14a: Résultat du bilan énergétique des complexes (enzyme-ligands) Kcal/mol.

| Mol | Energie de liaison (Kcal/mole) | Rmsd -refine | Energy -Conf | Energy Place | Energy Refine | RMSD |
|--------------------------|--------------------------------|--------------|--------------|--------------|---------------|-------|
| Hexanal | -2.995 | 3.099 | -5.978 | -30.237 | -6.069 | 0.060 |
| Z-hex-3-en-1-ol | -3.212 | 1.877 | 16.055 | -25.903 | -7.039 | 0.035 |
| β -citronelle | -3.119 | 1.951 | 22.989 | -28.863 | -7.362 | 0.054 |
| 6-methyl-hept-5-en-2-one | -3.233 | 1.389 | -13.936 | -31.409 | -7.485 | 0.091 |

| | | | | | | |
|--------------------------------|---------|-------|---------|---------|---------|-------|
| 1,8-Dihydro cineole | -3.236 | 3.617 | 56.560 | -19.787 | -3.236 | 0.964 |
| octanal | -3.300 | 2.294 | -2.944 | -18.824 | -7.818 | 0.036 |
| Nonal | -3.328 | 4.524 | 8.706 | -26.487 | -7.175 | 0.097 |
| α -Terpineol | -3.662 | 3.119 | 18.409 | -7.545 | -8.734 | 0.460 |
| theaspirane 1 | -3.855 | 2.107 | 36.675 | -17.982 | -8.6748 | 0.043 |
| cis-3-hexenyl tiglate | -3.823 | 1.502 | 24.584 | -35.636 | -7.214 | 0.314 |
| α -cubebene | -3.2146 | 1.769 | 43.839 | -28.440 | -7.631 | 0.050 |
| α -ylangene | -3.127 | 3.108 | 49.812 | -15.673 | -7.395 | 1.021 |
| α -Copaene | -2.992 | 2.247 | 52.798 | -22.361 | -7.083 | 0.032 |
| β -borbonene | -3.337 | 3.347 | 47.716 | 36.622 | -7.866 | 0.047 |
| Cyprene | -3.135 | 3.115 | 66.442 | 18.991 | -6.310 | 0.990 |
| cis- α - bergamotene | -3.134 | 2.411 | 48.391 | -31.669 | -7.557 | 0.106 |
| α -gurjunene | -3.554 | 3.400 | 38.933 | 26.525 | -6.757 | 1.067 |
| Aristolene | -3.367 | 3.118 | 58.719 | 8.070 | -7.860 | 1.001 |
| E- β - caryophyllene | -3.154 | 2.911 | 47.761 | -7.266 | -8.735 | 0.151 |
| β -copaene | -3.234 | 3.119 | 45.917 | 34.860 | -7.894 | 0.363 |
| β -gurjunene | -3.449 | 2.925 | 66.064 | 20.565 | -5.556 | 0.055 |
| nomadendrene | -3.081 | 2.897 | 24.397 | 74.377 | -5.253 | 0.126 |
| α -humulene | -2.927 | 4.158 | 62.965 | 199.949 | -5.524 | 0.132 |
| Allo- aromadendrene | -3.084 | 3.389 | 71.830 | 22.228 | -7.042 | 0.141 |
| γ -muurolene | -3.549 | 3.947 | 45.821 | 56.752 | -9.566 | 0.057 |
| germacrene D | -2.955 | 2.368 | 40.212 | 66.737 | -6.414 | 0.183 |
| α -muurolene | -3.634 | 3.691 | 45.824 | 14.940 | -10.259 | 0.174 |
| E,E- α -farnesene | -3.915 | 1.511 | -1.997 | -5.738 | -3.758 | 1.154 |
| γ -cadinene | -3.947 | 4.325 | 41.094 | 8.636 | -10.755 | 0.051 |
| α -cadinene | -3.463 | 2.752 | 43.859 | -12.451 | -7.455 | 1.014 |
| α -calacorene | -3.359 | 2.115 | 44.733 | -9.132 | -7.069 | 1.021 |
| β -calacorene | -3.360 | 2.116 | 44.733 | -9.132 | -7.071 | 1.039 |
| E-nerolidol | -3.752 | 2.877 | 32.588 | -3.852 | -8.647 | 1.142 |
| epi-globulol | -3.438 | 1.944 | 62.730 | -24.761 | -8.619 | 1.102 |
| caryophyllene oxyde | -3.370 | 2.137 | 54.1124 | 12.223 | -8.315 | 1.128 |
| Germacrene D-4- ol | -3.2506 | 3.089 | 33.487 | 28.563 | -7.371 | 1.028 |

| | | | | | | |
|---------------------------------|---------|-------|--------|---------|---------|-------|
| Globulol | -3.3849 | 2.333 | 23.513 | 8.360 | -8.326 | 0.443 |
| humuleneepoxyde | -3.497 | 4.006 | 66.206 | 15.868 | -7.562 | 1.085 |
| β -oploponone | -3.354 | 3.472 | 36.251 | -10.682 | -7.188 | 1.009 |
| viridiflorol | -3.677 | 2.241 | 62.639 | -3.894 | -8.881 | 1.051 |
| zingeberenol | -3.564 | 1.479 | 27.119 | -6.487 | -8.541 | 0.268 |
| 1,10-diepi-cubenol | -3.444 | 1.224 | 5.152 | -6.843 | -6.786 | 0.394 |
| Aromadendrene epoxyde | -3.686 | 2.888 | 63.107 | 35.337 | -8.540 | 0.228 |
| eudesma-4(15)-en-6-one | -3.470 | 2.992 | 54.987 | 23.426 | -6.505 | 1.002 |
| cadin-4-en-7-ol | -3.915 | 3.048 | 48.475 | 0.407 | -9.423 | 0.165 |
| Trans-murolol | -3.655 | 1.986 | 40.394 | -25.627 | -8.789 | 0.047 |
| β -eudesmol | -3.578 | 0.959 | 8.875 | -42.053 | -7.934 | 0.987 |
| α -cadinol | -3.075 | 2.841 | 35.815 | 14.554 | -6.960 | 0.079 |
| Z,Z-farnesol | -3.999 | 2.529 | 30.995 | -3.686 | -10.632 | 0.091 |
| α -eudesmol | -3.571 | 0.959 | 8.867 | -42.053 | -7.918 | 1.139 |
| β -bisabolol | -3.557 | 3.699 | 35.043 | 2.230 | -8.104 | 0.975 |
| ar-curcumen-15-al | -3.996 | 1.585 | 43.186 | -9.686 | -11.653 | 0.100 |
| eudesma-7,11-en-4- α -ol | -3.880 | 2.907 | 49.672 | -14.152 | -10.567 | 0.236 |
| Shyobunol | -3.433 | 2.084 | 70.682 | -18.550 | -7.232 | 0.070 |
| Ledol | -3.415 | 4.383 | 62.169 | 13.782 | -8.088 | 0.189 |
| heptadec-8,11,14-trien-2-one | -3.839 | 2.294 | 17.951 | 4.9099 | -6.093 | 1.133 |

Table 16a: Les énergies d'amarrage des inhibiteurs de la SOD.


| Compound | DE (kcal/mol) | DE (kcal/mol) | ETOR (kT) | EVDW (kcal/mol) | EIE (kcal/mol) |
|--------------------------|---------------|---------------|-----------|-----------------|----------------|
| Hexanal | -3.916 | -2.995 | 597.332 | 3342.402 | -8948.86 |
| Z-hex-3-en-1-ol | -4.009 | -3.212 | 596.126 | 3345.720 | -8943.17 |
| β -Citronellene | -4.838 | -3.119 | 592.205 | 3348.598 | -8936.64 |
| 6-methyl-hept-5-en-2-one | -4.538 | -3.233 | 598.320 | 3341.958 | -8936.61 |
| 1,8-Dihydro cineole | -4.540 | -3.236 | 627.105 | 27816.57 | -8907.44 |

| | | | | | |
|-----------------------------|--------|--------|----------|----------|----------|
| Octanal | -4.975 | -3.300 | 593.151 | 3346.690 | -8951.89 |
| Nonal | -4.900 | -3.328 | 589.330 | 3343.336 | -8941.91 |
| α -Terpineol | -4.065 | -3.214 | 601.468 | 3349.357 | -8932.99 |
| theaspirane 1 | -4.289 | -3.855 | 3749.304 | 613.157 | -8946.55 |
| cis-3-hexenyl tiglate | -5.025 | -3.823 | 647.190 | 6321.043 | -4319.38 |
| α -Cubebene | -4.689 | -3.214 | 610.306 | 3384.991 | -9032.64 |
| α -ylangene | -3.942 | -3.127 | 612.301 | 8762.842 | -8967.10 |
| α -Copaene | -6.484 | -2.992 | 601.373 | 3363.417 | -8934.37 |
| β -Bourbonene | -5.014 | -3.337 | 611.035 | 3347.124 | -8930.44 |
| Cyprene | -5.242 | -3.135 | 619.038 | 3277.628 | -8829.95 |
| cis- α -Bergamotene | -4.463 | -3.134 | 596.942 | 3361.550 | -8933.92 |
| α -gurjunene | -6.001 | -3.554 | 597.944 | 3267.282 | -8859.29 |
| Aristolene | -5.377 | -3.367 | 616.120 | 3336.980 | -8846.42 |
| (E)- β -Caryophyllene | -5.228 | -3.154 | 603.505 | 3357.169 | -8943.25 |
| β -Copaene | -5.889 | -3.234 | 603.198 | 3360.764 | -8940.74 |
| β -ghurjunene | -5.043 | -3.449 | 619.054 | 3317.916 | -8855.46 |
| Aromadendrene | -5.433 | -3.081 | 602.405 | 3341.775 | -8903.09 |
| α -Humulene | -5.263 | -2.927 | 592.172 | 3341.092 | -8893.32 |
| allo-Aromadendrene | -4.902 | -3.084 | 605.290 | 3332.595 | -8888.61 |
| γ -Muurolene | -6.295 | -3.549 | 584.796 | 3324.077 | -8877.05 |
| Germacrene-D | -4.843 | -2.955 | 591.711 | 3319.690 | -8875.09 |
| α -Murolene | -5.514 | -3.634 | 586.472 | 3324.826 | -8877.57 |
| Delta-cadinene | -5.914 | -3.033 | 587.266 | 3348.037 | -8915.92 |
| α -cadinene | -4.987 | -3.463 | 592.018 | 3329.039 | -8895.14 |
| α -calacorene | -5.442 | -3.359 | 612.513 | 3303.220 | -8867.23 |
| β -calacorene | -4.705 | -3.360 | 609.525 | 3344.146 | -8866.00 |

| | | | | | |
|---------------------------------|--------|--------|---------|----------|----------|
| E-nerolidol | -4.715 | -3.752 | 597.883 | 3307.371 | -8861.73 |
| epi-globulol | -5.053 | -3.438 | 615.302 | 3325.806 | -8872.41 |
| caryophyllene oxyde | -6.338 | -3.370 | 619.009 | 3288.317 | -8837.21 |
| Germacrene D-4-ol | -4.740 | -3.370 | 590.998 | 3318.289 | -8810.04 |
| Globulol | -6.213 | -3.384 | 617.196 | 3335.690 | -8905.43 |
| Humuleneepoxyde II | -5.772 | -3.497 | 597.785 | 3297.471 | -8816.91 |
| β -oplopenone | -4.720 | -3.354 | 602.155 | 3254.729 | -8748.37 |
| Viridiflorol | -4.340 | -3.677 | 638.614 | 3306.393 | -8864.61 |
| Zingiberenol | -5.201 | -3.564 | 593.478 | 3397.712 | -8993.73 |
| 1,10-diepi-cubenol | -4.519 | -3.444 | 6.790 | 0.272 | -6.263 |
| Aromadendreneepoxyde II | -4.398 | -3.686 | 612.929 | 3385.184 | -8991.95 |
| eudesma-4(15)-en-6-one | -3.240 | -3.470 | 601.660 | 193548.1 | -8878.01 |
| Cadin-4-en-7-ol | -4.211 | -3.915 | 593.034 | 3398.624 | -8982.18 |
| T-Muurolol | -4.519 | -3.479 | 593.141 | 3360.039 | -8960.44 |
| β -eudesmol | -5.398 | -3.578 | 599.518 | 3314.986 | -8820.03 |
| α -Cadinol | -5.310 | -3.075 | 600.368 | 3415.881 | -9012.55 |
| Z-Z-farnesol | -4.242 | -3.999 | 594.783 | 3420.311 | -9039.54 |
| α -eudesmol | -3.219 | -3.571 | 591.404 | 3298.312 | -8874.92 |
| β -bisabolol L60 | -4.367 | -3.557 | 605.394 | 3307.140 | -8804.15 |
| Curcumen-15-al | -3.040 | -3.996 | 599.566 | 3421.137 | -9028.64 |
| Eudesm-7(11)-en-4- α -ol | -4.105 | -3.880 | 606.410 | 3424.604 | -9031.10 |
| Shyobunol | -3.213 | -3.433 | 614.361 | 3404.770 | -8993.88 |
| Ledol | -3.105 | -3.415 | 614.888 | 3389.227 | -8993.63 |



Chemical composition/pharmacophore modelling- based, virtual screening, molecular docking and dynamic simulation studies for the discovery of novel superoxide dismutase (SODs) of bioactive molecules from aerial parts of *Inula Montana* as antioxidant's agents

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ABSTRACT

The accumulation of free radicals in the body develops chronic and degenerative diseases such as cancer, autoimmune diseases, rheumatoid arthritis, cardiovascular and neurodegenerative diseases. The first aim of this work was to study the chemical composition of *Inula Montana* essential oil using GC-FID and GC/MS analysis and the antioxidant activities using radical scavenging (DPPH) and the Ferric-Reducing Antioxidant Power (FRAP) tests. The second aim was to describe the assess the antioxidant activity and computational study of Superoxide Dismutase (SODs) and ctDNA inhibition. Sixty-nine compounds were identified in the essential oil of the aerial part of *Inula montana*. Shyobunol and α -Cadinol were the major compounds in the essential oil. The antioxidant power of the essential oil showed an important antioxidant effect compared to ascorbic acid and the methionine co-crystallized inhibitor. The results of the docking simulation revealed that E, E-Farnesyl acetate has an affinity to interact with binding models and the antioxidant activities of the ctDNA sequence and Superoxide Dismutase target. The penetration through the Blood-Brain Barrier came out to be best for E, E-Farnesyl acetate and E-Nerolidolacetate and was significantly higher than the control molecule and Lref. Finally, the application of ADMET filters gives us positive information on the compound E, E-Farnesyl acetate, which appears as a new inhibitor potentially more active towards ctDNA and SODs target. The active compounds, E,E-Farnesyl acetate can be used as templates for further development of more potent antioxidative agents.

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1. Introduction

Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources such the regular metabolism or external sources (Navidpour et al., 2007; Suthakaran et al., 2008). The action of free radicals is counteracted by free radicals endogenous or exogenous or synthetic route. Reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl, and nitric oxide radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases. The antioxidants are entities present at low concentration compared to that of oxidizable substrates (Halliwell, 1995). And which have the capacity to react with free radicals in order to delay or inhibit the oxidation of biological substrates, in other words, rendering them harmless (Al-Mamary et al., 2002). They have two origins, one endogenous represented by enzymes, and the other provided by food. On the other hand, synthetic antioxidants commonly used in

pharmacological and food applications are phenolic compounds such as butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA). However, following doubts about their carcinogenic and toxic effects, the latter were limited by legislative rules (Wichi, 1988). As a result, the interest that has been focused on identifying natural and safe alternative sources of natural antioxidants has continued to grow in recent years (Kabouche, 2014). Essential oils are a potential source of natural and bioactive molecules because their components have antioxidant power, and which could then be used as food preservatives to limit food spoilage. The *Asteraceae* family, also known by its compounds, is one of the most widely distributed in the plant kingdom and is particularly represented in temperate and cold regions of the globe. The latter has more than 13 tribes divided into 23,000 species and 1,500 genera whose Algerian soil includes 109 genera divided into 408 species, many of which are rich in producers of essential oils with biological properties (Adewole, 2002). The genus of *Inula* is among those

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belonging to the family of compounds and which holds a variety of about 90 abandoned species distributed in the Mediterranean basin (Kabouche, 2014). Traditional medicine has granted multiple therapeutic properties to species of the genus of *Inula*. Including the species that was the subject of our study *I. montana* which has an activity on the digestive system (Pascual & Morales, 2002). So, in order to identify possible sources of this plant and in the context of researching new bioactive substances, our study is based on the determination of the chemical composition of the essential oil of *I. montana* gathered in the north-west of Algeria using GC-FID and GC/MS analyzes as well as the evaluation of its antioxidant activity.

The main interest was to develop unique potential inhibitors of the Superoxide Dismutase and ctDNA interaction, to combat free radicals and protect the body from the damage caused by them. Additionally, Modeling and simulation techniques are becoming an important research method for investigating operational and organizational systems and in particular in Chemistry. They are often necessary when the real experience is too difficult, too dangerous and too expensive. Digital chemistry subsidizes a better understanding of the action of medicinal plants against diseases and offers high-level training, focused on the study of living things at the molecular level. In addition, represents a bridge between theory and experience. It makes it possible to represent, interpret and predict biomolecular structures and functions (Nadia et al., 2020, Fouzia et al., 2018). However, there are no reports on chemical composition and biological activities of *I. montana*. Therefore, this work was aimed to study for the first time, the chemical composition of *Aerial* parts and the intraspecies variations of essential oils from 7 locations using statistical analysis and the second study was to try to test the molecules of this oil for their antioxidant activity by interested at the interaction with binding models and the antioxidant activities of the ctDNA and Superoxide Dismutase target. It is an important antioxidant, active in endothelial cells, cytoplasm and mitochondrial intermembrane matrix (Oury et al., 1996). SOD protects cells against superoxide damage through catalyzing the dismutation of superoxide radicals into molecular oxygen and hydrogen peroxide (Marikovskiy et al., 2003; Oury et al., 1996) and thus inhibits peroxynitrite-mediated oxidative protein modification and cell membrane lipid peroxidation (Ischiropoulos et al., 1992).

On the other hand, we noted that in the last few years, the field of computer aided drug design and discovery (CADD) by using different methods such as: molecular docking, molecular dynamics and ADME prediction is a rapid wing area that has seen many successes to discover and develop new drugs in medicinal chemistry.

The inhibition of Superoxide Dismutase was theoretically investigated by two methods of computational chemistry: molecular docking analyzes and molecular dynamics simulations. In this contribution, a combined of two theoretical approaches by using drug likeness, pharmacokinetics, medicinal Chemistry and ADME Properties to explore potential inhibitors among compounds of essential oils of *Aerial* parts

of *I. montana* against enzyme: Superoxide Dismutase and ctDNA. The docking studies predicted that the constituent molecules of *Aerial* parts of *I. montana* possess more capability as inhibitors as compared to established drugs in the pharmaceutical industry.

2. Material and methods

2.1. Experimental procedures

2.1.1. Plant material and isolation of the essential oil

The aerial parts of *I. montana* were harvested at the flowering stage on May 2019 from 7 stations in Tlemcen (Algeria). The identification of the collected species was made by the laboratory of ecology and ecosystem management of the University of Tlemcen. The hydrodistillation of essential oils was done in a Clevenger type device for 5 h. The information of the habitats of the samples (places of harvest, altitudes and yield) is shown in (Table 1).

2.1.2. Identification of the oil components

2.1.2.1. Gas chromatography. The analyzes by gas chromatography (GC) were carried out using a Perkin Elmer Clarus 600 chromatograph (Walton, MA, United States), equipped with a Split/Splitless injector, two capillary columns in fused silica (60 m x 0.22 mm ID, film thickness 0.25 μ m), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Also equipped with a double flame ionization detection injector. The programming of the oven temperature consists of an increase from 60° C to 230° C at 2° C/min, then in a plateau of 35 min at 230° C. The essential oils were injected in split mode 1/50. With a volume of 0.2 μ L using hydrogen as carrier gas (0.7 ml min⁻¹). The pressure at the top of the column was 25 psi with a flow rate of 1 ml/min.

2.1.2.2. Gas chromatography/mass spectrometry. The samples were analyzed using a Perkin Elmer Autosystem XL chromatograph (Walton, MA, United States), equipped with an automatic injector and two columns (60 m x 0.22 mm ID, phase film thickness 0.25 μ m stationary), polar (Rtx-Wax) and non-polar (Rtx-1). Coupled with a Perkin Elmer Turbo Mass mass detector. The temperature of the ion source is 150° C, the ionization energy is 70 eV. The injection was made via split mode with a division ratio of 1/80. Mass spectra were acquired over the 35-350 Da mass range. And for the chromatographic conditions (temperature programming, carrier gas, etc.) are the same as those described above.

2.1.2.3. Component identification and quantification. The components were identified (i) by comparing their GC retention indices (RI) on non-polar and polar columns with those of standard standards (laboratory library "Aroma") or those reported in the literature. (ii) as well as by computer correspondence with the commercial mass of spectral libraries (Jennings & Shibamoto, 1980; National Institute of Standards and Technology, 1999) and comparison of the spectra with those of the laboratory's internal library. The quantification of the constituents was carried out via a flame ionization

Table 1. Data relative to harvest locations of *I. montana* from Algeria.

| Spieces | N° | Voucher codes | Localities | GPS coorodates | Yields | Alt |
|----------------------|----|---------------|-------------|-----------------------|--------|------|
| <i>Inula montana</i> | S1 | IM-1020-11 | Sidi wchaa | 35°06'55"N; 1°46'37"O | 0,09 | 54 |
| | S2 | IM-1020-12 | Bouzedjar | 35°34'42"N; 1°08'03"O | 0,02 | 26 |
| | S3 | IM-1020-13 | Ouchba | 34°54'00"N; 1°13'20"O | 0,06 | 780 |
| | S4 | IM-1020-14 | Werdania | 35°16'49"N; 1°30'13"O | 0,18 | 107 |
| | S5 | IM-1020-15 | Ain fezza | 34°52'26"N; 1°12'36"O | 0,1 | 780 |
| | S6 | IM-1020-16 | Oued tafna | 34°45'29"N; 1°34'04"O | 0,09 | 772 |
| | S7 | IM-1020-17 | Oum el Alou | 34°49'00"N ;1°16'15"O | 0.01 | 1022 |

detector by internal normalization of the peak areas calculated by integrating the response factors FID relative to tridecane (0.7g/100g) used as internal standard (Djabou et al., 2013).

2.1.2.4. DPPH free radical scavenging assay. The antioxidant power of the essential oil has been tested by the method that uses DPPH (2,2-Diphenyl Picryl-Hydrazyl). The reaction is carried out in a total volume of 100 µl of different concentrations included between [0.05-0.09]. Subsequently added to 25 µl of the methanolic solution of DPPH (0.5 mM) followed by incubation for 30 min in the dark and at room temperature. The absorbances were tested using a spectrophotometer at 517 nm. The same concentrations were prepared for ascorbic acid to serve as a positive control. The antioxidant activity is evaluated using the following equation:

$$AA\% = \frac{(\text{Abscontrol} - \text{Abstest})}{\text{Abscontrol}} \times 100 \quad \text{Eq. (1)}$$

Where: AA: antioxidant activity, Abs: absorbance. The IC₅₀'s was calculated graphically by the linear regression formula of the inhibition percentages as a function of different concentrations of the sample tested.

2.1.2.5. Ferric-Reducing antioxidant power assay. The reducing power of iron (Fe³⁺) was evaluated using the Oyaizu technique (Oyaizu, 1986). The different concentrations of the essential oil (from 0.5 to 50 mg/L) were mixed with 2.5 ml of the phosphate buffer solution (0.2 M, pH 6.6) and 2.5 ml of to 1% solution of potassium ferricyanide K₃Fe (CN)₆. Then the whole was incubated for 20 min at 50 °C. Once the incubation was completed 2.5 mL of trichloroacetic acid (10%) was added in order to stop the reaction. The whole was centrifuged at 3000 revolutions for 10 min. We followed the same technique of (El Omari et al., 2019), at the end 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water. Absorbances were measured at 700 nm using a double visible beam spectrophotometer against ethanol as blank. Ascorbic acid was used as a positive control. The analyzes were carried out in triplicate.

2.2. Theoretical background and comptional details

2.2.1. Preparation and optimization of both enzyme and inhibitors

In this study, the structures of all inhibitors were downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>) (Wang et al., 2017) and ChEMBL database

(<https://www.ebi.ac.uk/chembl>) The PDB database (<https://www.rcsb.org/>) was worm to obtain the complete structure of Superoxide Dismutase (PDB ID: **2wz5** (Antonyuk et al., 2010)). In general, the protein structure with a resolution between 1.5 and 2.5 Å have an excellent quality for further studies (Clément & Slenzka, 2006; Didierjean & Tête-Favier, 2016), whereas, the resolution value of Superoxide Dismutase and B-DNA belongs to this interval. The 3 D structures of Superoxide Dismutase enzyme and B-DNA sequence were obtained by X-ray diffraction with resolution 1.50 Å, 1.90 Å and R-Value Free 0.255, 0.178 respectively (Fig.1). The RMSD obtained by molecular docking varies between 0.116 and 1.154 according to table 5; the ligands tested are superimposed by the addition of the reference ligand (co-crystallized), which proves the correct choice of the enzyme. In addition, the structure of the co-crystal (reference ligand) of this SOD1 enzyme resembles the molecules of our plant.

Amyotrophic lateral sclerosis (ALS) is the most common degenerative disease of motor neurons in adults and has also been shown to be a type of conformational disease associated with improper folding and dysfunction of proteins.

- To date, over 150 distinct genes have been shown to be associated with ALS, of which *Superoxide Dismutase 1* (SOD1) is the first and most studied and is classified as mutant human SOD1 (Huai & Zhang, 2019). It has been well established that the toxicity mediated by SOD1 mutants is caused by gain in function rather than loss of the detoxifying activity of SOD1.

A large body of evidence has shown that SOD1 mutants can adopt a complex conformation profile and interact with a wide range of client proteins.

The Protein data bank (PDB) format of ligand and complexes was obtained from Chemdraw (MM2) energy minimized file and by converting its file using the Mercury software. The crystal data of the B-DNA dodecamer d (CGCGAATTCGCG)₂ (PDB ID: **1BNA**) (Drew et al., 1981) were downloaded from the Protein Data Bank. The water molecules were removed from the 1BNA using MOE before performing docking calculations.

2.2.2. Molecular docking

Molecular docking is a structure-based drug designing approach used to find out the best orientation and key interactions between ligand and receptor. Molecular docking simulation was performed on the X-ray crystal structures of Superoxide Dismutase (PDB code: **2wz5**) and B-DNA

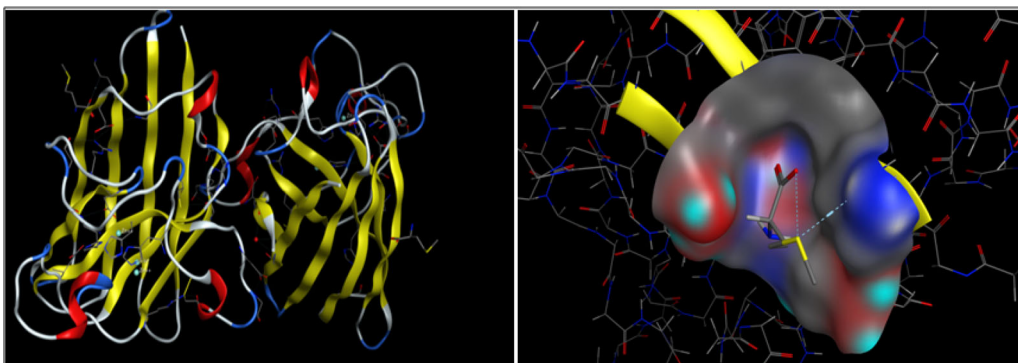


Figure 1. Enzymatic cavity with the residues of the active site for the target: 2wz5 detected by site finder (MOE).

sequence utilizing Molecular Operating Environment (MOE) software.

In this study, the software package MOE (Molecular Operating Environment) was introduced to study the modeling applications between essential oils of *Aerial parts of I. montana* compounds and the Superoxide Dismutase enzyme. MOE runs on Microsoft windows, mac Os and Linux (Molecular Operating Environment (MOE), 2013). The wash setting was applied at pH 6.0 and 300K, hydrogen atoms were added, and protonation 3D was assigned. The minimum energy configuration was performed using the MMFF94x force field. OPLS-AA force field was used (Jorgensen et al., 1996). To assign atom type and partial charges in receptor structure. The number of interactions varies between (0, n) where n is 10, the *cut-off* for coulomb interaction and *Vander Waal* interaction was 30 Å with the ability to study the hydrogen-electrostatic in the total active site of Superoxide Dismutase was optimized and the results were discussed. The predicted orientations and binding modes of essential oils of *Aerial parts of I. montana* compounds into the target Superoxide Dismutase were then visualized using software package MOE. For molecular docking calculations, we followed the same steps (same protocol) used in our previous studies (Chenafa et al., 2021; Daoud et al., 2018) and the default parameters are: Placement: Triangle Matcher; Rescoring 1: London dG (the scoring function was employed to estimate the lowest free energy of the complex with the best pose of ligand tested). During the docking process the ligand was considered structurally rigid while the target was set as completely flexible. At first, we minimized the energy of the enzymes and the ligands and their geometries were conducted using Hamiltonian AM1 implanted in MOE software and then isolation of the active site of the targets was carried out. The most stable conformation of each compound was minimized by the *same* semi-empirical method (AM1) (Stewart, 2007). All simulations were run by using all explicit solvation models using TIP3P water. (Semaoui et al., 2020) After that, the RMSD value was used to compare the differences between the atomic distances of the docked poses and the ligand molecule of reference pose, where a threshold of 2.0 Å corresponding to better solutions (Cross et al., 2009). In the end, positioning the ligands into the enzyme's active site was done using the docking module implanted in MOE software and, the binding energy (score) between ligands and targets was calculated using molecular mechanics (Molecular

Operating Environment (MOE), 2013) and based on molecular mechanics (Halgren, 1996; 1999). The results of the top-scoring docking poses were constructed and the best scoring complexes in the active site were selected for the further MD simulation study (Dal Ben et al., 2013).

2.2.3. Molecular dynamics simulation, ADMET, PASS cytochromes P450 and pharmacophore mapping

The top compound which has high binding affinity (Score) and one almost a stable interaction) with the Superoxide Dismutase target was subjected to Molecular Dynamics Simulations. Molecular dynamics was run by NAMD for 20 – 100 ns achieved for both the complex (2wz5-ligand) was carried out through MOE software (Molecular Operating Environment (MOE), 2013). Dynamics simulation needs the following parameters: sample time: 1 and time step: 0.001 for running simulation. The Langevin equation (Toda et al., 1991) is used in NAMD to generate the Boltzmann distribution for (canonical NVT, isobar-isotherm NPT) units and simulations. The Brünger-Brooks-Karplus (BBK) method (Brünger et al., 1984) is used to integrate the Langevin equation. The stochastic equations of motion (position and velocity) are described by Fokker-Planck (Wang & Skeel, 2003). The detailed analysis of (MD) simulation results of complex L69 with target Superoxide Dismutase is summarized in (See Figures 6 and .7). Moreover, the stable conformation obtained in the MD simulation between the best two ligands and Superoxide Dismutase target was conducted by iMODS. Internal coordinate's normal mode analysis server (iMODS) has been used for many years in online modeling of protein flexibility to investigate the values of deformability, eigenvalues, variance, co-variance map and elastic network. The software package MOE (Molecular Operating Environment) has proven its performance in several recent studies and has been invoked, for example in: Fouzia & Salim, (2019), Daoud et al., (2018), and Chenafa et al., (2021). That's why was used for our research. Among the 69 selected compounds the molecular structures of the best compounds were analyzed using a SWISSADME server (<http://www.swissadme.ch/>). The results of Absorption, Distribution, Metabolism and Excretion for selected compounds are listed in Table 11. These results prescribe that the ADMET-score would be a comprehensive index to estimate chemical drug-likeness. The drug score associate druglikeness, cLogP, logs, molecular weight and toxicity risks in one handy value than may be attuned to judge the compound's overall potential for

quality for a drug (Filimonov et al., 2014 & Geronikaki et al., 1999 and Lipinski et al., 1997). In this research, prediction and significant descriptors of druglikeness such as mutagenicity, toxicological dosage level for different tissues and pharmacologically relevant properties of the compounds were predicted using a PreADMET server (<http://preadmet.bmdrc.org/>) and admetSAR server (<http://lmm.d.ecust.edu.cn:8000/>) (Chen et al., 2012). The PASS (Prediction of Activity Spectra for Substances) may be efficiently used to find new targets for some ligands and, conversely, to reveal new ligands for some biological targets, and there are also many research where PASS predictions were approved by biological samples, subsequent synthesis and immunological analysis and cell test (Filimonov et al., 2014; Singh et al., 2014). The PASS predictions of the two best selected ligands were performed using the PASS-Way2Drug server. The results of the PASS prediction studies are summarized in Table 12. Cytochromes P450 are key enzymes involved in the metabolism of various endogenous or exogenous molecules. The results of The P450 sites of metabolism (SOM) of the two best selected compounds were determined by online tool, RS-WebPredictor 1.0 (Release, 2018) and listed in (See [Supplementary Materials](#) Table S7). The process of deriving pharmacophore is known as pharmacophore mapping. The pharmacophore mapping study of the best ligands was carried out by online server PharmMapper. It consists to identify a common binding element that is responsible for the biological activity and determining the 3D relationship between pharmacophore elements in each conformation generated. The pharmacophore mapping study of the two best ligands was carried out by online server PharmMapper (Parr, 1989). The pharmacophore modelling was done for the best ligand molecule among the 69 selected ligand molecules is summarized in Fig. 9. The conformations of the dataset were generated by using default settings in software MOE version 2013.08. MOE is comprehensive drug discovery software used ligand and structure-based pharmacophore modeling. However, in this study, we used ligand-based pharmacophore modeling to reveal the chemical features important for the compounds activity against IGF-1R. The Scientific Vector Language (SVL) command-line is provided by MOE that integrates with database browsers which helps in the formation of packed conformation dataset (Chen & Foloppe, 2008). The conformational search module in MOE was used to generate minimum energy conformations of each active and inactive compound.

A conformation training dataset of 20 chemical compounds was implemented in MOE to generate pharmacophore hypothesis. The dataset consisted of 20 active and 4 (α -Curcumene, Zingiberene, E, E- α -farnesene and trans-calamenene inactive compounds were employed to influence the quality of pharmacophore models depending on the two basic values are specificity and sensitivity. Active compounds have well known pharmacokinetics properties such as drug absorption, distribution, metabolism and excretion that directly bind to target receptors while inactive compounds had a poor binding affinity (Chen & Foloppe, 2008). In the present study, only active compounds were selected with common features such as HBDs, HBAs, Aro, HyD, number of non-polar atoms and rotatable bonds. The developed

pharmacophore model was selected based on molecular sequence and low RMSD (see Table 5). However, the P450 sites of metabolism (SOM), Prediction of Activity Spectra for Substances, pharmacophore mapping, druglikeness prediction and ADMET- calculations were carried out to determine and compare the biological activities of the two best ligands.

3. Results and discussion

3.1. Experimental approach

3.1.1. Chemical composition of *I. montana* essential oils

The hydrodistillation of dry leaves of *I. montana* collected from seven different regions for 4 h has resulted in essential oils of yellowish color. The essential oil yields for each station are mentioned in Table 1. The essential oil yields vary from 0.01% to 0.18% (w/w), from one station to another. The highest essential oil yields were obtained in the stations of Wardania (0.18%) (S4) and Ain fezza (0.1%) (S5) with altitudes of 107 m to 780 m respectively, the stations of Sidi wchaa (S1) which is located at 54 m and Oued tafna (S6) which is located at 772 m posted the same yield which is in the order of (0.9%). For the station of Ouchba (S3) which is located at an altitude of 780 m, the yield was (0.06%). While the lowest yields were observed in the stations of Bouzadjar (0.02%) (S2) and oum el alou (0.01%) (S7) with altitudes varying from 26 m to 780 m.

The components of the essential oil were identified by comparing their mass spectra (EI-MS) and their retention indices (RI) with those of the mass spectra library (Table 2). The essential oil of *I. montana* demonstrated the predominance of sesquiterpene components (99.2%) from the identified content. The oxygenated sesquiterpenes were the most dominate with a percentage of 74.3%. The main ingredients were Shyobunol (19.2%), α -cadinol (12.2%), tau-Cadinol (6.7%), δ -Cadinene (8.2%), Veridiflorol (3.5%), Caryophyllene oxide (3.2%), Z, E-Farnesyl acetate (6.4%) and ar-Curcumen-15-al (3.4%) (Table 2). It should be noted that the essential oil of the aerial part of *I. montana* has never been studied before. However, the chemical composition of the essential oils of other *Inula* species has been widely studied; according to geographical and botanical origins of the samples, monoterpenes (p-Cymene, Thymol, Bornylacetate ... etc) have been reported as the main component classes, but when we compare our results with those of other *Inula* species from different regions which were widely studied we notice a difference in the chemical composition, but the class of monoterpenes remains the predominant one (Table 13).

The major component of essential oil of leaves of *I. ensifolia* and *I. salicina* collected in Bulgaria (Trendafilova et al., 2020), and *I. verbascifolia* collected in Croatia (Giamperi et al., 2010) consist mainly of hexadecanoic acid (8.1%, 8.3%, and 10.4%) respectively. While bornyl acetate was detected as the main constituent in essential oil of *I. oculus-christi* from Turkey (21.3%) (Kilic, 2014), in *I. viscosa* from Algeria (39.81%) (Boumaza, 2011) and *I. graveolens* from France (54%) (Boumaza, 2011). On the other hand, p-Cymene was detected as the main component of essential oil of *I. crithmoides* collected in Tunisia (27.3%) (Jallali et al., 2014), Spain (53.8%) (Tsoukatou & Roussis, 1999), central Italy (30.1%)

Table 2. Chemical composition of the essential oil of leaves of *I. montana*.

| No | Components | IRI _a ^b | Rla ^c | Rl _{pd} | S1 | S2 | S3 | S4 | S5 | S6 | S7 | Identification |
|---------------------------|------------------------------|-------------------------------|------------------|------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|----------------|
| 1 | Hexanal | 773 | 770 | 1055 | 0,1 | 0,2 | 0,1 | tr | tr | tr | 0,2 | Rl, MS |
| 2 | Z-hex-3-en-1-ol | 834 | 831 | 1380 | 0,2 | tr | 0,3 | tr | tr | tr | 0,5 | Rl, MS |
| 3 | β-citronelle | 938 | 940 | 1031 | tr | 0,1 | 0,1 | tr | 0,1 | tr | 0,1 | Rl, MS |
| 4 | 6-methyl-hept-5-en-2-one | 961 | 963 | 1337 | 0,2 | tr | tr | tr | tr | 0,1 | 0,1 | Rl, MS |
| 5 | 1,8-Dihydro cineole | 979 | 979 | 1180 | 0,1 | tr | 0,3 | 0,1 | 0,6 | 0,1 | 0,4 | Rl, MS |
| 6 | Octanal | 981 | 982 | 1169 | 0,2 | 0,1 | 0,2 | 0,1 | 0,5 | 0,1 | 0,2 | Rl, MS |
| 7 | Nonal | 1082 | 1083 | 1394 | 0,1 | 0,1 | tr | 0,4 | 0,3 | 0,2 | 0,1 | Rl, MS |
| 8 | α-Terpineol | 1179 | 1175 | 1688 | 0,2 | tr | 0,4 | tr | 0,1 | 0,1 | 0,1 | Rl, MS |
| 9 | Theaspirane 1 | 1293 | 1292 | 1480 | 0,1 | tr | 0,1 | 0,1 | 0,1 | 0,1 | tr | Rl, MS |
| 10 | cis-3-Hexenyl tiglate | 1305 | 1304 | 1641 | 0,1 | tr | 0,1 | 0,1 | 0,1 | 0,1 | 0,1 | Rl, MS |
| 11 | Theaspirane 2 | 1308 | 1307 | 1517 | 0,1 | tr | 0,1 | 0,1 | tr | 0,1 | 0,1 | Rl, MS |
| 12 | α-Cubebene | 1348 | 1350 | 1452 | 0,1 | tr | 0,1 | 0,2 | tr | tr | 0,1 | Rl, MS |
| 13 | α-Ylangene | 1375 | 1372 | 1476 | 0,2 | 0,1 | 0,2 | 0,2 | 0,2 | 0,1 | 0,2 | Rl, MS |
| 14 | α-Copaene | 1379 | 1379 | 1488 | 0,1 | tr | 0,2 | 0,1 | tr | tr | 0,2 | Rl, MS |
| 15 | β-Borbonene | 1383 | 1385 | 1515 | 0,2 | 0,1 | tr | 0,3 | 0,2 | 0,1 | 0,1 | Rl, MS |
| 16 | Cyprene | 1402 | 1406 | 1525 | 0,3 | 0,3 | 0,3 | tr | tr | 0,5 | 0,7 | Rl, MS |
| 17 | cis-α-Bergamotene | 1409 | 1411 | 1562 | 0,3 | tr | 0,2 | tr | 0,1 | tr | 0,2 | Rl, MS |
| 18 | α-Ghurjunene | 1413 | 1410 | 1524 | 0,2 | 0,4 | 0,4 | 0,9 | 0,9 | 1,1 | 1,6 | Rl, MS |
| 19 | Aristolene | 1420 | 1416 | 1560 | 0,1 | 0,1 | 0,1 | tr | 0,1 | tr | tr | Rl, MS |
| 20 | E-β-Caryophyllene | 1424 | 1418 | 1524 | 0,3 | 0,3 | 0,9 | 0,1 | 0,1 | 0,4 | 1 | Rl, MS |
| 21 | β-Copaene | 1426 | 1431 | 1581 | 0,1 | 0,4 | 0,3 | tr | 0,2 | tr | 0,1 | Rl, MS |
| 22 | β-Ghurjunene | 1439 | 1439 | 1591 | 0,3 | 0,2 | 0,2 | 0,1 | 0,2 | 0,2 | 0,3 | Rl, MS |
| 23 | E-β-Farnesene | 1448 | 1448 | 1660 | 0,3 | 0,4 | 0,2 | 0,3 | 0,6 | 0,2 | 0,1 | Rl, MS |
| 24 | Nomadendrene | 1452 | 1450 | 1581 | 0,2 | tr | 0,1 | tr | 0,1 | tr | 0,3 | Rl, MS |
| 25 | α-Humulene | 1456 | 1451 | 1611 | 0,4 | 0,1 | 0,1 | 0,6 | 0,1 | 0,4 | 0,4 | Rl, MS |
| 26 | Allo-aromadendrene | 1462 | 1463 | 1476 | 0,2 | 0,1 | 0,5 | 0,3 | 0,8 | 0,4 | 0,4 | Rl, MS |
| 27 | α-Curcumene | 1471 | 1468 | 1465 | 0,2 | 0,1 | 0,6 | 0,3 | 0,3 | 0,1 | 0,4 | Rl, MS |
| 28 | γ-Murolene | 1471 | 1471 | 1681 | 0,2 | 0,1 | 0,3 | 0,5 | 0,3 | 0,5 | 1 | Rl, MS |
| 29 | Germacrene D | 1480 | 1480 | 1704 | 0,3 | 0,5 | 0,6 | 1,1 | 1,8 | 0,3 | 0,1 | Rl, MS |
| 30 | Zingebreene | 1486 | 1489 | 1717 | 0,1 | 0,2 | 0,1 | 0,3 | tr | 0,2 | 0,3 | Rl, MS |
| 31 | α-Murolene | 1496 | 1994 | 1719 | 0,3 | 0,1 | 1,5 | 1,1 | 2,7 | 0,2 | 1 | Rl, MS |
| 32 | E,E-α-Farnesene | 1498 | 1500 | 1740 | 0,3 | tr | tr | 0,1 | 0,6 | 0,1 | 0,1 | Rl, MS |
| 33 | γ-Cadinene | 1507 | 1507 | 1752 | 1 | 1,4 | 2,1 | 2,1 | 1,7 | 1,2 | 1 | Rl, MS |
| 34 | trans-Calamenene | 1512 | 1511 | 1816 | 0,3 | tr | 0,5 | 0,9 | 0,5 | 0,5 | 0,3 | Rl, MS |
| 35 | δ-Cadinene | 1516 | 1516 | 1752 | 9,7 | 9,2 | 7,7 | 8,4 | 8,9 | 5,1 | 3 | Rl, MS |
| 36 | α-Cadinene | 1535 | 1530 | 1740 | 0,3 | 0,5 | 0,7 | 0,4 | 0,7 | 0,3 | 1,3 | Rl, MS |
| 37 | α-Calacorene | 1531 | 1531 | 1895 | 1,1 | 0,2 | 0,5 | 0,4 | 0,7 | 0,2 | 1 | Rl, MS |
| 38 | β-Calacorene | 1548 | 1547 | 1936 | 0,5 | 0,3 | 0,2 | 0,4 | 0,2 | 0,2 | 0,2 | Rl, MS |
| 39 | E-Nerolidol | 1546 | 1551 | 2037 | 0,4 | 1,3 | 1,7 | 1,4 | 1,6 | 1,1 | 2,1 | Rl, MS |
| 40 | epi-Globulol | 1558 | 1559 | 2010 | 0,6 | tr | 0,3 | 0,6 | 0,3 | 0,4 | 0,4 | Rl, MS |
| 41 | Caryophyllene oxyde | 1576 | 1571 | 1980 | 3,2 | 1,4 | 2,1 | 3,1 | 2,1 | 2,5 | 3,5 | Rl, MS |
| 42 | Z-Nerolidol | 1518 | 1521 | 1987 | 0,3 | 0,1 | 0,2 | 0,4 | 0,5 | 0,2 | 0,3 | Rl, MS |
| 43 | Germacrene D-4-ol | 1573 | 1566 | 2020 | 0,7 | 4,4 | 2,5 | 1,8 | 1,1 | 3,4 | 1,2 | Rl, MS |
| 44 | Globulol | 1580 | 1576 | 2067 | 0,4 | 0,5 | 1,9 | 0,7 | 1,3 | 1,7 | 0,4 | Rl, MS |
| 45 | Humulene epoxyde | 1592 | 1601 | 2044 | 1,1 | 0,4 | 0,7 | 0,5 | 0,4 | 0,1 | 1 | Rl, MS |
| 46 | β-Oplopenone | 1593 | 1590 | 2017 | 1,2 | 2,6 | 1,1 | 4,1 | 1,3 | 2,2 | 3,2 | Rl, MS |
| 47 | Viridiflorol | 1591 | 1591 | 2089 | 4 | 4,5 | 3,9 | 2,6 | 3,1 | 5,8 | 4,5 | Rl, MS |
| 48 | Zingiberenol | 1598 | 1599 | 2019 | 0,5 | 0,8 | 0,6 | 0,4 | 0,3 | 0,1 | 1 | Rl, MS |
| 49 | 1,10-diepi-Cubenol | 1608 | 1608 | 2025 | 0,4 | 1,1 | 1,3 | 1,3 | 1,2 | 1,2 | 0,8 | Rl, MS |
| 50 | Aromadendrene epoxyde | 1615 | 1617 | 2002 | 0,4 | 1,2 | 1,3 | 1,5 | 1,1 | 0,8 | 1,4 | Rl, MS, Ref |
| 51 | Eudesma-4(15)-en-6-one | 1616 | 1614 | 2039 | 1,4 | 0,3 | 1,2 | 1,5 | 1,1 | 1,3 | 0,5 | Rl, MS |
| 52 | Cadin-4-en-7-ol | 1627 | 1627 | 2096 | 1,1 | 0,5 | 0,6 | 1,6 | 1,2 | 1,6 | 1,1 | Rl, MS |
| 53 | Trans-Murolol | 1630 | 1630 | 2134 | 0,6 | 0,5 | 0,4 | 0,2 | 0,5 | 0,6 | 1,2 | Rl, MS |
| 54 | tau-Cadinol | 1632 | 1632 | 2169 | 6,7 | 9,5 | 8,4 | 6,6 | 9,1 | 5,6 | 6,7 | Rl, MS |
| 55 | β-Eudesmol | 1638 | 1644 | 2234 | 0,5 | 0,3 | tr | tr | tr | 0,3 | 2,4 | Rl, MS |
| 56 | α-Carinol | 1644 | 1645 | 2231 | tr | tr | tr | tr | tr | tr | 0,2 | Rl, MS |
| 57 | α-Cadinol | 1645 | 1642 | 2231 | 12,2 | 12,3 | 16,1 | 15,2 | 18,8 | 15,3 | 12,2 | Rl, MS |
| 58 | Z,Z-Farnesol | 1648 | 1653 | 2167 | 1,2 | 1 | 0,9 | 0,7 | 0,2 | 0,5 | 2 | Rl, MS |
| 59 | α-Eudesmol | 1653 | 1649 | 2220 | 1,8 | 1,8 | 1,1 | 1,6 | 1 | 1,8 | 1,1 | Rl, MS |
| 60 | β-Bisabolol | 1656 | 1653 | 2140 | 1,2 | 0,8 | 0,9 | 0,7 | 0,5 | 1,2 | 1,3 | Rl, MS, Ref |
| 61 | ar-Curcumen-15-al | 1681 | 1675 | 2164 | 3,4 | 1,5 | 1,9 | 1,6 | 1 | 3 | 3,9 | Rl, MS |
| 62 | Eudesma-7,11-en-4-α-ol | 1683 | 1683 | 2300 | 0,6 | 0,3 | 0,1 | 0,5 | 0,2 | tr | 2 | Rl, MS |
| 63 | Shyobunol | 1687 | 1688 | 2218 | 19,7 | 23,9 | 21,1 | 20,5 | 19,1 | 19,5 | 10,5 | Rl, MS |
| 64 | Ledol | 1696 | 1693 | 2050 | 2,1 | 0,3 | 2 | 0,7 | 1,8 | 1,1 | 4 | Rl, MS |
| 65 | E-Nerolidol acetate | 1732 | 1738 | 2269 | 5,4 | 2,7 | 3,2 | 1,4 | 3,1 | 4,9 | 0,9 | Rl, MS, Ref |
| 66 | β-Acoradienol | 1797 | 1792 | 2221 | 6,4 | 0,9 | 2,1 | 4,3 | 2,1 | 1,1 | 2,1 | Rl, MS |
| 67 | Z,E-Farnesyl acetate | 1840 | 1832 | 2356 | 0,4 | 0,2 | 1,5 | 0,4 | 0,3 | 0,2 | 6,4 | Rl, MS |
| 68 | Heptadec-8,11,14-trien-2-one | 1871 | 1871 | 2330 | 1,6 | 4,1 | 1,3 | 0,4 | 0,5 | 3,7 | 0,4 | Rl, MS |
| 69 | E,E-Farnesyl acetate | | | | 1,6 | 4,1 | 1,3 | 0,4 | 0,7 | 5,4 | 1,2 | |
| Total Identification % | | | | | 99,2 | 98,6 | 99,6 | 98,7 | 99,3 | 99,3 | 97,2 | |
| Yields % (w/w) | | | | | 0,09 | 0,02 | 0,06 | 0,18 | 0,1 | 0,09 | 0,01 | |
| Hydrocarbon compounds | | | | | 18 | 15,8 | 17,3 | 18,2 | 21,4 | 12,9 | 16,7 | |
| Sesquiterpenehydrocarbons | | | | | 18 | 15,8 | 17,3 | 18,2 | 21,4 | 12,9 | 16,7 | |

(continued)

Table 2. Continued.

| No | Components | IRI _a ^b | Rla ^c | Rlpd | S1 | S2 | S3 | S4 | S5 | S6 | S7 | Identification |
|----|----------------------------------|-------------------------------|------------------|------|------|------|------|------|------|------|------|----------------|
| | Oxygenated compounds | | | | 75,3 | 75,6 | 76,5 | 72,2 | 73,3 | 80,6 | 80,5 | |
| | Oxygenated monoterpenes | | | | 0,5 | tr | 0,9 | 0,3 | 0,8 | 0,4 | 0,6 | |
| | Oxygenated sesquiterpenes | | | | 74,3 | 75,4 | 74 | 71,4 | 72 | 79,9 | 79,9 | |
| | Non-terpene oxygenated compounds | | | | 0,5 | 0,2 | 1,6 | 0,5 | 0,5 | 0,3 | 0,6 | |

Table 3. % Inhibition of DPPH• of aerial part of *I. montana* essential oil and ascorbic acid at different concentrations.

| Samples | Antioxidant activity | | | | | | |
|---------------|--------------------------------------|-------------|-------------|-------------|--------------|-------------|--------------|
| Essential oil | Concentration (mL/L) | 0.04 | 0.05 | 0.06 | 0.075 | 0.09 | |
| | DPPH Radical scavenging activity [%] | 33.06 | 42.01 | 49.75 | 63.6 | 78.57 | |
| | IC ₅₀ (mL/L) | | | | | | 0.059 |
| Ascorbic acid | Concentration (mL/L) | 0.04 | 0.05 | 0.06 | 0.07 | 0.08 | |
| | DPPH Radical scavenging activity [%] | 36.03 | 47.95 | 61.27 | 75.32 | 90.7 | |
| | IC ₅₀ (mL/L) | | | | | | 0.051 |

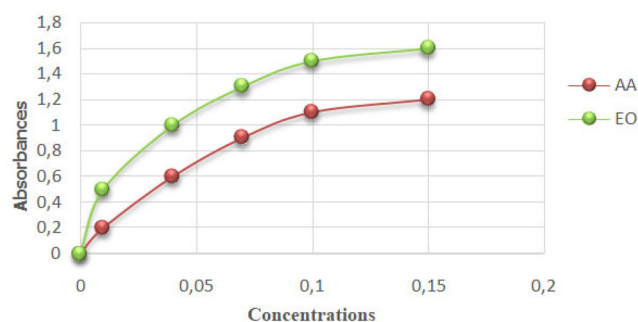
(Giamperi et al., 2010) and Malta (26.2%) (Tsoukatou & Roussis, 1999). Then that of Greece (Tsoukatou & Roussis, 1999) by β -Phellandrene (30.7%) and that of Sicily (Italy) (Fontana et al., 2014) by thymol (11.8%). The essential oil of *I. germanica* and *I. bifrons* collected in Bulgaria (Trendafilova et al., 2020) was composed by cis-Carvyl acetate (20.7%) and Muurola-4,10 (14) dien-1-ol (8.6%) respectively, and that of *I. graveolens* from Turkey (Kilic, 2014) was constituted by 1,8-cineole (22.4%). Note that the variation in the composition of essential oils could be due to several factors; the climatic conditions of the season of sample collection, the water supply, the nature and composition of the soil as well as insect attacks that can influence the chemical composition of essential oils (Baser & Buchbauer, 2010). From our results cited in Table 2, we were able to trace the variation in the contents of the different chemical classes of essential oils from other *I. Montana*.

The histogram (See Supplementary Materials Figure S1) showed that the essential oil is rich in oxygenated sesquiterpenes (74.3%), the percentage of hydrocarbon sesquiterpenes is relatively lower (18%) compared to oxygenated sesquiterpenes. However, oxygenated monoterpenes (0.5%) and oxygenated non-terpene components (0.5%) constitute the lowest percentage classes.

3.1.2. Evaluation of the antioxidant activities of essential oil

The Antioxidant power was performed by the DPPH free radical scavenging and the ferric reducing antioxidant power (FRAP) tests using ascorbic acid as a positive control. The comparison of the DPPH scanning activity of the essential oil of *I. montana* with that expressed by ascorbic acid showed good antioxidant activity. Indeed, the essential oil has the ability to reduce the DPPH radical to 78.57% with a concentration of around 0.09 mL/L (Table 3).

Figure 2 represent the absorbance values as a function of different concentrations of essential oil obtained by the FRAP method. The antioxidant power of the essential oil increases with increasing concentration. However, at 0.15 mL/L, the antioxidant activity of the essential oil was greater than of the synthetic antioxidant (ascorbic acid) (Fig.2).

**Figure 2.** Reducing power activities of *I. montana* essential oil (EO), and Ascorbic acid.

3.2. Theoretical and computational methods

The ligands of essential oils from the Aerial parts of *I. montana* minimized toxicity and energy obtained by MOE software are shown in (Table 4). Properties for other compounds (See Supplementary Materials Table S1)

As stated in the table above. We find that the molecules Z,E-Farnesyl acetate, E-Nerolidol acetate, E, E-Farnesyl acetate, and E- β -Farnesene have a high value of Log P and Log S compared to other molecules and also the results obtained show that these ligand molecules (Z,E-Farnesyl acetate, E,E-Farnesyl acetate, E-Nerolidol acetate and E- β -Farnesene have a high value of torsion angle relative to other compounds. This shows that these compounds L67, L69, L65, L23 and L56 are more flexible. As well, it is noted that the growth of the torsion angle depends on the binding number of the molecule.

3.2.1. Binding affinity of compounds with superoxide dismutase target

3.3. Evaluation of molecular docking

3.3.1. Interaction with superoxide dismutase

We note that the result obtained (Table 5, 6), out of the best compounds studied E,E-Farnesyl acetate (Ligand 69) (Fig. 3) was predicted to be the strongest Superoxide Dismutase binder that forms a complex with the most stability with the lowest energy -4.401 /mol. The ligands that interact with

Table 4. Some properties of best compounds For Antioxidants Drug.

| Ligand compound | Toxic | LogP | Energies (Kcal/mol) | LogS | Hdon + Hacc | Flexibility |
|------------------------------|-------|------|---------------------|-------|--------------|-------------|
| L32 E,E- α -Farnesene | No | 5.20 | 2.18401e + 001 | -6.01 | don:0; acc:0 | 2 out 2 |
| L27 α -Curcumene | No | 4.84 | 3.01079e + 001 | -5.22 | don:0; acc:0 | 4 out 4 |
| L30 Zingibrene | No | 4.89 | 2.59488e + 001 | -4.87 | don:0; acc:0 | 4 out 4 |
| L34 trans-Calamenene | No | 4.69 | 4.34534e + 001 | -5.80 | don:0; acc:0 | 1 out 1 |
| L42 Z-Nerolidol | No | 4.40 | 2.11435e + 001 | -3.93 | don:1; acc:1 | 7 out 7 |
| L56 α -Carinol | No | 1.23 | 8.54556e + 001 | -2.09 | don:5; acc:7 | 9 out 9 |
| L65 E-Nerolidol acetate | No | 4.97 | 2.66094e + 001 | -4.55 | don:0; acc:1 | 9 out 9 |
| L66 β -Acoradienol | No | 3.70 | 4.03789e + 001 | -4.64 | don:1; acc:1 | 2 out 2 |
| L67 Z,E-Farnesyl acetate | No | 4.97 | 1.57696e + 001 | -4.87 | don:0; acc:1 | 9 out 9 |
| L69 E,E-Farnesyl acetate | No | 4.97 | 1.53201e + 001 | -4.87 | don:0; acc:1 | 9 out 9 |

Results of Energy balance of best complexes formed with SODs target are given in (Table 5). Energy for other compounds (See Supplementary Materials Table S2).

Table 5. Results of Energy balance of best complexes formed with Antioxidants Drug molecules.


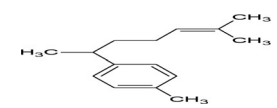
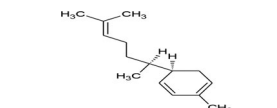
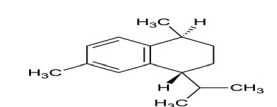
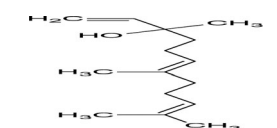
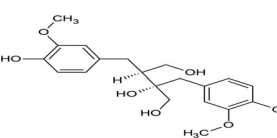
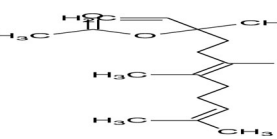
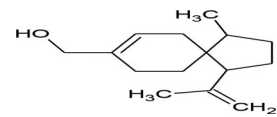
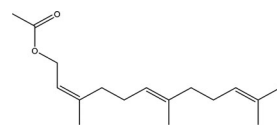
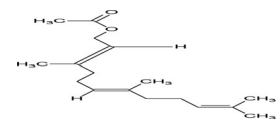
| | Chemical structure | Binding energy (Kcal/mole) | Rmsd -refine | Energy-conf | Energy- place | Energy-refine | RMSD |
|--------------------------|---|----------------------------|--------------|-------------|---------------|---------------|-------|
| E,E- α -Farnesene |  | -3.915 | 1.511 | -1.997 | -5.738 | -3.758 | 1.154 |
| α -Curcumene |  | -4.093 | 1.503 | 35.082 | -0.102 | -12.536 | 0.214 |
| Zingibrene |  | -4.032 | 2.021 | 36.990 | 25.109 | -13.224 | 0.198 |
| Trans- -Calomenene |  | -4.100 | 2.831 | 44.135 | -33.581 | -9.968 | 0.434 |
| Z-Nerolidol |  | -4.000 | 3.403 | -10.987 | -1.967 | -10.604 | 0.116 |
| α -Carinol |  | -4.150 | 3.192 | 3.192 | 75.237 | -17.291 | 0.984 |
| E-Nerolidolacetate |  | -4.226 | 5.681 | 33.046 | -2.144 | -10.626 | 1.101 |
| β -Acoradienol |  | -4.011 | 1.236 | 38.401 | -12.797 | -12.301 | 0.476 |
| Z,E-Farnesyl acetate |  | -4.175 | 2.665 | 23.760 | -14.341 | -10.152 | 1.007 |
| E,E-Farnesyl acetate |  | -4.401 | 3.665 | -22.011 | 1.348 | -11.109 | 0.539 |

Table 6. Results of bonds between atoms of compounds and active site residue of the Superoxide Dismutase (SODs).

| N° | Compounds | S-score | Bonds between atoms of compounds and residues of the active site | | | | Type of interaction bond | Distances (Å) | Energies (kcal/mol) |
|---------------------------|---------------------------|---------|--|-------------------------|----------------------------|-------------------|--------------------------|---------------|---------------------|
| | | | Atom of compound | Involved receptor atoms | Involved receptor residues | | | | |
| Superoxide Dismutase(SOD) | | | | | | | | | |
| L27 | α -Curcumene | -4.093 | 6-ring | O | HOH 2043 | pi-H | 4.24 | -0.7 | |
| L54 | tau-Cadinol | -4.087 | O1 1 | OG | SER 98 | H-acceptor | 2.91 | -1.6 | |
| L56 | α -Carinol | -4.150 | 6-ring | CD | LYS 30 | pi-H | 4.57 | -0.6 | |
| L66 | β -Acoradienol | -4.011 | O1 1 | O | HOH 2051 | H-donor | 2.52 | 0.7 | |
| | | | O1 1 | O | HOH 2042 | H-acceptor | 2.43 | 5.1 | |
| L2 | Z-Hex-3-en-1-ol | -3.212 | O1 1 | 5-ring | TRP 32 | H-pi | 3.81 | -0.7 | |
| L6 | Octanal | -3.300 | O1 1 | ND2 | ASN 19 | H-acceptor | 3.15 | -1.3 | |
| L20 | E- β -Caryophyllene | -3.154 | O1 1 | NE1 | TRP 32 | H-acceptor | 3.50 | -0.5 | |
| L39 | E-Nerolidol | -3.752 | O1 1 | O | HOH 2130 | H-donor | 2.61 | -0.6 | |
| L40 | epi-Globulol | -3.438 | O1 1 | O | HOH 2128 | H-acceptor | 2.73 | -1.1 | |
| L46 | β -Oplophenone | -3.354 | O1 1 | OG | SER 98 | H-acceptor | 2.48 | 2.9 | |
| L47 | Viridiflorol | -3.677 | O1 1 | O | HOH 2126 | H-acceptor | 2.53 | -0.1 | |
| L53 | Trans-Murolol | -3.655 | O1 1 | OG | SER 98 | H-acceptor | 2.90 | -1.6 | |
| L55 | β -Eudesmol | -3.578 | O1 1 | O | HOH 2043 | H-acceptor | 2.97 | -0.6 | |
| L59 | α -Eudesmol | -3.571 | O1 1 | O | HOH 2043 | H-acceptor | 2.97 | -0.6 | |
| L63 | Shyobunol | -3.433 | O1 1 | NZ | LYS 75 | H-acceptor | 3.34 | -1.5 | |
| L65 | E-Nerolidol acetate | -4.226 | H2 | O2 | HOH2043 | WaterH-bond | 2.10 | -0.9 | |
| | | | C14 | O | LYS30 | Alkyl Interaction | 4.83 | -1.2 | |
| | | | C18 | O | LYS75 | Alkyl Interaction | 2.87 | -0.5 | |
| | | | C19 | O | LYS75 | Alkyl Interaction | 4.87 | -1.1 | |
| | | | C15 | O | LYS30 | Alkyl Interaction | 4.83 | -1.2 | |
| L67 | Z,E-Farnesyl acetate | -4.175 | C15 | O | LYS30 | Alkyl Interaction | 4.83 | -1.2 | |
| L69 | E,E-Farnesyl acetate | -4.401 | H1 | O2 | HOH2043 | Water H-bond | 2.21 | -0.6 | |

H = Conventional hydrogen bond, C = Carbon hydrogen bond, Aa = Alkyl-alkyl, Ap = Alkyl-Pi, Aps = Amide-Pi stacked, Ppt = Pi-pi T-shaped, X = Halogen.

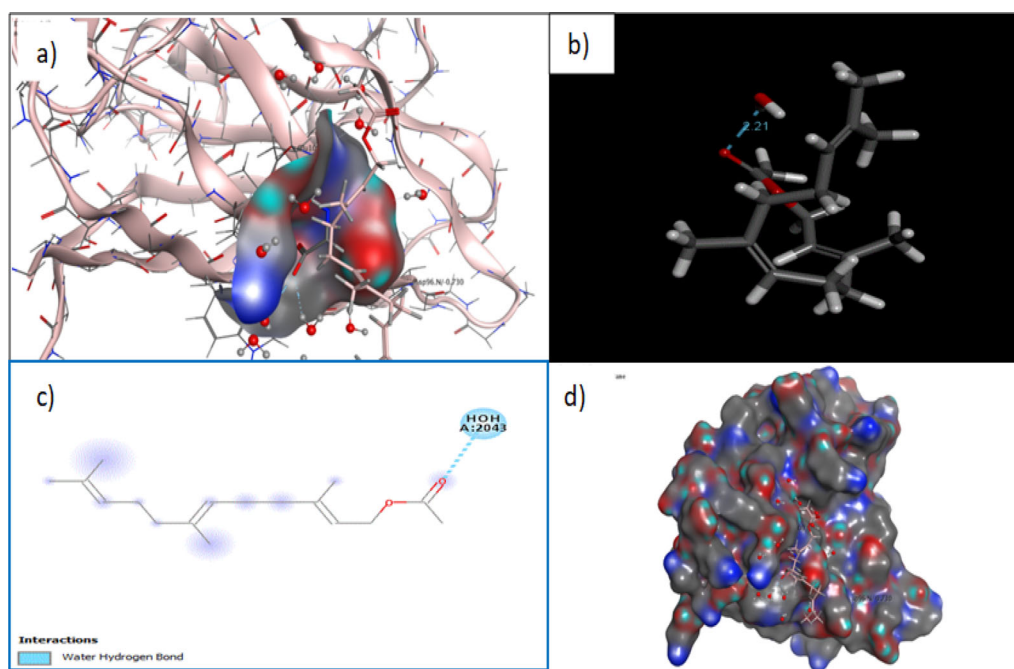


Figure 3. Displaying the Superoxide Dismutase binding site cavity 3 D interaction diagram of E,E-Farnesyl acetate with pocket(a) and without pocket (b) ; Binding mode of E,E-Farnesyl acetate as 2 D diagram (c) ;The binding patterns E,E-Farnesyl acetate in the active site of Superoxide Dismutase, in which E,E-Farnesyl acetate is disappeared in stick model with pink while the surface of SOD is shown in blue red green (d).

Superoxide Dismutase are: α -Curcumene Ligand L27 interacts with HOH 2043 at a distance of 4.24 Å strong with energy of -0.7 and tau-Cadinol ligand L54 interacts with one amino acid SER 98 H- acceptor at a distance of 2.91 Å strong and energy binding of -1.6, similarly, the β -Acoradienol ligand L66 interacts with HOH 2051 HOH 2042, (H-acceptor, H-donor) at a distance of (2.52,2.43)Å respectively strong, low. Z-Hex-3-en-1-ol Ligand L2 interacts with TRP 32H-pi at a distance of 3.81 Å weak and energy binding of -0.7 and methyl-hept-5-

en-2-one Ligand L46 interacts with one amino acid TRP 32 H-acceptor at a distance of 3.30 Å strong and energy binding of -0.7. It is noted that the interactions between the residues of the active site of 2wz5 and E, E-Farnesyl acetate ligand form a stable complex.

The second-best binder was E-Nerolidol acetate (Ligand65) with the energy of -4.226 Kcal/mol) that interacts with HOH 2043 H-acceptor at a distance of 3.05 Å strong interaction and energy binding of -0.9 Kcal/mol (Fig. 4). The ligands that interact

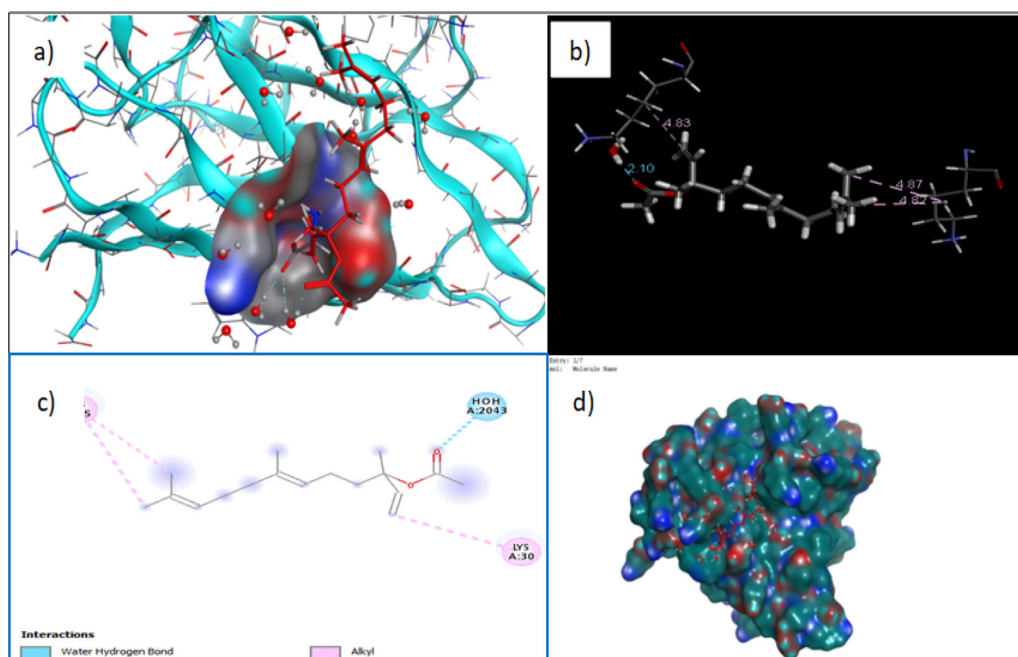


Figure 4. Displaying the Superoxide Dismutase binding site cavity 3D interaction diagram of E-Nerolidolacetate with pocket(a) and without pocket (b) ; Binding mode of E-Nerolidolacetate as 2D diagram (c) ;The binding patterns E-Nerolidolacetate in the active site of Superoxide Dismutase, in which E-Nerolidolacetate is disappeared in stick model with pink while the surface of SOD is shown in blue red green (d).

Table 7. The docking energies of DNA inhibitors.

| | Compound | DE*(kcal/mol) ctDNA | DE*(kcal/mol) Enzyme | ETOR (kT) | VDW (kT) | EIE (kT) |
|----------------|--------------------------|---------------------|----------------------|-----------|----------|----------|
| Control ligand | Ascorbic acid | -4.383 | -3.329 | 345.417 | 553.157 | -1639.53 |
| Lref | L-methionine | -4.277 | -3.388 | 331.046 | 412.988 | -1941.78 |
| L69 | E,E Farnesyl acetate | -6.855 | -4.401 | 331.636 | 412.865 | -1941.94 |
| L67 | E,Z Farnesyl acetate | -6.336 | -4.175 | 331.503 | 412.724 | -1941.62 |
| L56 | α -Carinol | -6.276 | -4.150 | 330.732 | 413.030 | -1942.17 |
| L65 | E-Nerolidol acetate | -6.087 | -4.226 | 345.417 | 553.157 | -1639.53 |
| L32 | E,E- α -Farnesene | -6.084 | -3.915 | 331.636 | 412.864 | -1941.94 |
| L27 | α -Curcumene | -5.727 | -4.093 | 330.612 | 413.093 | -1942.17 |
| L42 | Z-Nerolidol | -5.569 | -3.980 | 330.570 | 412.976 | -1942.21 |
| L34 | trans-Calamenene | -5.498 | -4.100 | 330.567 | 412.975 | -1942.22 |
| L30 | Zingebrene | -5.176 | -4.032 | 330.174 | 413.373 | -1942.50 |
| L66 | β -Acoradienol | -4.742 | -4.011 | 330.719 | 412.932 | -1942.20 |

*DE: Docking Energy; ETOR: torsionEnergy; VDW: Van der Waals; EIE: Electrostatic Interaction Energy.

with Superoxide Dismutase are: E- β -Caryophyllene, Trans-Murolool and Shyobunol interacts with one amino acid ASN19, TRP3, 2 SER98 and LYS75 H-acceptor at a distance of 3.15,3.50,2.48 and 3.34 Å averages, strong interaction and energy binding of -1.3, -0.5,2.9 and -1.5 Kcal/mol And interaction with E-Nerolidol, epi-Globulol, Viridiflorol, β -Eudesmol and α -Eudesmol with HOH 2130, HOH 2128, HOH 2126 and HOH 2043 respectively. H-donor and H-acceptor at a distance of 2.61,2.73,2.53 and 2.97 Å strong, low, average interaction and energy binding of -0.6,-1.1,-0.1 and -0.6 Kcal/mol respectively. This suggests that E-Nerolidol acetate can inhibit Superoxide Dismutase. Results of docking calculations and bonds between atoms of compounds and residues of the active site are given in Table 6.

Binding affinities and non-bonding interactions are listed in Table 3. The additions of halogen not only increase the physicochemical properties but also binding affinity and speciality (Nair et al., 1994). The incorporation of -CF₃ group increased inhibiting and medicinal properties (Abu Saleh et al., 2016; Plano et al., 2016). Group carbon trifluoride has great

significant applications in the field of agronomical dyes, pigments, pharmaceuticals, polymers and material science when it is incorporated to different organic molecules due to the strong electronegative and hydrophobic characteristics, which can be used in drug design to improve the selective functionality (Lishchynskiy et al., 2013; McClinton & McClinton, 1992).

In our study, all the compounds show several significant hydrogen bonds. Some recent studies reported that, halogen bonding similar to hydrogen bonding plays crucial role for biological and chemical system (Sarwar et al., 2010; 2013). The binding affinity of L69, L65, L67, L56, L27 and L54 have considerably increased to -4.401, -4.226, -4.175, -4.150, -4.093, and -4.087 kcal/mol respectively of E,E-Farnesyl acetate, E-Nerolidol acetate, Z,E-Farnesyl acetate, α -arinol α -Curcumene, and tau-Cadinol. Improved hydrogen bond is observed in L69 and L65 not only contribute in increasing binding affinity but also enhance the binding specificity (Bissantz et al., 2010; Sarwar et al., 2013). This observation helped to confirm that (Oxygenated sesquiterpenes) and

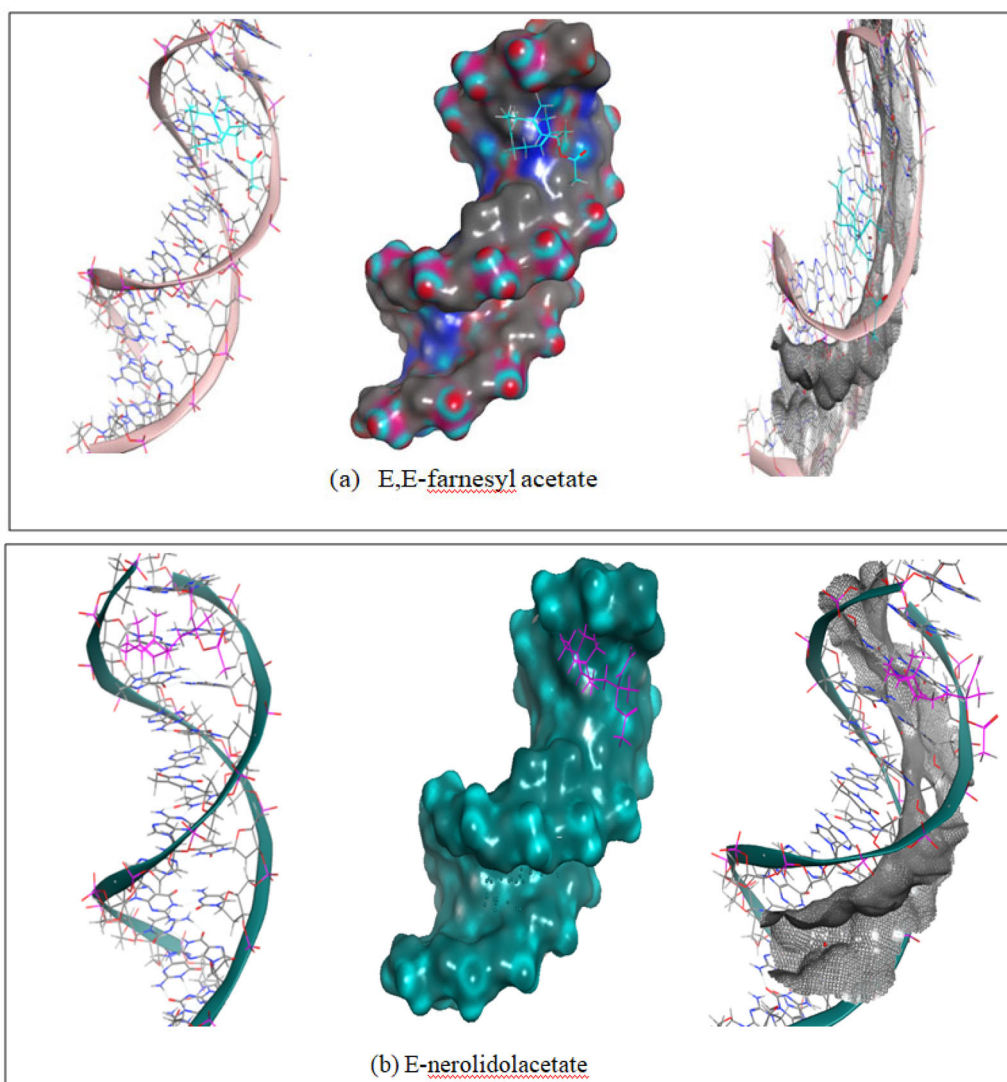


Figure 5. Molecular docked model of the most favorable binding site of compounds (a) L69 and (b) L65 with DNA dodecamer duplex of sequence d (CGCGAATTCGCG)2 (PDB ID: 1BNA).

compounds (D67, D56 and D27) are binding at the desired binding site of receptor protein after molecular docking.

3.3.2. Interaction with DNA sequence

Free radicals, especially OH° , can attack DNA. They react with nucleotides. They can lead, for example, to changes in nitrogen bases, DNA fragmentation, strand breaks or base bridging. Therefore, they would be needed in the development of diseases such as cancer, neurodegenerative diseases and the pathogenesis of viral infections. The accumulation with age of radicals and therefore the damage they generate would be one of the factors explaining cellular aging. The drug was successively docked with DNA duplex of sequence d(CGCGAATTCGCG)2 dodecamer (PDBID:1BNA) and is shown in (Table 7)

Our results demonstrated that the four compounds: E- β -Farnesene (Ligand 23), α -Carinol (Ligand 56), E-Nerolidol acetate (Ligand 65) and E;E Farnesyl acetate (Ligand 69) were the best interacting compounds (see Table 7). The calculated docking energies for these compounds were respectively -6.009 , -6.276 , -6.087 and $-6.114 \text{ kcal.mol}^{-1}$. With the

exception of tau-Cadinol and β -Acoradienol, other compounds are located in the small cDNA groove.

The results of molecular docking showed that tau-Cadinol and β -Acoradienol existed in the main groove region. The calculated docking energy of this compound was -4.708 and -4.742 respectively kcal.mol^{-1} (see Table 5).

Binding energy is the primary parameter which is generated as a result of molecular docking. It gives us the idea of strength and affinity of the interaction between the ligand and the receptor. The greater the binding energy is, the weaker the interaction is and vice versa. Thus, during any docking study, we intend to look for the ligand which displays the least binding energy, thus the best affinity among the test molecules (Nisha et al., 2016). Among the test candidates in this study, E,E Farnesyl acetate and E-Nerolidol acetate displayed the lowest binding energy of -6.865 and -6.087 kcal/mol for DNA sequence and -4.733 and -4.226 kcal/mol for enzyme. The binding energy of the Lref (-3.388 kcal/mol for the enzyme and the control ligand; ascorbic acid (-4.383 kcal/mol for DNA sequence and -3.329 kcal/mol for the enzyme) were much higher than E, E Farnesyl acetate, as found in our study; thus Farnesyl acetate and E-Nerolidol acetate

Table 8. Thermodynamic properties calculated in reels units.

| Stage | Method | H | U | EKT | P | V |
|-----------------|-----------|-----------|-----------|----------|----------|------------|
| SP ₁ | PEROX1NVT | -165.222 | -1121.029 | 2397.107 | -259.305 | 21731.382 |
| | PEROX2NPT | 36.306 | -2679.731 | 1027.389 | -57.269 | 417.362 |
| | PEROX1NVT | 1.584 | -2233.072 | 1930.936 | -184.288 | 21731.382 |
| | PEROX2NPT | 1.320 | -2874.760 | 1199.694 | -58.531 | 218.488 |
| | PEROX1NVT | -1.367 | -2258.180 | 1976.864 | 296.146 | 21731.382 |
| | PEROX2NPT | -0.943 | -2929.258 | 1269.987 | -52.646 | 501.078 |
| SP ₂ | PEROX1NVT | -2273.905 | 1975.170 | 1297.141 | 286.447 | 21057.382 |
| | PEROX2NPT | 0.835 | -2909.660 | 1252.199 | -57.083 | 601..500 |
| | PEROX1NVT | 2.523 | 2356.218 | 2016.582 | 114.555 | 21757..382 |
| | PEROX2NPT | 92.991 | -2923.494 | 1358.883 | -49.508 | 129..876 |
| | PEROX1NVT | 3.171 | -2332.877 | 2332.877 | 148.306 | 21761.382 |
| | PEROX2NPT | -1903.034 | -5548.395 | 1990.654 | -58.157 | 700.555 |
| SP ₃ | PEROX1NVT | 1986.618 | -2372.214 | 1986.618 | -125.724 | 21731.382 |
| | PEROX2NPT | -1993.422 | -5682.353 | 2034.241 | -59.258 | 680.882 |
| | PEROX1NVT | 2.226 | -2339.252 | 1965.961 | -136.010 | 21731.382 |
| | PEROX2NPT | -3045.507 | -2253.922 | 2253.925 | -57.256 | 351.001 |
| | PEROX1NVT | 1.678 | -2429.067 | 1953.394 | -132.638 | 21731.382 |
| | PEROX2NPT | -5154.32 | -5839.098 | 3174.523 | -53.748 | 143.483 |

Pressure $P = P^* \epsilon / \sigma^3$. Energy of configuration $U = U^* N \epsilon$. Translation Kinetic Energy $EKT = EKT^* N \epsilon$ and Enthalpy $H = H^* N \epsilon$.

displayed much better binding than the Lref and control molecule; ascorbic acid (See [Supplementary Materials Figure S2](#)).

The more negative relative binding energy for E,E Farnesyl acetate indicated greater binding tendency of this compound with DNA. Thus the results obtained from docking molecular studies were found to be consistent with molecular dynamics simulation studies, which can validate our simulation findings. Both docking and dynamics studies suggest that compound E,E Farnesyl acetate has a strong DNA-binding affinity than compound E-Nerolidol acetate. The additional acetate group of compound E,E Farnesyl acetate makes specific hydrophobic interactions with nucleotide bases of DNA. Moreover, docking studies also suggested that a large part of binding energy is derived from hydrophobic interactions. From these results, it should be noted that several factors, notably the variability of the ligand structures and the capacity to build covalent and/or non-covalent bonds, could affect their binding affinity to the small cDNA groove.

There are even other factors such as the distribution of electrostatic charges with DNA and also the base pairs A: T (Neidle, 2001). Studies have suggested that minor groove binding ligands carry a cationic charge, complementing the potential in A: T regions. Our results are fully consistent with previous reports and the compounds studied interacted with A: T base pairs. E, E Farnesyl acetate, and E-Nerolidol acetate displayed much better binding. (see [Fig.5](#)).

3.3.3. Interaction with endogenous enzymatic antioxidant systems

3.3.3.1. The case of superoxide dismutase (SODs).

Antioxidants can be classified into fat soluble or water soluble molecules. According to their physicochemical characteristics, they will have a preferential cellular localization: the cell membranes for the liposoluble substances and the cytosol and/or the extracellular medium for the water-soluble substances. They will be particularly effective on the free radicals present in each type of medium, respectively (See [Supplementary Materials Figure S3](#)). The results of docking energies of Superoxide Dismutase (SODs) best inhibitors are shown in (See [Supplementary Materials Table S3](#)). And results of other compounds are shown in (see [supplementary Table S3.a](#)).

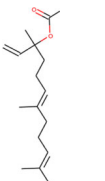
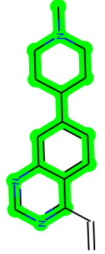
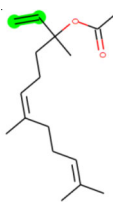

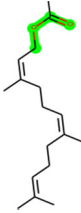
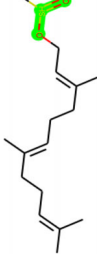
The exogenous defenses (*Superoxide dismutases*, *Catalase*, *Glutathione peroxidase*) seem the easiest to support since they could be strengthened by the intake of food supplements. *Superoxide dismutases*: Converts free radicals to less damaging substances, such as Hydrogen peroxide. *Catalase*: Removes hydrogen pyroxide from the body. *Glutathione peroxidase*: Removes hydrogen peroxide. Molecular docking results revealed that trans-Calamenene (Ligand 34), α -Carinol (Ligand 56), Zingebrene (Ligand 30) E-Nerolidol acetate (Ligand 65), Z,E-Farnesyl acetate (Ligand 67) and E,E-Farnesyl acetate (Ligand 69) were the best compounds interacting with the suspected binding residues at the active site Superoxide Dismutase (See [Supplementary Materials Figure S4](#)). The calculated docking energies of these molecules were respectively -4.100, -4.150, -4.322, -4.226, -4.175 and -4.401 kcal.mol⁻¹. Z-Nerolidol and E,E- α -Farnesene was the weakest interacting compound with this receptor. The calculated docking energy of these compounds is: -3.980 and -3.915 kcal.mol⁻¹, respectively. The docking parameters include free binding energy, intermolecular energy and torsional free energy values were calculated. We observed that E,E- α -Farnesene (Ligand 32), α -Curcumene (Ligand 27), α -Carinol (Ligand 56), and E-Nerolidol acetate (Ligand 65) E,E-Farnesyl acetate (Ligand 69) and Z, E-Farnesyl acetate (L67) showed a binding affinity for interacting with receptors for cDNA, and trans-Calamenene (L34), α -Carinol (Ligand 56), Zingebrene (Ligand 30), E-Nerolidol acetate (Ligand 65) E,E-Farnesyl acetate (Ligand 69) and Z, E-Farnesyl acetate (Ligand 67) showed a binding affinity for interacting with receptors for Superoxide Dismutase. 3D representations of the best pose interactions are shown in (See [Supplementary Materials Figure S4](#)). The results given, we can conclude that the best inhibition this product by two molecules E-Nerolidol acetate (Ligand 65) and E,E-Farnesyl acetate .

3.4. Evaluation of molecular dynamics

3.4.1. Thermodynamic properties

Thermodynamic properties using the MD simulation approach, we have studied the evolution thermodynamic

Table 9. In silico Bioisosteric Replacement based on similarity comparison method.

| Molecule | Replacable group | Transformation in previous studies | Analogue structure | Smiles |
|----------|---|---|---|--|
| L65 |  | // | 1-Analog L65  | <chem>CN1CCC(C2=CC=C3C(C=NN=C3C)=C2)CC1</chem> |
| |  | J. Med. Chem., 2015, 58, 9625-9638 J. Med. Chem., 2014, 57, 1208-1224 Eur. J. Med. Chem., 2014, 80, 295-307 | 2-Analog L65  | <chem>O=C(C)OC(C(NH3+))C(C)C/C=C(C)/CC/C=C=C(C)/C</chem> |
| L69 |  | // | 1-Analog L69  | <chem>O=[SH]C(C)OC/C=C(C)/CC/C=C=C(C)/CC/C=C=C(C)/C</chem> |

properties of best ligand, E,E-Farnesyl acetate in NVT and NPT ensemble (Table 9). We performed energy minimizations of the best complex after docking of 600ps. Then carried out simulations up to (MD production cycles) 100 ns in three stages under constraints (see table 8).

Initiation stage sp1 20 ns Heating (thermalization) from 0 to 300 K for example

Equilibration stage sp2 20 ns -40ns Normal simulation, but the system is not in equilibrium. 40 ns-60ns relaxation (adaptation) of water on the surface of the protein

Production stage sp3 60 ns-100ns we saved the trajectories

Analyzes Finally, the analysis of the results and validation by contributing to the score (energy) obtained by docking.

The results represented in table 7 revealed that the kinetic energies of translation and the internal energy for the E,E-Farnesyl acetate (Ligand 69) in NVT and NPT ensemble. In the *Superoxide dismutases (SODs)* enzyme in NVT units, are low compared to the *Superoxide dismutases (SODs)* receptors in NPT units. And the fluctuation in pressure for the complex receiver is significant. Therefore, E,E-Farnesyl acetate (Ligand 69) are predicted to be the most interactive system. These results are in total agreement with the Docking prediction results(see Tables 4-5). We can show the detailed analysis of MD simulation results of only compound E,E-farnesyl acetate (Ligand 69) with target *Superoxide dismutases (SODs)* enzyme (Fig. 6 and 7).

3.4.2. Structural dynamics properties

Structural Dynamic properties using the MD simulation approach, we have studied the evolution structural dynamics of the best ligand molecule L69 (See Table 5) by IMODS.

The normal mode analysis (NMA) of the prepared E,E-Farnesyl acetate- Superoxide Dismutase(SODs) complex was illustrated in (Fig. 8a) From the molecular dynamics study of the prepared E,E-Farnesyl acetate - Superoxide Dismutase(SOD) complex, it was clear that the prepared enzyme-ligand complex had a quite high eigenvalue of 2.446366e-04 the eigenvalue is illustrated in (Fig. 8b). However, the variance map showed a high degree of cumulative variances than individual variances (Fig. 8c). The co-variance and elastic network map also produced quite satisfactory results (Fig. 8d, and Fig. 8e) respectively. The deformability graphs of the complex (E,E-Farnesyl acetate-Superoxide Dismutase(SODs)) illustrate the peaks in the graphs correspond to the regions in the protein with deformability (Fig. 8f). The two selected ligand molecules can be used as potential agents to deplete DPPH and free radicals. Overall, in our study, E,E-Farnesylacetate emerged as the most potent anti- Superoxide Dismutase(SOD) agent. However, more *in vitro* and *in vivo* researches should be performed on the E,E-Farnesyl acetate best ligands to finally confirm the findings of this study.

3.5. The OSIRIS property explorer

A computational study of best compounds was performed for the assessment of OSIRIS properties (See Supplementary Materials Table S4) Prediction results are valued and color coded.(Nalini et al., 2011). Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are

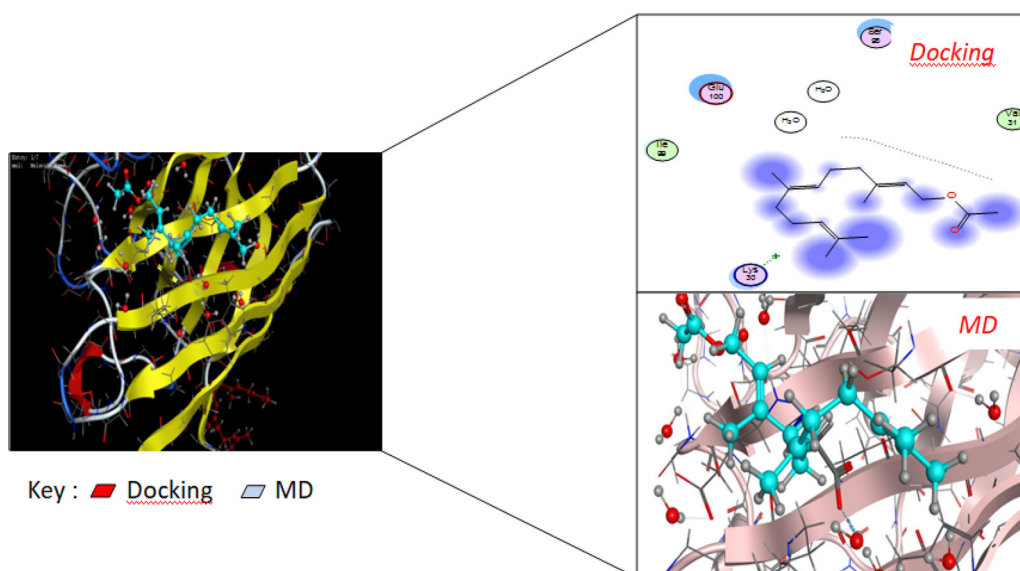


Figure 6. The compound – 69; E,E-Farnesyl acetate docked without water well into the binding site of SOD and has the highest dock score; there is also a clear difference between the final ligand pose and the docking pose after a molecular dynamics (MD) simulation in NVT.

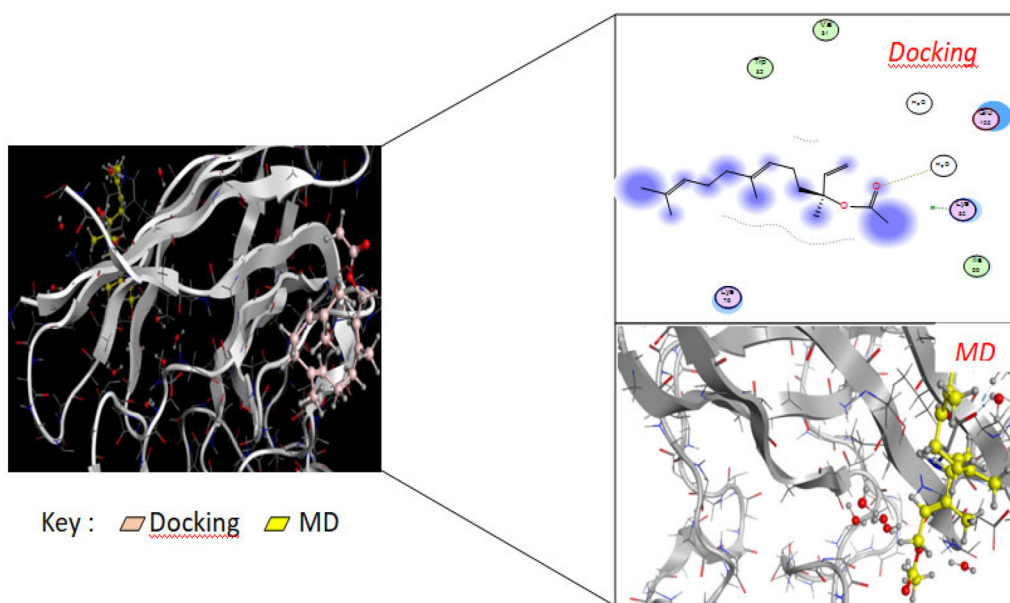


Figure 7. The compound – 69; E,E-Farnesyl acetate docked without water well into the binding site of SOD and has the highest dock score; there is also a clear difference between the final ligand pose and the docking pose after a molecular dynamics (MD) simulation in NPT.

shown in red. Whereas a green color indicates drug-conform behavior. The results exposed in table 8 revealed that compounds (L32, L34 and L56) are the best ligands and L42 and L66 are only irritating. What interests us most are ligands L65 and L69 because the latter have well complementary with the enzyme. We see that L69 is irritant and moderately tumorigenic reproductive effective and L65 is tumorigenic. It is moderately irritant. So the solution is to replace a fragment.

3.6. Bioisosteric replacement

In order to solve the problems associated with the pharmacokinetics of drugs and eliminate unwanted side effects such

as toxicity and metabolic responsibilities. We used, for our best ligands L65 and L69, (see table 9) Molopt (A web server for drug design using bioisosteric transformation) which automatically generates analog lists by replacing molecular substructures with chemical groups with similar biological properties. The resulting set of transforming analogs can be evaluated for future synthesis in the future.

We could not carry on further with the *in vitro* and *in vivo* testing due to lack of the required facilities, and thus this study needs further *in vitro* and *in vivo* animal studies for the development and authentication of these probable potent inhibitors of E,E-Farnesyl acetate for reduction of oxidative stress.

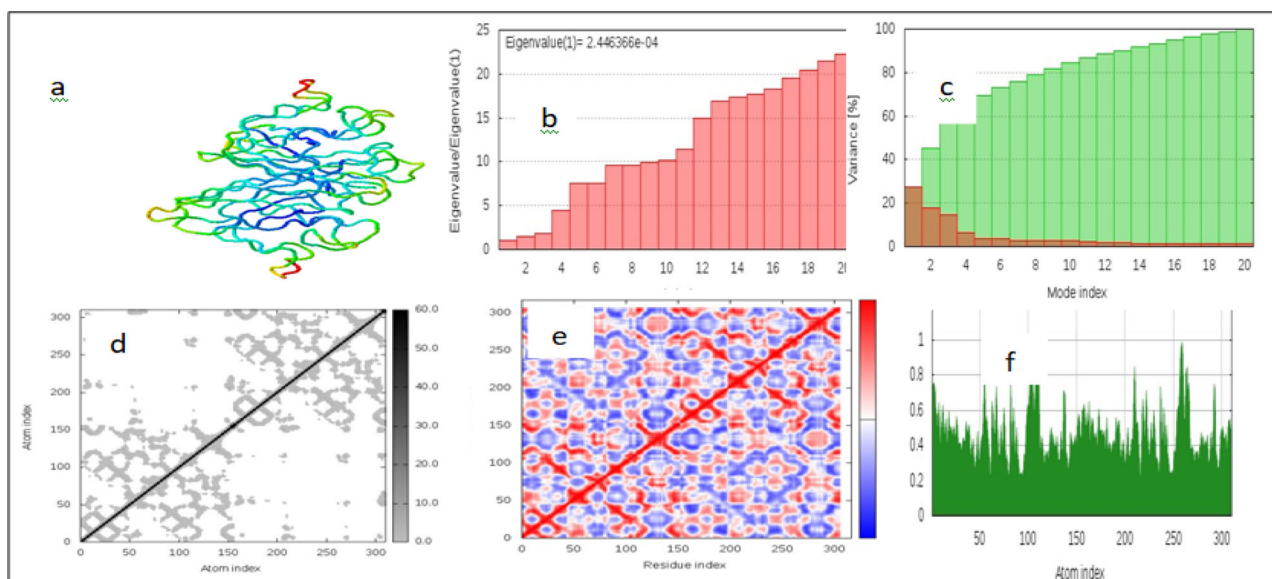


Figure 8. Results of molecular dynamics simulation of E,E-farnesyl acetate - Superoxide Dismutase(SOD) docked complex. (a) NMA mobility, (b) eigenvalue, (c) variance (red color indicates individual variances and green color indicates cumulative variances), (d) elastic network (darker grey regions indicate stiffer regions) of the complex, (e) co-variance map (correlated (red), uncorrelated (white) or anti-correlated (blue) motions) and (f) deformability.

Table 10. Drug-likeness prediction through OSIRIS Property Explorer of best inhibitors (ligands).

| S. number | Ligand | $c \log P$ | Solubility $\log S$ | Molecular weight | TPSA \AA^2 | Druglikeness | Drug score |
|-----------|---------------|------------|------------------------|------------------|---------------------|--------------|------------|
| | Ascorbic acid | -2.46 | -0.35 | 176.12 | 107.22 | 0.02 | 0.75 |
| | Lref | -2.39 | -1.05 | 149.21 | 88.62 | -13.32 | 0.50 |
| 01 | L27 | 5.36 | -3.81 | 202.34 | 00.00 | -4.90 | 0.17 |
| 02 | L30 | 5.06 | -3.38 | 204.36 | 00.00 | -3.02 | 0.08 |
| 03 | L32 | 6.40 | -3.31 | 204.36 | 00.00 | -7.39 | 0.29 |
| 04 | L34 | 5.19 | -4.22 | 202.34 | 00.00 | -8.18 | 0.36 |
| 05 | L42 | 5.40 | -3.13 | 222.37 | 20.23 | -6.38 | 0.21 |
| 06 | L56 | 1.66 | -2.09 | 378.42 | 119.61 | -1.63 | 0.52 |
| 07 | L65 | 5.89 | -3.54 | 264.41 | 26.30 | -9.30 | 0.15 |
| 08 | L66 | 3.70 | -3.16 | 220.35 | 20.23 | -24.21 | 0.26 |
| 09 | L69 | 6.14 | -3.28 | 264.41 | 26.30 | -2.70 | 0.07 |
| | 1-Analog L65 | 2.5 | -2.72 | 241.34 | 29.02 | 6.15 | 0.94 |
| | 2-Analog L65 | 1.12 | -2.90 | 268.42 | 53.94 | -4.86 | 0.39 |
| | 1-Analog L69 | 5.78 | -3.62 | 285.47 | 26.30 | -2.94 | 0.20 |

MW: molecular weight, MLogP: logarithm of partition coefficient of the compound between water and n-octanol: n-OHND: number of hydrogen bonds donors. n-ON: number of hydrogen bond acceptors. n-ROTB: number of rotatable bonds.

Table 11. Pharmacokinetic and toxicity evaluated parameters of best compounds.

| | | 65 | Analog structure L65 | Analog structure L65 | 69 | Analog structure L69 |
|--------------|----------------------------------|---------|-------------------------|-------------------------|---------|-------------------------|
| Absorption | Human intestinal | 93.999 | 95.602 | 95.251 | 93.643 | 92.869 |
| | Skin permeability | -1.93 | -2.496 | -2.444 | -1.896 | -2.1 |
| | Caco-2 permeability | 1.64 | 1.646 | 1.501 | 1.632 | 1.609 |
| | Surface Area | 117.785 | 107.829 | 117.449 | 117.785 | 120.841 |
| Distribution | VD _{ss} (human) | 0.273 | 0.917 | 0.377 | 0.275 | 0.154 |
| | Fraction unbound (human) | 0.176 | 0.314 | | 0.157 | 0.173 |
| | BBB permeability | 0.57 | 0.173 | -0.201 | 0.621 | 0.637 |
| | CNS permeability | -2.133 | -2.065 | -2.482 | -1.973 | -1.823 |
| Excretion | Total clearance | 1.752 | 0.869 | 0.958 | 1.749 | 1.957 |
| | Renal Organic Cation transporter | No | No | No | No | No |
| Toxicity | Oral rat acute toxicity (LD50) | 1.631 | 2.648 | 2.331 | 1.633 | 2.541 |
| | AMES toxicity | No | No | No | No | No |
| | Tetrahymena Pyriformis Toxicity | 2.251 | 1.211 | 1.053 | 2.344 | 2.527 |

green = good, yellow = tolerable, red = bad.

Active components: 65- E-nerolidolacetate, 69- E-E- Farnesylacetate. BBB: Blood-brain barrier. ADMET: Absorption. Distribution. Metabolism and Excretion and Toxicity.

VD_{ss}: < -0.15 low, >0.45 high, BBB: >0.3 cross BBB, <-1 poorly distributed to the BBB, CNS: > -2 penetrate CNS, < -3 unable to penetrate CNS, Low skin permeability: > -2.5, Caco-2 permeability: > 0.9, Human intestinal absorption: > 90.

Table 12. The PASS prediction results showing the biological activities of the best two molecules.

| Sl no | Biological activities | E,E-Farnesyl acetate L69 | | E-Nerolidol acetate L65 | |
|-------|--|--------------------------|-------|-------------------------|-------|
| | | Pa | Pi | Pa | Pi |
| 01 | Mucomembranous protector | 0.973 | 0.002 | 0.976 | 0.002 |
| 02 | CYP2E1 inhibitor | 0.824 | 0.004 | 0.600 | 0.006 |
| 03 | Macrophage colony stimulating factor agonist | 0.729 | 0.010 | 0.582 | 0.037 |
| 04 | Chemoprotective | 0.562 | 0.006 | 0.496 | 0.015 |
| 05 | Antioxidant | 0.547 | 0.005 | 0.404 | 0.012 |
| 06 | Antiulcerative | 0.807 | 0.004 | 0.694 | 0.010 |
| 07 | TNF expression inhibitor | 0.779 | 0.004 | 0.610 | |
| 08 | Antiviral (Rhinovirus) | 0.754 | 0.002 | 0.734 | 0.002 |
| 09 | ANTIFUNGAL | 0.598 | 0.019 | 0.554 | 0.023 |
| 10 | Membrane permeability agonist | 0.540 | 0.008 | 0.588 | 0.094 |

3.7. In silico assessment of the ADME properties and drug-likeness

A computational study of best compounds was performed for the assessment of OSIRIS properties (Table 10).

The results exposed in table 10 revealed that compounds (L69 and L65) have high absorption with a low molecular weight of order 264.41. Also, we can note that these compounds comply with Lipinski's rule of 5, Veber's rule and Egan's rule. MW range 264.41 (<500), A log S value indicates solubility; the lesser the log S value, the higher the solubility, which would enhance the absorption log S for E-E Farnesyl acetate and E-Nerolidol acetate are -3.28 and -3.54 respectively. A lower molecular weight would again enhance the absorption rate and thus most of the drugs are tried to be kept at the lowest possible molecular weight, suggesting that these compounds would not be expected to cause problems with oral bioavailability and thus showing the possible utility of both compounds for developing the compound with good drug like properties. Therefore, admetSAR will facilitate medicinal chemists to design and optimize lead compounds with better ADMET properties. We will continue to improve and extend the computational models for the prediction of chemical ADMET properties to make admetSAR a practical platform for drug discovery and other chemical research. Results of ADMET profiles of test ligand are given in (See Supplementary Materials Table S5). To date, it has been widely applied to drug discovery and environmental risk assessment, and has been cited by 207 times (Patel et al., 2018; Yang et al., 2018). It has also been embedded in Drug Bank since version 4.0 in 2014 (Law et al., 2014).

From the table 14 ADMET properties, as derived from admetSAR server (Cheng et al., 2012) reveal that L69, L65 and L42 had better Human Intestinal Absorption (HIA) score than the Lref and ascorbic acid (0.9703, 0.9703 and 0.9645 versus 0.4786 and 0.8150). If a compound with the HIA% is less than 30%, it is labeled as HIA-, otherwise it is labeled as HIA+. Greater HIA denotes that the compound could be better absorbed from the intestinal tract upon oral administration. The penetration through the Blood-Brain Barrier (BBB) came out to be best for L69, L65 and L42 and was significantly higher than the control molecule and Lref (0.9932 and 0.9859 versus 0.9785 and 0.9384, resp.). L69 and L42 were a non substrate/non inhibitor while ascorbic acid was a non substrate/non inhibitor. But L65 was substrate/non inhibitor. A non inhibitor of CYP450 means that the molecule will not

hamper the biotransformation of drugs metabolized by CYP450 enzymes. The AMES toxicity test is employed to know whether a compound is mutagenic or not. Similar to the control molecule and Lref, all the test ligands displayed negative AMES toxicity test which means that the ligands are non mutagenic. Carcinogenic profile also revealed that the ligands were non carcinogenic similar to the control molecule and Lref, except for L32 which present **Carcinogenicity**. Acute oral toxicity was found to be highest for ascorbic acid and Lref. All other test ligands had low and almost similar oral toxicity (between 1.3 and 3.2). Important information obtained from admetSAR server was the computed LD50 dose in a rat model. Comparing the LD50 doses, a compound with lower dose is more lethal than the compound having higher LD50. From our observation, we found that L69, L65 and L42 had the highest LD50, compared to the ascorbic acid (1.981, 2.276 and 2.768, resp.). Lref and ascorbic acid had the lowest LD50 of 1.235 and 1.318.

Apropos the absorption parameters compounds L65; and L69 present a promising oral availability, due to the optimal Caco-2 cell permeability and HIA (> 0.9 and Human intestinal > 90%. Respectively, Table 11).

All compounds passed the AMES tests. The volume of distribution (VDs) for our two best ligands L65 and L69 are, respectively, 0.273 and 0.275 suggest that the drug will be distributed in plasma since the values of the VDss < -0.15. So, VDss describes the extent of drug distribution and the fraction unbound describes the portion of free drug in plasma that may extravasate. The compounds L65 and L69 may be interesting more potent antioxidative agents. The synthetic antioxidant ascorbic acid and the methionine co-crystallized inhibitor are entirely unable to penetrate the central nervous system (CNS).

The ligands antioxidant ascorbic acid and the methionine showed gastrointestinal absorption within acceptable limits. The absorption and distribution parameters, respectively, have been graphically represented by the extended and renewed version of the Egan-Egg model named Brain Or Intestinal Estimated (BOILED) permeation predictive model (BOILED-Egg). Egan-Egg model named Brain Or Intestinal Estimated (BOILED) permeation predictive model (BOILED-Egg). The overall lecture of (Table 11) highlights that compounds L69 could be an excellent candidate as drugs. However, lead to further studies and manipulations. Ligand 65, Ligand 69 and their analogue compounds are not substrates of the renal organic cation transporter 2 (OCT2). The

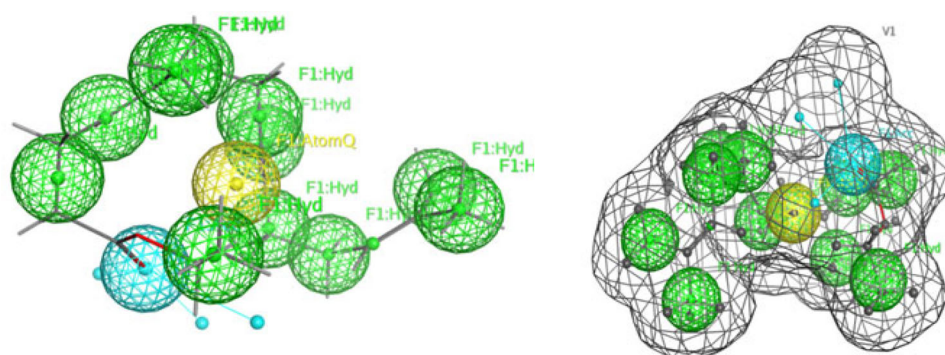


Figure 9. Pharmacophore Mapping of the E,E-Farnesyl acetate L69, Here, cyan color-hydrogen bond acceptor, link source and green color-hydrophobic.

Table 13. Energy balance of complexes formed with Superoxide Dismutase (SOD) under potent clinical antioxidant, some species of *Inula* genus and our results for essential oils of the *I. montana*.

| Some species of <i>Inula</i> genus | | | | |
|------------------------------------|--------|--|----------------------------------|-----------------------------|
| Other experiments | | | | |
| Country | Score | Species | Major components | Reference |
| Bulgaria | -3.738 | <i>I. germanica</i> | cis-Caryylacetate 20.7% | (Trendafilova et al., 2020) |
| | -3.265 | <i>I. bifrons</i> | Muurola-4,10 (14) dien-1-ol 8.6% | |
| | -3.866 | <i>I. ensifolia</i> | Hexanoicacid 8.1% | |
| Turkey | -3.135 | <i>I. salicina</i> | Hexanoicacid 8.3% | (Kilic., 2014) |
| | | <i>I. graveolens</i> | 1,8-cineole 22.4% | |
| | | <i>I. oculus-christi</i> | Bornylacetate 21.3% | |
| Algeria (Oran) | -3.601 | <i>I. viscosa</i> | Bornylacetate 39.81% | (Boumaza, 2011) |
| France | | <i>I. graveolens</i> | Bornylacetate 54% | |
| Italy (Sicily) | -3.674 | <i>I. crithmoides</i> | Thymol 11.8% | (Fontana et al., 2014) |
| Tunisia | -3.450 | | p-Cymene 27.3% | |
| Italy | | | p-Cymene 30.1% | |
| Spain | | | p-Cymene 53.8% | (Tsoukatou & Roussis, 1999) |
| Malta | | | p-Cymene 26.2% | |
| Greece | -3.142 | | β -Phellandrene 30.7% | (Giamperi et al., 2010) |
| Croatia (Badija Island) | -4.051 | <i>I. verbascifolia</i> | Hexadecanoic acid 10.4% | |
| <i>Clinical</i> | | | | |
| Acide Ascorbic | -3.329 | | | |
| <i>Ligand of reference</i> | | | | |
| Methionine (Native) | -3.388 | | | |
| <i>Ours Results</i> | | | | |
| E,E-Farnesyl acetate L69 | -4.401 | <i>Aerial parts of Inula Montana</i> | | |
| E-Nerolidol acetate L65 | -4.226 | | | |
| Analog L65 | -3.952 | Best analogue of compound L65 and L69 from Aerial parts of <i>I. montana</i> | | |
| Analog L69 | -3.878 | | | |

absorption and distribution parameters, respectively, have been graphically represented by the extended and renewed version of the Edan-Egg model named Brain Or Intestinal Estimated (BOILED) permeation predictive model (BOILED-Egg (See [Supplementary Materials Figure S5](#)))

3.8. Pharmacokinetics and medicinal chemistry properties

The results Medicinal Chemistry and Pharmacokinetics revealed that all compounds have High GI absorptions. We notice that there was a complement between our results for assessment of ADME properties (Table 9) and the predicted results in medicinal chemistry and pharmacokinetics (See [Supplementary Materials Table S6](#)).

E,E-Farnesyl acetate and E-Nerolidol acetate essential oils of *Aerial parts of I. montana* (Oxygenated sesquiterpenes). (Ligand 69) and Ligand 65) is predicted to be characterized

by a high lipophilicity and high coefficient of skin permeability $\log K_p$ by providing Z-Nerolidol (Ligand 42), β -Acoradienol (Ligand 66) and α -Carinol (Ligand 56) (Oxygenated sesquiterpenes). We can explain that the more negative the $\log K_p$ (with K_p in cm/s), the less the molecule is absorbed to the skin, which explains the reliability of our study. We cite the works which have valid the stability of complexes and their affinities by MOE software (Molecular Operating Environment (MOE), 2013; Mesli et al., 2013).

$$\text{Log}P_{o/w}L42 > \text{Log}P_{o/w}L65 > \text{Log}P_{o/w}L69.$$

So, Ligand L69 represents high affinity with Superoxide Dismutase (SODs) and ctDNA. Synthetic accessibility (SA) is a major factor to take into account in this selection process an acceptable value between 3.27, 3.43 and 3.53 for the ligands (L69, L65 and L42) respectively, these are more encouraging compounds which can be synthesized or subjected to bioassays or other experiments. Our previous research has revealed that oils from our region have better biological

activities (Belabbes et al., 2017; Benyoucef et al., 2020). We cite the works which have valid the stability of complexes and their affinities by MOE software (Molecular Operating Environment (MOE), 2013; Mesli et al., 2013).

3.9. Pass (prediction of activity spectra for substances) and P450 site of metabolism (SOM) prediction

The predictions of activity spectra for substances (PASS prediction) study for best ligands were conducted to predict 10 intended biological activities. To carry out the PASS prediction experiment, $Pa > 0.7$ was kept, since this threshold give highly reliable prediction (Release, 2018). The PASS prediction results of all the best selected ligands are listed in Table 12. However, at $Pa > 0.7$.

Prediction tests were also carried out for 10 biological activities, the results of which are in Table 12. We note that the compounds (E,E-Farnesyl acetate and E-Nerolidol acetate) shows some biological activities among the 10 tested.

3.10. Pharmacophore mapping

The possible sites of metabolism by CYPs1A2, 2A6, 2B6, 2C19, 2C8, 2C9, 2D6, 2E1 and 3A4 of E,E Farnesylacetate L69 are summarized in (See Supplementary Materials Table S7). The possible sites of a chemical compound, where the metabolism by the isoforms of CYP 450 enzymes may be taking place, are illustrated by the circles on the chemical structure of the compound (Zaretski et al., 2013).

The P450 SOM predictions showed that E,E-farnesyl acetate L69 had 4 sites of metabolism (SOMs) for the CYP450 1A2, 450 2B6 enzyme, CYP450 2C8, CYP450 2C9, CYP450 2C19 and 3 sites for CYP450 2A6.

The Pharmacophore Mapping is conveyed for the E,E-Farnesyl acetate L69 best oral ligand of the (Oxygenated sesquiterpenes) showed 1 hydrogen acceptor bonds, 11 Hydrophobic groups and one Link source. It also generated a good number of good contacts with the Pharmacophore of (Superoxide Dismutase) (see Figure 9). The Pharmacophore of E,E-Farnesyl acetate generates a hypothesis which can be applied successfully in biological screening for further experiments (Dixon et al., 2006).

Validation of our results, for essential oils of the Aerial parts of *I. montana*, the synthetic antioxidant ascorbic acid and the methionine co-crystallized inhibitor and some species of *Inula* genus is mentioned in (Table 13).

Our molecular docking results with Superoxide Dismutase (SOD) coincide with clinical results; The Oxygenated sesquiterpenes compounds were the most dominant.

Ascorbic acid or vitamin C is involved in the main functions of the body: defense against viral and bacterial infections, protection of the wall of blood vessels, iron assimilation, antioxidant action (capture of free radicals), healing. The effect of ascorbate on the cell can be either protective or toxic. Epidemiological studies have shown that ascorbic acid sub-deficiencies can generate, in the long term, pathologies associated with oxidative stress. Research by (Jackson et al., 1998) has shown that normal extracellular

concentrations of ascorbic acid (30 to 150 micromoles/L) are not likely to prevent the interaction of NO (nitric oxide) with superoxide in physiological conditions.

Our ligands natural E,E-Farnesyl acetate L69 better stabilizes the system with its energy of Superoxide Dismutase $-4.401 \text{ Kcal.mol}^{-1}$ we compare with the components of Clinical Drugs (see Table 13). E,E-Farnesyl acetate could be an excellent candidate as drugs because represents better energy (See Supplementary Materials Table S3) for all ligand molecules and the volume of distribution (VDss) suggest that the drug will be distributed in plasma. And again from the (see table 5) E,E-Farnesyl acetate was characterized by a high lipophilicity and high coefficient of skin permeability LogKp. Therefore, we propose E,E-Farnesyl acetate as the best ligand which allows the inhibition of Superoxide Dismutase and ctDNA. And in the meantime, we suggest E,E-Farnesyl acetate present in (Oxygenated sesquiterpenes) with its validated activity Score (-4.401 , -6.855) respectively for (Superoxide Dismutase and ctDNA) as a *new oral ligand*. The present bio-informatic analysis, molecular dynamics simulations used to scrutinize novel (Oxygenated sesquiterpenes). E,E-Farnesyl acetate inhibitor of enzyme (Superoxide Dismutase). Preceding studies have indicated that E,E-Farnesyl acetate has been shown to have antioxidant activity.

In vitro, many studies were focused on the inhibitory effect of the polyherbal compound (Bhāraṅgyādi), on key enzymes linked to investigate antioxidant activity, Superoxide Dismutase.

The result of (Kajaria et al., 2012) proved that polyherbal composed of three herbal drugs, namely (*Clerodendrum serratum*, *Hedychium spicatum* and *Inula racemosa*) formulation to be a good potential for antioxidant activity. The superoxide free radical showed a maximum scavenging activity of 62.45 ± 1.86 with EC_{50} 774.70 ± 5.45 . Anti-lipidperoxidation free radicals showed a maximum absorption of 67.25 ± 1.89 with an EC_{50} of 700.08 ± 6.81 . Ascorbic acid was used as standard with an IC_{50} value of $4.6 \mu\text{g/ml}$. The mountain *Inula* is in the same family as the mountain arnica, Asteraceae. Mountain Arnica contains essential oils, thymol, sesquiterpene lactones and flavonoids. These plant molecules therefore act against inflammation through their ability to inhibit the production of prostaglandins. Extracts of *A. montana* flowers have a long history of use in European traditional medicine as a remedy to treat several ailments (Willuhn, 1998), including pain, injuries, hematomas, dislocations, sprains, bruising, edema associated with fractures, rheumatic muscle and joint complaints, inflammations of the mucous membranes of the mouth and throat, furuncles, inflamed insect bites and surface phlebitis (Klaas et al., 2002). (Klaas et al., 2002) have proved that the inhibition of the transcription factor NFkB (underline the importance of Arnica preparations as anti-inflammatory remedies. (Morales Valverde et al., 2002) have proved that *Inula Montana* has an activity on the digestive system. (Haihaywanshi et al., 2017) have proved that *Arnica montana* also has anti-inflammatory and anti dandruff properties which assist its hair growth property.

In our research the software platform that integrates visualization, modeling and simulations (MOE) does not detect any mark of the hydrophobic interactions between E,E-Farnesyl acetate and both the Superoxide Dismutase; what may be connected to the large size of this compound and the high number of torsion angles. The results are identified to have inhibitory activities against novel Superoxide Dismutase. Of these ligands, E,E-Farnesyl acetate has a stronger bond and high absorption in the intestines with good bioavailability. Consequently, the study carried out in this research reveals many secrets conveyed by the use of magic plants. At the end of our study, we retain that all biological activity depends on the presence of certain metabolites inside the tissues of the plant. The results obtained in this study reveal that E,E-Farnesyl acetate and E-Nerolidol acetate have potential antioxidant ability in receptor (Superoxide Dismutase.) via ROS generation. Thus, E,E-Farnesyl acetate and E-Nerolidol acetate may be used for more analyses in order to further evaluate their efficiency in the reduction of oxidative stress and as a possible antioxidant to be used in the pharmaceutical industry.

4. Conclusion

The present research aimed at the chemical and biological investigation of the essential oil of the aerial part of the species *I. montana* belonging to the Asteraceae family in the hope of finding natural alternatives. Indeed, the chemical characterization of the essential oil revealed the predominance of oxygenated sesquiterpenes. On the other hand, the evaluation of antioxidant power has shown promising antioxidant activity, who could to be used as a natural food preservative and therefore as an alternative to synthetic chemicals. The inhibition of Superoxide Dismutase receptor was theoretically investigated by two methods of computational chemistry: molecular docking analyzes MD simulations, ADME properties and pharmacological knowledge. The results revealed that ligand natural inhibitor – E,E-Farnesyl acetate L69 and E-Nerolidol acetate L65 of (Essential oils from Aerial parts of *I. montana*) has an affinity to interact with cDNA and superoxidase. Although two compounds L65 and L69 have a potent binding affinity with Superoxide Dismutase in the docking simulation. Compound E,E-Farnesyl acetate showed better antioxidant, scavenging activity than other compounds. Molecular docking simulation and molecular dynamic (M.D) simulations indicated that the superior activity of compound E,E-Farnesyl acetate among other compounds might be attributed to the alkyl elongation at acetate chain. Moreover, the in silico assessment of the ADME properties and BOILED-Egg plot validate the compound 65 and 69 pass the brain barrier and have high lipophilicity and high coefficient of skin permeability in the intestines with good bioavailability. E,E-Farnesyl acetate (Oxygenated sesquiterpenes) has the highest binding affinity among all the inhibitors, it is proposed as a *natural orally active* drug and it may also be a good candidate for further biological and pharmacological investigations. All methods of computational have been able to identify promising oxygenated

compounds that might represent future solutions in critical areas of human health. E,E-Farnesyl acetate Can be potential candidates for better performance. The docking results revealed that groove mechanism is followed by E,E-Farnesyl acetate to bind with DNA. The results of molecular docking provide useful information about drug-DNA interaction, which are valuable for the rational design of drugs as well as for understanding mechanism of these drugs at the molecular level. The results obtained in this study reveal that L65 and L69 has potent antioxidant ability in at least two receptors (Superoxide Dismutase and DNA sequence) via ROS generation. Thus, E,E-Farnesyl acetate and E-Nerolidol acetate may be used for more analyses in order to further evaluate their efficiency in the reduction of oxidative stress and as a possible antioxidant to be used in the pharmaceutical industry.

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Article

Chemical Composition and Antioxidant, Anti-inflammatory and Antimicrobial Activities of the Essential Oil and its Major Component (Carlina oxide) of *Carlina hispanica* Roots from Western Algeria

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Abstract: The purpose of this work was to study the chemical composition and *in vitro* antioxidant, antimicrobial and anti-inflammatory activities of essential oil and carlina oxide isolated to *Carlina hispanica* roots. The *in vitro* combinatory effect of carlina oxide with BHT, EDTA and diclofenac to reduce their minimum effective dose and minimize their side effects was also tested. The essential oil obtained from the roots has been studied using GC and GC/MS. Antioxidant activity was assessed using radical scavenging activity (DPPH), the β -carotene bleaching test using the BHT as a positive control, and the ferrous Iron chelating using EDTA as a positive control. The activity of anti-inflammatory was assessed by the protein denaturation method using the diclofenac as a positive control. The antimicrobial activity was evaluated against six microbial strains using the Disc Diffusion Assay and the microplate method to determine the MIC. The essential oil of *C. hispanica* was represented mainly by an acetylenic compound carlina oxide (81.1 %) which was isolated and identified by spectroscopic ¹H NMR, ¹³C NMR and IR methods. The results of the biological activities showed that both essential oil and carlina oxide had an interesting antioxidant and anti-inflammatory power. Likewise, the combinations of positive controls with carlina oxide showed better activities with a substantial reduction in inhibitory concentrations and dose of the antioxidants and anti-inflammatories synthetics. Further studies are needed to provide these positive control combinations with carlina oxide in the pharmaceutical industry.

Keywords: *Carlina hispanica*, essential oil, carlina oxide, biological activities.

Introduction

Since prehistoric times, mankind has always used plants, which are a huge reservoir of compounds from secondary metabolites for healing¹. Plants are the main source of discovery of new active ingredients, indeed around 170000 bioactive molecules have been identified such as: artemisinin, morphine, colchicine and quinine². In recent years, the use of synthetic antioxidants in the fields of pharmacy, agro-food and cosmetics has been thought to provide solutions to the various pathological mechanisms in which oxidative stress is involved, but their uses are suspected of having a long-lasting carcinogenic effect, tetragens and mutagens³. On the other hand, the problem of resistance of microorganisms to antibiotics continues to challenge the health sector⁴. The use of antimicrobial agents which are chemicals has led to the emergence of serious infectious diseases⁵. Moreover, the majority of anti-inflammatory drugs on the market are based on chemicals, although these are quite powerful, their long-term administration causes side effects such as gastric intolerance⁶. In this way, the substitution of these chemical treatments by natural remedies from plants such as essential oils with effects identical to the drugs synthesized seems interesting. The essential oils are natural substances biosynthesized by aromatic plants⁷ rich in bioactive compounds with powerful biological and therapeutic effects, particularly as antioxidant, anticancer, anti-inflammatory, antimicrobial, antiviral, pain reliever and insecticides⁸⁻¹³. Essential oils are used to extend the shelf life of food products and as an antioxidant¹⁴. Their antioxidant and immunostimulatory actions will reduce bacterial attacks. On the other hand, these natural essences are widely applied in various fields such as pharmacy, aromatherapy, food processing and perfumery⁷. The family of Asteraceae, also called compound or more rarely compositaceous, is an important, large family and one of the most evolved. It is present all over the world with a predominance in temperate regions. It includes nearly 23000 species¹⁵ divided into 1500 described genus, of which 750 are endemic¹⁶. Among the plants of this economically important family some provide insecticides as

species of the genus *Pyrethrum*, and some have medicinal properties such as *Inula montana*¹⁷. The genus *Carlina* is among those belonging to the Asteraceae family. Traditional medicine has granted multiple therapeutic properties to species of this genus, yet in many countries, they have been used to treat many skin disorders such as cancer¹⁸. The species that is the subject of our study was *C. hispanica* commonly known as Djrinza. The exhaustive bibliographical research carried out of this species has shown us that until now it has not been the subject of study before and in order to identify possible sources and potentials of this plant. Our study was based to (i) isolate the essential oil of *C. hispanica* and major components, (ii) evaluating the in-vitro antimicrobial, antioxidant and anti-inflammatory activities of essential oil and its major component and (iii) evaluating the combinatory activities of the major component of the essential oil with reference molecules.

Material and methods

Plant material and essential oil isolation

The roots of *C. hispanica* were harvested at the flowering stage in May 2019 from Ain Fezza Forest in Tlemcen (Algeria). The authentication (C.H.05.19) of the collected plant was made by the Pr Hassani A of the laboratory of ecology and ecosystem management of the University of Tlemcen. To obtain essential oil, the fresh root parts (500 g) were subjected to hydrodistillation for 4-5 h using a Clevenger-type apparatus advocated by the European Pharmacopoeia **that allows the recycling of the aqueous phase of the distillate by cohobage. The extractions were performed in triplicate.**

Isolation of components

Collective essential oil (1 g) was obtained by the mixture of all oil samples, which was then subsequently submitted to Column chromatography for separation, using silica gel column chromatography (FC, silica gel 200-500 µm) and eluted with 100 % Hexane.

Gas chromatography (GC)

The analyzes by gas chromatography were carried

out via a Perkin Elmer Clarus Autosystem XL GC apparatus equipped with double flame ionization, and two capillary columns made of silica (60 mx 0.22 mm ID, 0.25 μm stationary phase film thickness), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). The oven temperature was programmed for an increase from 60°C to 230°C at 2°C/min and then kept isothermal for 35 min at 230°C. For the injector and detector, the temperatures were maintained at 280°C. The pressure at the top of the column is 25 psi with a flow rate of 1 ml/min. the sample was injected with a volume of 0.1 μL . using hydrogen as carrier gas (0.7 ml/min). Retention indices (RI) of the compounds were determined relative to the retention times of the series of n-alkanes (C_5 to C_{30}) with linear interpolation, using the Van den Dool and Kratz (2003) equation and software from Perkin-Elmer ²¹.

Gas chromatography-mass spectrometry (GC-MS)

The essential oil was analyzed by gas chromatography-mass spectrometry using a Perkin Elmer Turbo Mass detector (quadrupole) coupled to a Perkin Elmer Auto system XL equipped with an automatic injector and two columns (60 mx 0.22 mm D.I., film thickness of stationary phase 0.25 μm), polar (Rtx-Wax) and non-polar (Rtx-1). The ionization energy was 70 eV, the temperature of the ion source was 150°C, and the mass range with which mass spectra were acquired was 35-350 Da. The injection of the sample was done by a divided mode with a division ratio of 1/80 with a volume of pure essential oil of 0.2 μL ²².

Component identification and quantification

The components were identified via (i) comparison of the retention indices (RI) calculated respectively on polar and apolar columns with those of standard compounds (laboratory library "Aroma") or those reported in the literature. (ii) Computer correspondence of mass spectra (electronic impact) with those of the laboratory's internal library ^{23,24}. The quantification of the constituents was carried out using a flame ionization detector by internal standardization using the response factor calculated with respect to the tridecane (0.7 g.100 g⁻¹) used as internal standard ⁹.

DPPH Free Radical Scavenging Assay

The antioxidant power of the essential oil and carlina oxide has been tested by the method that uses DPPH (2,2-Diphenyl Picryl-Hydrazyl)⁹. 20 g/L of essential oil and carlina oxide stock solution was prepared in a series of different concentrations included between [0.5-15 g/L]. 1000 μl of each concentration was then mixed with 1000 μl of the ethanolic solution of DPPH (0.5 mM). followed by incubation for 30 minutes in the dark and at room temperature. The absorbances were tested using a spectro-photometer at 517 nm. The same concentrations were prepared for BHT to serve as a positive control. The antioxidant activity was calculated in the following way:

$$AA\% = \frac{[(Abs_{\text{control}} - Abs_{\text{test}})]}{Abs_{\text{control}}} \times 100$$

Where: AA: antioxidant activity, Abs: absorbance. The IC_{50} s were calculated graphically by the linear regression formula of the inhibition percentages as a function of different concentrations of the sample tested.

Ferrous iron chelating

The ferrous ion-chelating effect of the essential oil and Carlina oxide by Fe^{2+} ferrozine test system was estimated by the method of Chua *et al.* ²⁵. The solutions of the samples and the reference antioxidant EDTA (500 μl) were initially mixed with 100 μl of FeCl_2 (0.6 mM in distilled water) and 900 μl of ethanol. After 5 min, 100 μl of Ferrozine (5 mM in ethanol) were added to the reaction medium. The mixture was stirred well and then left to react for 10 min at room temperature. The negative control contains all the reagents except the test sample which is replaced by an equal volume of Ethanol. The absorbance of the reaction was measured at 562 nm and the ratio of inhibition of ferrozine Fe^{2+} complex formation was calculated as follows:

$$\%I = [(Ac-As) / Ac] \times 100$$

Where: Ac: Absorbance at 562 nm of control, As: Absorbance at 562 nm of samples.

The IC_{50} s was calculated graphically by the linear regression formula of the inhibition percentages as a function of different concen-

trations of the sample tested.

β-Carotene bleaching test

The antioxidant capacity of the essential oil and carlina oxide was determined by measuring the inhibition of oxidative degradation of β-carotene (discoloration) by the oxidation products of acid linoleic according to the method described by Bougateg *et al.*²⁶. The β-carotene / linoleic acid emulsion was prepared by dissolving 2 mg of β-carotene in 10 ml of chloroform, 25 μl of linoleic acid and 200 mg of Tween 40 were mixed, the chloroform was completely evaporated at 40°C with the rotavapor, then 100 ml of water distilled saturated with oxygen were added, the resulting emulsion was stirred vigorously. A volume of 2.5 ml of the previous emulsion was transformed into test tubes containing 1ml of extracts in Ethanol at different concentrations. Heated thereafter at 50°C in a double boiler. The absorbance was measured for all samples at 470 nm, immediately (t = 0) and after the time of 120 min, using a spectrophotometer against blanc consisting of an emulsion without β-carotene. The percentage inhibition was calculated according to the following formula:

$$A\% = [1 - (A_{E(0)} - A_{C(120)}) / (A_{C(0)} - A_{C(120)})] \times 100$$

Where: A_{E120} : Absorbance at 470 nm of the samples at t = 120 min.

A_{C0} : Absorbance at 470 nm of the control at t = 0 min.

A_{C120} : Absorbance at 470 nm of the control at t = 120 min.

The IC_{50} s was calculated graphically by the linear regression formula of the inhibition percentages as a function of different concentrations of the sample tested.

Anti-inflammatory activity

The activity of anti-inflammatory *in vitro* of essential oil and carlina oxide was assessed by the protein denaturation method. Diclofenac, a powerful anti-inflammatory drug is used as a reference. The reaction mixture consists of 2 ml of the different dilutions of the essential oil, Carlina oxide or of the control (distilled water) and 2.8 ml of the phosphate-buffered saline (PBS,

pH 6.4) mixed with 0.2 ml of egg albumin (fresh), then the mixture is incubated at 37°C for 15 minutes. The denaturation of albumin is induced in a water bath at 70°C for 5 min. After cooling, the absorbance was measured at 660 nm^{27,28}. The percentage inhibition of denaturation was calculated according to the following formula:

$$\% \text{ inhibition} = [(Ac - At) / Ac] \times 100$$

Where: Ac: Absorbance at 660 nm of control, At: Absorbance at 660 nm of samples

Antimicrobial activity

Microbial strains

The antimicrobial activity of the essential oil and carlina oxide was evaluated against six microbial strains: two Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), two Gram-positive bacteria: *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* SAMS (ATCC 25923), and two yeasts: *C. albicans* IPP444 and *C. albicans* (ATCC 10231).

The bacterial strains were cultured in Muller-Hinton Agar at a temperature of 37°C for 24 h, on the other hand, the yeast was cultivated in Sabouraud Agar (Merck) at a temperature of 37°C for 48 h. After incubation, the microbial suspensions were made in sterile physiological water (0.85 %) and adjusted to a final concentration corresponding to 10⁷-10⁸ CFU / mL for bacteria and 1 to 5 × 10⁶CFU / mL for yeast.

Disc diffusion assay

The disk method makes it possible to determine the antimicrobial power of these compounds with respect to the test microorganisms using Muller-Hinton Agar (MHA, Oxoid) medium for bacteria and Sabouraud Dextrose Agar (SDA, Merck) medium for yeasts. This technique was performed according to the recommendations of the Clinical and Laboratory Standards Institute²⁶. The sterile filter paper discs of 05 mm (Whatman, no.2) were impregnated with 10 μl of each then placed on the surface of the appropriate agar (MHA or SDA) previously inoculated by swabbing, the dishes are then incubated for 37°C for 24 to 48 hours. Gentamicin (10 μg) and amphotericin B (0.2 mg),

a commercial antibiotic and antifungal were used as positive controls for bacteria and yeasts respectively. The diameters of the zone of inhibition (mm) were measured. This test was done in duplicate, the mean and the standard deviation were calculated with the PAST VERSION 3.22 software.

Micro-well dilution assay

The Minimum inhibitory concentrations (MICs) of essential oil and carlina oxide which is the lowest concentration of the antimicrobial agent that inhibits the growth of microorganisms according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2012). Were performed using the method of the microplate (96 wells)²⁹. In each line of the microplate, 100 µl of Mueller Hinton broth or 100 µl of Sabouraud Dextrose broth was deposited. Then, 100 µl of each compound was introduced into the 1st well. After having thoroughly mixed the contents of the 1st well, 100 µl were taken, then placed in the 2nd well, until reaching the 10th well; the remaining 100 µl were then eliminated. Therefore, a dilution of ½ between each well will be obtained. The last two wells represent negative controls: well n°11 contains the culture medium

and inoculum and well n°12 contains only the culture medium.

Finally, 100 µl of the bacterial (5×10^5 CFU / ml) or yeast (1 to 5×10^4 cells / ml) inoculum were added to each well. The microplates were sealed and incubated at $37 \pm 2^\circ\text{C}$ for 24 hours. The minimum inhibitory concentration (MIC) was determined to be the lowest concentration at which no growth occurred in the well.

Results and discussion

Chemical composition of the essential oil

The hydrodistillation of the essential oil of *C. hispanica* roots for 5 hours has resulted an essential oil of yellow-orange color with a yield of 0.14 % (0.7 g). The essential oil components were identified by comparing their mass spectra (EI-MS) and retention indices (RI) with those of the mass spectra library (Table 1). Routine GC-RI and GC-MS analysis of root essential oil led to the identification of 6 components. However, our spectral libraries were found to be non-operative in identifying the major component of the essential oil (N°6 of Table 1). Column chromatography allowed us (see material and method section) to separate the major compound (Carlina oxide) with a mass of 0.80 g. The

Table 1. Chemical composition of essential oil of the roots of *C. hispanica*

| No ^a | Components | Retention time | RI ^b | RI ^c | Concentration % | Identification ^d |
|-----------------|--|----------------|-----------------|-----------------|-----------------|-----------------------------|
| 1 | <i>trans</i> -Caryophyllene | 43.92 | 1424 | 1431 | 0.1 | RI. MS |
| 2 | α -Curcumene | 48.24 | 1471 | 1470 | 2.3 | RI. MS |
| 3 | <i>trans</i> - β -Bergamotene | 48.37 | 1480 | 1477 | 4.4 | RI. MS |
| 4 | Zingibrene | 49.42 | 1489 | 1496 | 4.5 | RI. MS |
| 5 | β -Sesquiphellandrene | 52.32 | 1516 | 1514 | 1.8 | RI. MS |
| 6 | Carlina oxide | 57.64 | 1563 | 1572 | 81.1 | RI. MS |
| 7 | Z,Z-Farnesal | 58.74 | 1653 | 1661 | 4.5 | RI. MS |
| | Total identification % | | | | 98.7 | |
| | % Sesquiterpene hydrocarbons (1,2,3,4,5) | | | | 13.1 | |
| | % Oxygenated sesquiterpenes (7) | | | | 4.5 | |
| | % Acetylenic compound (6) | | | | 81.1 | |

a: Order of elution is given on apolar column (Rtx-1)

b: Retention indices of literature (RI^b)

c: Retention indices on the apolar Rtx-1 column (RI^c)

d: RI: Retention Indices; MS: Mass Spectra in electronic impact mode

identification of carlina oxide was done by other complementary analyzes such as ^1H NMR, ^{13}C and the IR spectra. The comparison of the compound obtained with the data described in the literature²⁰ led to the identification of carlina oxide also known as benzyl-2-furylacetylene (Fig. 1).

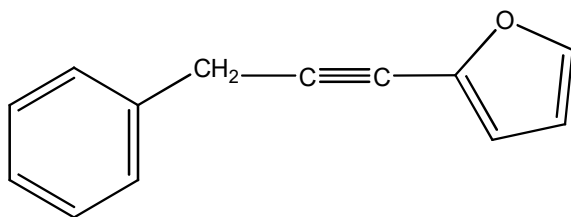


Figure 1. Carlina oxide

The identification of carlina oxide was done by other complementary analyzes such as ^1H NMR, ^{13}C and the IR spectra.

IR Spectrum (KBr, cm^{-1}): (C=C) 2216; (C-H Ar) 3086; (C=C Ar) 1453, 1487, 1494, 1573; (C=C furano) 1603; (C-H, furano) 984; (C-H Ar) 740.

^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.93 (2H, s, CH₂); 6.44 (1H, dd, J₁ = 3.24, J₂ = 1.8, CH furano); 6.62 (1H, d, J = 3.62, Furane C-H); 7.3-7.5 (6H, m, CH phenyl + CH furano).

^{13}C NMR (100 MHz, CDCl_3 , δ ppm): 143.05, 137.4, 135.9, 128.7, 128.1, 126.9, 114.3, 110.8, 92.1, 73.1, 25.8.

The comparison of the compound obtained with the data described in the literature²⁰ led to the identification of carlina oxide also known as benzyl-2-furylacetylene.

^1H NMR spectrum (200 MHz, CDCl_3 , δ , ppm): 3.82 (2H, s, CH₂), 6.34 (1H, dd, J₁ = 3.4, J₂ = 1.8, CH furano), 6.51 (1H, d, J = 3.4, CH furano), 7.2-7.4 (6H, m, CH phenyl + CH furano).

^{13}C NMR spectrum (50 MHz, CDCl_3 , δ ppm): 142.9, 137.3, 135.9, 128.6, 127.9, 126.7, 114.2, 110.7, 91.9, 72.9, 25.7.

C. hispanica essential oil demonstrated the predominance of acetylenic compounds (81.1%) from the identified content. Sesquiterpenes were the most dominant class after acetylenic compounds with a percentage of (17.6 %). The main component were carlina oxide (81.1 %) zingibrene (4.4 %), *Z,Z*-farnesal (4.5 %) and *trans*- β -bergamotene (4.4%).

The essential oil of the roots of *C. hispanica* has never been the subject of study before, therefore we had gathered the results of identification of the chemotypes of essential oils of the roots of a few species of *Carlina* genus (Table 2). The essential oils of *C. vulgaris* and *C. gummefera* harvested in the region of Tlemcen (Algeria) are mainly represented by Carlina oxide (33.7 % and 63.2 %, respectively). The essential oil of *C. acaulis* from Italy was dominated by Carlina's oxide with a percentage of 94.6 %. On the other hand, in the essential oil of *C. radix* it was detected with a percentage of 98.9 %. Carlina oxide still constitutes the majority molecule in the essential oil of *C. acanthifolia*, in fact, it was detected with a percentage of 91.5 % (Table 2).

Table 2. The main component of essential oil of some species of *Carlina* genus

| Country | Species | Major components |
|--|------------------------|---|
| Italy (Bagnacavallo) ³⁰ | <i>C. acaulis</i> | Carlina oxide 94.6 %, aromatic benzaldehyde 3.1 % and ar-curcumene 0.4 % |
| Algeria (Tlemcen-Sidi Djilali) ³¹ | <i>C. vulgaris</i> | Carlina oxide 33.7 %, Hexadecanoic acid 15.0 % and Neophytadiene 13.4 % |
| Serbia ³² | <i>C. radix</i> | Carlina oxide 98.9 % and ar-Curcumene 0.2 %. |
| Serbia (Suva Planina) ³² | <i>C. acanthifolia</i> | Carlina oxide 91.5 % and β -Sesquiphellandrene 2.8 % |
| Algeria (Tlemcen- Ouchebeba) ³³ | <i>C. gummefera</i> | Carlina oxide 63.2 %, (E)- β -Caryophyllene 8.5 % and α -Bisabolol 5.5 % |

Evaluation of the antioxidant activities

DPPH free radical scavenging assay

The antioxidant power was performed according to the DPPH free radical scavenging method, the FeCl_2 iron chelation method and the β -carotene bleaching method using BHT and EDTA as a positive control (Fig. 2-4).

The half-maximal inhibitory concentration (IC_{50}) providing 50 % inhibition of DPPH in the test solution was calculated. The scavenging ability of all samples has shown antioxidant activity. The Carlina oxide-BHT combination indicated the strongest activity in the quenching of the DPPH \cdot radical with an IC_{50} of 2.5 g/L, i.e., approximately 4.5 times higher than the synthetic antioxidant used as a reference, BHT (IC_{50} = 6.9 g/L; Fig 2), followed by BHT (IC_{50} = 6.9 g/L), carlina oxide (IC_{50} = 7.8 g/L) and essential oil (IC_{50} = 11.3 g/L) (Fig. 2).

Metal chelating assay

The antioxidant activity (IC_{50} s) evaluated by the ferrous iron chelation method of the essential oil, carlina oxide and the combination of carlina oxide-EDTA is shown in Figure 3. The combination of carlina oxide with the reference EDTA showed greater antioxidant activity (IC_{50} = 3.2 g/L) than that of carlina oxide (IC_{50} = 9.7 g/L) and essential oil (IC_{50} = 12.9 g/L) but which remains lower than the antioxidant synthetic EDTA (IC_{50} = 0.8 g/L) (Fig. 3).

β -Carotene Bleaching assay

The results obtained demonstrated that the essential oil and carlina oxide have very good activity against peroxide radicals generated by the oxidation of linoleic acid (Fig. 4). Carlina oxide showed more interesting free radical scavenging activity than essential oil with IC_{50} values of 5.1

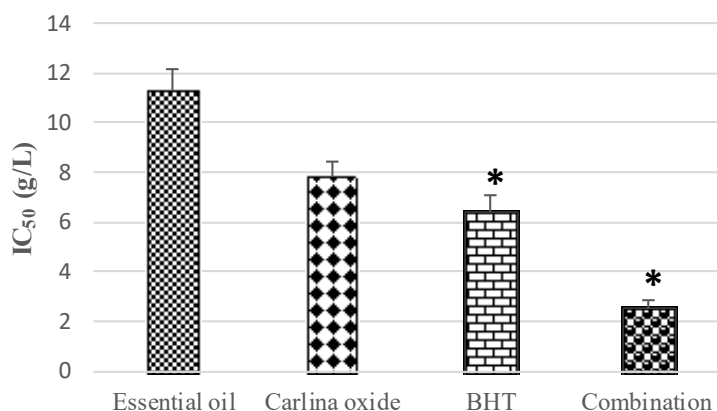


Figure 2. IC_{50} of essential oil, Carlina oxide and the combination Carlina oxide-BHT at different concentrations by the DPPH \cdot test

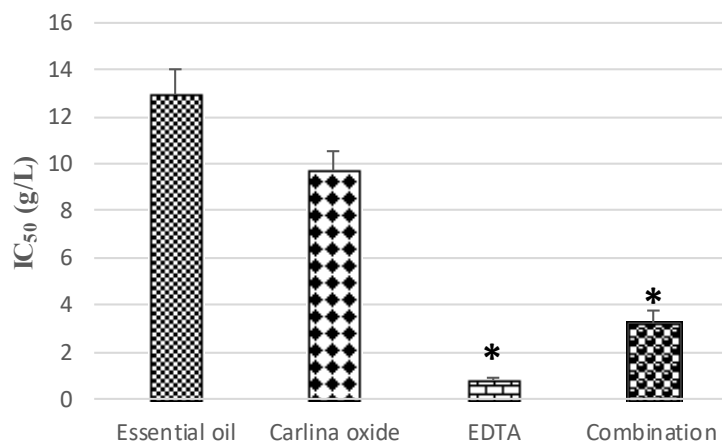


Figure 3. Antioxidant activity of the essential oil, carlina oxide and the combination Carlina oxide-EDTA by the ferrous iron chelation test

g/L and 12.6 g/L respectively. For the synthetic antioxidant the IC_{50} was about 4.9 g/L. However, the combination of carlina oxide-BHT has been shown to have a synergistic effect with IC_{50} of 2.4 g/L, which is about 2 times stronger than that of the BHT alone (Fig. 4).

Evaluation of the anti-inflammatory activity

The *in-vitro* anti-inflammatory activity of essential oils, carlina oxide, diclofenac sodium and combination (carlina oxide-diclofenac) were done using the protein denaturation method (Table 3). From the results of Table 3, concentration-dependent inhibition of protein denaturation (albumin) was observed by the samples. Diclofenac sodium was used as the reference drug at the same concentration. The results showed that the oil and carlina oxide has a very good inhibitory

effect, with percentages of 75.1 % and 77.9 %, at a concentration of 15 g/L compared to Diclofenac (60.7 %) respectively. However, the combination between carlina oxide and Diclofenac showed high inhibition (83.9 %) at the same concentration (15 g/L) (Table 3).

Evaluation of the antimicrobial activity

The antimicrobial power of the essential oil of *C. hispanica* was evaluated against six microorganisms: 4 bacteria [two Gram-positive and two Gram negatives] and two yeasts. The activity was performed qualitatively (disk method) and quantitatively (MIC method). The results are shown in Tables 4 and 5 respectively. The results obtained by the disk diffusion method showed that for *Enterococcus faecalis* ATCC 49452, both essential oil and carlina oxide

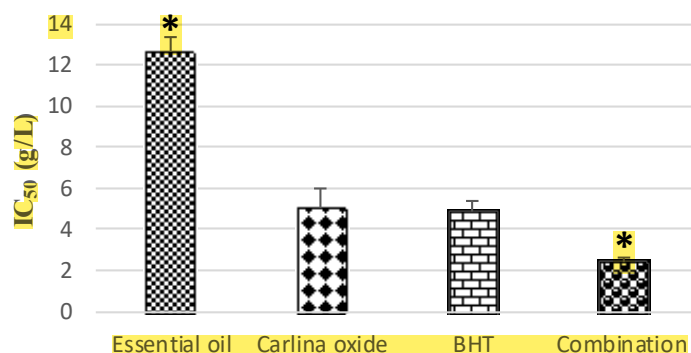


Figure 4. Antioxidant activity of the essential oil, Carlina oxide and the combination Carlina oxide-BHT for the bleaching assay method of β -carotene/linoleic acid at different concentration

Table 3. Percentages of inhibition of protein denaturation of *C. hispanica* essential oil, carlina oxide, sodium diclofenac and their combination at different concentrations

| Concentrations (g/L) | % Inhibition (%) | | | |
|----------------------|-----------------------|---------------|---------------|-------------|
| | Diclofenac sodium (%) | Essential oil | Carlina oxide | Combination |
| 1 | 8.5±0.1 | 4.9±0.1 | 10.7±0.1 | 11.2±0.2 |
| 2 | 17.6±0.1 | 10.2±0.3 | 12.1±0.1 | 18.1±0.1 |
| 3.5 | 22.2±0.2 | 20.5±0.2 | 27.7±0.3 | 37.1±0.1 |
| 5 | 34.2±0.8 | 37.6±0.6 | 56.3±1.3 | 58.3±0.2 |
| 10 | 44.5±0.6 | 70.3±1.6 | 73.4±0.8 | 75.6±0.9 |
| 15 | 60.7±1.1 | 75.1±0.8 | 77.9±0.5 | 83.9±1.1 |

Samples and positive control were done in triplicates (n=3)

SD= standard deviation

Combination: Carlina oxide+Diclofenac

Table 4. Inhibition Zones of *C. hispanica* essential oil and carlina oxide

| A. Gram-positive bacterial strains | | | | | |
|---|-------------------------------|--------------------|--|-------------------------------|--------------------|
| <i>Staphylococcus aureus</i> ATCC 25923 | | | <i>Enterococcus faecalis</i> ATCC 49452 | | |
| Zones of inhibition (mm) | | | | | |
| EO (10 µL/disc) | Carlina oxide (10 µL/disc) | Gent (10 µg) | EO (10 µL/disc) | Carlina oxide (10 µL/disc) | Gent (10 µg) |
| 11.5±1.5 | 9±1 | 33 | 7.5±0.5 | 7.5±0 | 13 |
| B. Gram-negative bacterial strains | | | | | |
| <i>Escherichia coli</i> ATCC 25922 | | | <i>Pseudomonas aeruginosa</i> ATCC 27853 | | |
| EO (10 µL/disc) | Carlina oxide (10 µL/disc) | Gent (10 µg) | EO (10 µL/disc) | Carlina oxide (10 µL/disc) | Gent (10 µg) |
| 10.5±0.5 | - | 23 | - | - | 25 |
| Yeast | | | | | |
| <i>Candida albicans</i> ATCC 10231 | | | <i>Candida albicans</i> IPP444 | | |
| EO (10 µL/disc) | Carlina oxide (10 µL/disc) | AmphB 0.2 µg/mL | EO (10 µL/disc) | Carlina oxide (10 µL/disc) | AmphB 0.2 µg/mL |
| 19.5±0.5 | 13.5±0.5 | 30±0.0 | 16.66±1.2 | 11.66±1.20 | 30±0.0 |

Table 5. Minimum inhibitory concentration of essential oil of *C. hispanica* and carlina oxide

| A. Gram-Positive Bacterial Strains | | | | | |
|---|-------------------------------|---------------------|--------------------------------|-------------------------------|--------------------|
| <i>Staphylococcus aureus</i> ATCC 25923 | | | | | |
| Minimum inhibitory concentration | | | | | |
| EO (10 µL/disc) | Carlina oxide (10 µLdisc) | Gent MIC µg/mL | | | |
| 1.562±0.00 | - | 0.5±0.000 | | | |
| Yeast | | | | | |
| <i>Candida albicans</i> ATCC 10231 | | | <i>Candida albicans</i> IPP444 | | |
| EO (10 µL/disc) | Carlina oxide (10 µL/disc) | AmphB MIC µg/ mL | EO (10 µL/disc) | Carlina oxide (10 µL/disc) | AmphB MIC µg/mL |
| 0.39±0.00 | 3.125 0.00 | 8 | 0.39±0.00 | 1.562± 0.00 | 4 |

demonstrated the same efficacy with an inhibition zone of 7.5 mm. For *Staphylococcus aureus* ATCC 25923 the diameters were 11.5 mm and 9 mm for the essential oil and carlina oxide respectively. For *Escherichia coli* ATCC 25922, the activity was displayed only by the essential oil with an inhibition zone of 10.5 mm. However, for *Pseudomonas aeruginosa* ATCC 27853 no activity was obtained. While for yeasts the essential oil was more effective than carlina oxide with zones of inhibition of 19.5 µg/ml against *Candida albicans* ATCC 10231 and 16.66 µg/ml against *Candida albicans* IPP 444. For carlina oxide, the zones of inhibition were 13.5 µg/ml

and 11.66 µg/ml respectively (Table 4). This means that it is the synergy of molecules of the essential oil that is responsible for the antifungal activity and not only carlina.

The minimum inhibitory concentration (MIC) was obtained using the microplate method (96 wells). The MIC value of the essential oil was 1.562 µg/ml for *Staphylococcus aureus* ATCC 25923, while for *Enterococcus faecalis* ATCC 49452 and gram-negative bacteria didn't demonstrate any efficiency. However, carlina oxide has not been active for all bacteria which is in agreement with the disk test. For yeasts, the MIC values of the essential oil were 0.39 µg/ml

for both yeasts. For carlina oxide, the MIC was 3.125 µg/ml for *Candida albicans* ATCC 10231 and 1.562 µg/ml for *Candida albicans* IPP 444 (Table 5).

Conclusions

This is the first report on the chemical composition and biological activities of the essential oil from the root part of *C. hispanica*. The Chemical characterization has shown that essential oil was rich in acetylenic compound. The *in vitro* combination of carlina oxide with BHT and EDTA showed better activity which led to a substantial

reduction of inhibitory concentration. Essential oil and carlina oxide had good activity anti-inflammatory, but the combination of carlina oxide with diclofenac was better. From the results, we observe that the combination of carlina oxide with the positive controls can reduce the minimum effective dose, which in turn can help to minimize side effects and deliver them with similar potency. Therefore, other evaluations of the therapeutic efficacy of the essential oil, carlina oxide as well as the combinations with *in vivo* tests in the context of further research should be carried out.

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Chemical composition variability and vascular endothelial growth factor receptors inhibitory activity of *Inulaviscosa* essential oils from Algeria

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Chemical composition variability and vascular endothelial growth factor receptors inhibitory activity of *Inulaviscosa* essential oils from Algeria

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ABSTRACT

Angiogenesis therefore appears to be a complex phenomenon, finely regulated by various activators (pro-angiogenic factors) and inhibitors (anti-angiogenic factors). Among the pro-angiogenic factors, VEGF (Vascular Endothelial Growth Factor) seems to be one of the main players in tumor angiogenesis. It exerts its pro-angiogenic activity by attaching to the surface of receptors with tyrosine kinase activity (VEGFR). The aim of this research was the bioinformatical study of VEGFR inhibition by essential oils of the *Inula viscosa*.

Analyses of essential oils obtained by hydrodistillation from the aerial parts of the plant were performed using GC and GC/MS analysis. We used molecular modeling approaches as molecular mechanics to theoretical investigation VEGF receptors by natural inhibitors.

Nineteen compounds were identified, constituting 90.1–98.8% of the total essential oils. The main components of the plants were (E)-nerolidol (15.5–20.2%), caryophyllene oxide (10.6–18.1%), (E)-Z-farnesyl acetone (13.2–25.1%) and (E)-β-farnesene (1.5–5.6%). Essential oil samples were clustered into two groups according to their chemical compositions. The molecular dynamics study was conducted for the best inhibitors. A few key residues were identified at the binding site of VEGFR. The Pharmacokinetics was justified by means of lipophilicity and high coefficient of skin permeability. The *in silico* evaluation of ADME revealed that L19 has high absorption. The essential oil of *I. viscosa* presents a significant variability. This study revealed that (E)-Z-Farnesylacetone is a functional inhibitor of VEGF activities and subsequently can be the best inhibitors candidate to be scrutinized *in vivo* and *in vitro*.

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Inulaviscosa; cancer cells; pharmaco-informatics; molecular dynamic; MOE (molecular operating environment)

1. Introduction

Cancer is a complex disease whose generic term covers different pathologies: There are around 200 types of tumors that can affect all the tissues of the body. In recent years, we have witnessed considerable growth in cancer therapies. Radiotherapy and chemotherapy act mainly against cancer by triggering an overproduction of free radicals in cells (Arruebo et al., 2011). These free radicals constitute reactive oxygen species (ROS). ROS are a family of chemical entities grouping together non-radical derivatives whose toxicity is significant (anion peroxide (O_2^{2-}), hydrogen peroxide (H_2O_2), peroxy nitrite ($ONOO^-$)) and free radicals oxygenate which interests our subject (superoxide anion ($O_2^{\bullet-}$), hydroxyl radical (OH^{\bullet}), alkoxy radical (RO^{\bullet}), peroxy radical (ROO^{\bullet}), nitrogen monoxide (NO^{\bullet}), nitric oxide (NO^{\bullet}) and nitrogen dioxide (NO_2^{\bullet})) (Novelli, 1997). These are aggressive molecules, 'carnivores' you could say, which damage cells and can cause their death. But it is an advantage when it comes to cancer cells, which we try to destroy. This is how chemotherapy and

radiotherapy act, at least in part, to shrink the size of tumors. The problem is that these therapies do not just target cancer cells. They destroy all the rapidly dividing cells (Lesgards et al., 2014). The study of natural products is one of the strategies for the discovery of new drugs that can be used in cancer therapy. Essential oils have the advantage of being well absorbed by the body. They can be administered in different ways: oral, respiratory (inhalation, olfaction, diffusion), rectal and cutaneous (massage), which gives them great bio-availability (Salim et al., 2017). Numerous *in vitro* studies in mice, rats and hamsters have been carried out to study the effect of essential oils on cancer. A very large number of studies suggest that natural terpenoids like limonene are a new class of anticancer drugs with the ability to cause tumor regression with low toxicity (Lesgards et al., 2014). In addition, numerous studies have also shown that the terpenoids of essential oils could act in synergy with conventional chemotherapy. Antitumor effects have been observed in combination with chemotherapy (Balusamy et al., 2018). For example, the combination of geraniol (essential oil extract)

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with 5-fluorouracil (chemotherapy product) reduces the volume of colon cancer in mice by 53%, while chemo alone has no effect and that geraniol alone reduces it by only 26% (Arruebo et al., 2011). *Inulaviscosa* or *Dittrichia viscosa* belongs to the Asteraceae family. The genus includes more than 90 species distributed in the Mediterranean regions, Spain, France, Asia, Turkey and Africa (Morocco, Egypt and Algeria) (Bouyahya et al., 2018). The viscous inule (*I. viscosa*) contains several secondary metabolites in the aerial parts. It is very rich in volatile compounds (terpenoids) (Bouyahya et al., 2018). The pharmacological properties of *I. viscosa* have been extensively studied. The extracts and essential oils of this plant have shown different pharmacological activities such as anti-inflammatory, antiviral and antitumor activity (Bouyahya et al., 2018). Isocostic acid isolated from the essential oil of *I. viscosa* exhibited an antityrosinase activity comparable to the positive control (kojic acid). Moreover, the calculated bioactivity and drug likeness scores showed also significant binding interaction proven with molecular docking analysis (Aissa et al., 2019). The essential oils from leaves and flowers of *I. viscosa* showed a significant antifungal activity against dermatophytes even at low concentrations (0.01 mg/mL). However, the leaf essential oil exhibited the greatest antifungal efficacy (Cafarchia et al., 2002). The therapeutic effects of this plant have been very diverse and have been known for a long time in traditional medications. It is a plant that is widely used in traditional medicine for its inflammatory, antipyretic and antimicrobial properties (Talib & Mahasneh, 2010). *I. viscosa* is also used to treat gastroduodenal disorders (Al-Dissi et al., 2001; Chahmi et al., 2015) and intestinal disorders. The essential oils are extracted from it for the treatment of various diseases such as bronchitis, diabetes, rheumatism, wounds and diseases of the urinary and digestive system (Al-Dissi et al., 2001; Talib et al., 2012). The study by Rozenblat et al. (2008) revealed the presence of different biologically active sesquiterpenes in *I. viscosa* and their ability to induce apoptosis in cancer cells. Furthermore, modeling and simulation have become standard practices in many scientific and technical fields and in particular in Chemistry. They are often necessary when the real experience is too difficult, too dangerous and too expensive. Computational and theoretical chemistry subsidizes to better comprehension of medicinal plants action against diseases and is being important and crucial to wet laboratory experiment, permitting studying structures and functions of bimolecular (Mesli et al., 2019). To our knowledge, this is the first study that describes the intraspecific variations of essential oils of *I. viscosa* from Algeria from 10 locations using statistical analysis and the structure–activity relationship (SAR). The second objective of this work was to study the essential oils of *I. viscosa* as an inhibitor for VEGF receptors in order to study their mechanism of enzymatic inhibition. Given that vascular endothelial growth factor (VEGF) increases the phosphorylation of tyrosine kinase FAK (Walker, 1996), we set ourselves the goal of inhibiting it in order to decrease phosphorylation. The essential oils of the aerial parts of the *Inulaviscosa* inhibitors were the subject of our investigation. These were used to target the intracellular part of the VEGF receptors (the tyrosine

kinase domain), knowing that the two receptors (VEGFR1 and VEGFR2) have different affinities for VEGF and induce different cellular and biological effects. The main interest was to develop new potential inhibitors of the VEGF/VEGFR interaction and finally discuss with the bioactivity scores, drug likeness, pharmacokinetics, medicinal chemistry, molecular docking and molecular dynamics (MD) analysis of major components. The more we know about these interactions, the more we can do with that knowledge. However, many efforts have been made to produce the natural and reliable treatment during the first stage of cancerous cells.

2. Material and methods

2.1. Experimental procedures

2.1.1. Plant material and essential oil extraction

Plant material used (Aerial parts) of *I. viscosa* was collected at the flowering stage in May 2019 from 10 locations (S1–S10) widespread in the regions of Tlemcen (Algeria) (Table 1). The plant material was botanically authenticated by the Laboratory of Ecology and Ecosystem Management of University of Tlemcen, Algeria. Voucher specimens (see Table 1) were deposited in the herbarium of the Natural and Bioactive Substances Laboratory, Tlemcen University. To obtain essential oils, 400–500 g to aerial parts was subjected to hydrodistillation for a period of 5 h using a Clevenger-type apparatus according to the European Pharmacopoeia. For the chemical analysis, essential oils were stored in dark glass bottles at 4 °C. The essential oil yields were expressed in percent (w/dw) through the weight of dried plant material. The geographical origin, yields and the voucher number of each sample are presented in Table 1.

2.1.2. Analysis conditions

2.1.2.1. Gas chromatography (GC). GC analyses were carried out using a Perkin Elmer Autosystem Clarus 600 GC apparatus (Germany) equipped with a dual flame ionization detection system and fused Rtx-1 silica capillary columns (60 m × 0.22 mm i.d., 0.25- μ m film thickness; polydimethylsiloxane). The oven temperature was programmed to increase from 60–230 °C at 2 °C/min and was then held isothermally at 230 °C for 35 min. Injector and detector temperatures were maintained at 280 °C. The essential oils were injected in the split mode (1/50), and the injection volume was 0.2 μ L. The retention indices (RI) of the compounds were determined from Perkin Elmer software.

2.1.2.2. Gas chromatography-mass spectrometry (GC-MS).

Essential oils were analyzed using a Perkin Elmer Turbo mass detector (quadrupole) coupled to a Perkin Elmer Autosystem XL equipped with Rtx-1 fused silica capillary columns and Rtx-Wax (poly-ethyleneglycol) (ion source temperature, 150 °C; ionization energy, 70 eV). Ionization energy MS were seized over a mass range of 35–350 Da (scan time, 1 s). Other GC conditions were the same as interpreted for GC, except the split was 1/80.

Table 1. Data relative to harvest locations of *I. viscosa* from Algeria.

| Samples | Locations | GPS coordinates | N°. Voucher codes | Yields | Altitudes (m) |
|---------|-------------|-----------------------|-------------------|--------|---------------|
| S1 | SidnaYoucha | 35°7'0"N; 1°46'60"O | I.V-0518-DMA7 | 0.06 | 5 |
| S2 | Beni saf | 35°18'8" N;1°23'1"O | I.V-0518-DMA9 | 0.08 | 25 |
| S3 | Rachgoun | 35°19'26"N; 1°28'47"O | I.V-0518-DMA10 | 0.06 | 36 |
| S4 | Ghazaouet | 35°05'38"N;1°51'37"O | I.V-0518-DMA6 | 0.05 | 118 |
| S5 | Souahlia | 35°1'60" N; 1°52'60"O | I.V-0518-DMA8 | 0.1 | 318 |
| S6 | Terny | 4°47'45"N; 1°21'29"O | I.V-0518-DMA4 | 0.09 | 854 |
| S7 | Tlemcen | 34°52'41"N; 1°18'53"O | I.V-0518-DMA3 | 0.08 | 811 |
| S8 | Beni snous | 34°38'35"N; 1°33'41"O | I.V-0518-DMA1 | 0.16 | 1500 |
| S9 | Tafna | 34°52'38"N; 1°14'07"O | I.V-0518-DMA2 | 0.2 | 1600 |
| S10 | EL Aricha | 34°13'22"N; 1°15'21"O | I.V-0518-DMA9 | 0.2 | 1148 |

2.1.2.3. Component identification and quantification.

Identification of individual components was accomplished by comparing their GC retention indices (RIs) on nonpolar and polar columns, determined relative to the retention time of a series of n-alkanes with linear interpolation, with those of authentic compounds or literature data (Jennings & Shibamoto, 1980; Joulain & König, 1998; König et al., 2001) and through computer matching with commercial mass spectral libraries (Mc Lafferty & Stauffer, 1988; National Institute of Standards and Technology, 2008) and also by comparing the spectra obtained with those of the in-house laboratory library. The quantification of essential oils and blend was performed using peak normalization (%) abundances calculated by integrating FID response factors relative to tridecane (0.7 g/100 g), used as an internal standard.

3. Theoretical background and computational details

3.1. Selection of receptor and ligand

In this study, the interactions of essential oils of the aerial parts of *I. viscosa* from compounds as described in Table 2 were investigated. The structures of inhibitors were downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>).

The PDB database (<https://www.rcsb.org/>) were used to obtain the complete structure of VEGF receptors (VEGFR-1) (PDB ID: **3HNG** [Tresaugues et al., 2013]), VEGFR-2 (PDB ID: **2XIR**), VEGF (PDB ID: **5t89** was obtained by X-ray diffraction).

3.2. Molecular docking

Virtual screening is advised as an alternative method for experimental screening and has a marked up success rate in the drug discovery process. It is a computational analogue of biological screening and has become increasingly popular in the pharmaceutical industry for lead identification (Rasouli et al., 2017). Here, the docking procedure provides specification of the ligand binding site in a receptor and then the docked ligands in the specified site (Rasouli et al., 2017).

3.3. Drug-likeness prediction

Properties analyzed are TPSA, clogP calculation, logS calculation, molecular weight, fragment based drug-likeness, and drug score (Nisha et al., 2016).

3.4. ADME prediction

ADMET, which constitutes the pharmacokinetic profile of a drug molecule, is very essential in evaluating its pharmacodynamic activities (Nisha et al., 2016). In this study, we have used the SwissADME online property calculation from all these parameters for the best scoring lead compounds (Daina et al., 2017).

3.5. MD simulation

MD aims to numerically simulate condensed phases of a molecular system in order to understand, predict and calculate the properties of a studying system (Champagnat et al., 2013).

The best conformer of VEGF receptors with ligands was subjected to MD simulations was performed for both the complexes (3HNG, 2XIR, 5t89) using the MOE software (Al-Hader et al., 1993). MOE dynamics simulation uses the Nose Poincare-Andersen (NPA) equations of motion (Bond et al., 1999; Sturgeon & Laird, 2000). The Berendsen thermostat is an algorithm to rescale the velocities of particles in MD simulations to control the simulation temperature (Berendsen et al., 1984). The coordinates were stored every 0.2 ps to get an accurate view of molecular movement. In all simulations, the van der Waals cutout distance was set to 8 Å. Energy minimization process was applied by using MMFF94x force field (Parikesit et al., 2015). We have shown the detailed analysis of MD simulation results of only compound L19 with target VEGF receptors (Figures 13–15) because this compound showed better binding affinity for both VEGF receptors. In the end and according to the MD simulation analysis among these two compounds, the most active compounds were L4 and L19 in VEGF receptors.

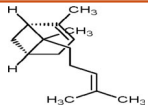
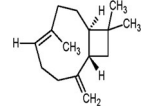
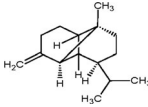
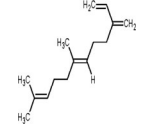
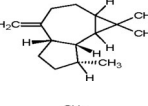
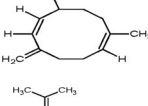
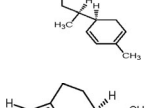
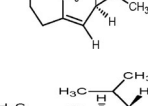
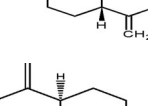
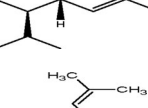
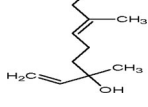
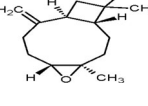
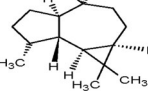
4. Results and discussion

4.1. Experimental

4.1.1. Yields and chemical compositions of *I. viscosa* essential oils

The hydrodistillation of dry leaves of *I. viscosa* of different stations led to the isolation of yellowish oils. The essential oil yields of populations, collected from study areas, are shown in Table 1. Essential oil yields varied from 0.05% to 0.2% (w/w), among stations. The highest yields of essential oils were obtained in the stations of Tafna (0.2%) (S9), EL Aricha (0.2%) (S10) and Beni snous (0.16%) (S8), with altitudes above 1000 m, while the lowest (0.05%–0.1%) were observed in the

Table 2. Various drugs used in the *in silico* docking studies. 'Adopted from online PubChem database (accessed on 07.01.2014). Adopted from online CHEMBL database (accessed on 13.11.2013)'.

| No. | Anti-angiogenic drug | IUPAC Name | CID/ N ^o | M.W. (g/mol) | Molecular Formula | Structure |
|-----|-----------------------------|--|---------------------|--------------|-----------------------------------|---|
| 1 | cis- α -Bergamotene | (1S,5S,6S)-2,6-dimethyl-6-(4-methylpent-3-enyl)bicyclo[3.1.1]hept-2-ene | 6429303 | 204.35 | C ₁₅ H ₂₄ |  |
| 2 | (E)- β -Caryophyllene | [(5Z)-6,10-dimethyl-2-methylidene-10-bicyclo[7.2.0]undec-5-enyl]methanol | 5352484 | 220.35 | C ₁₅ H ₂₄ O |  |
| 3 | β -Copaene | 1,3-dimethyl-8-propan-2-yltricyclo[4.4.0.0.2,7]dec-3-ene | 19725 | 204.35 | C ₁₅ H ₂₄ |  |
| 4 | | (3E,6E)-3,7,11-trimethyldodeca-1,3,6,10-tetraene | 5281516 | 204.35 | C ₁₅ H ₂₄ |  |
| 5 | allo-Aromadendrene | (4aS,7R,7aR)-1,1,7-trimethyl-4-methylidene-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulene | 42608158 | 204.35 | C ₁₅ H ₂₄ |  |
| 6 | Germacrene-D | (1Z,6Z,8S)-1-methyl-5-methylidene-8-propan-2-ylcyclodeca-1,6-diene | 91723653 | 204.35 | C ₁₅ H ₂₄ |  |
| 7 | Zingibrene | (5R)-2-methyl-5-[(2S)-6-methylhept-5-en-2-yl]cyclohexa-1,3-diene | 92776 | 204.35 | C ₁₅ H ₂₄ |  |
| 8 | Bicyclgermacrene | (1R,2E,6E,10S)-3,7,11,11-tetramethylbicyclo[8.1.0]undeca-2,6-diene | 11820258 | 204.35 | C ₁₅ H ₂₄ |  |
| 9 | γ -Cadinene | (1S,8aR)-4,7-dimethyl-1-propan-2-yl-1,2,3,5,6,8a-hexahydronaphthalene | 441005 | 204.35 | C ₁₅ H ₂₄ |  |
| 10 | δ -Cadinene | (1S,4aR,8aR)-7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene | 6432404 | 204.35 | C ₁₅ H ₂₄ |  |
| 11 | (E)-Nerolidol | (6E)-3,7,11-trimethyldodeca-1,6,10-trien-3-ol | 5284507 | 222.37 | C ₁₅ H ₂₆ O |  |
| 12 | Caryophyllene oxide | (1R,4R,6R,10S)-4,12,12-trimethyl-9-methylidene-5-oxatricyclo[8.2.0.0.4,6]dodecane | 1742210 | 220.35 | C ₁₅ H ₂₄ O |  |
| 13 | Globulol | (1aR,4R,4aR,7R,7aS,7bS)-1,1,4,7-tetramethyl-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulene-4-ol | 12304985 | 222.37 | C ₁₅ H ₂₆ O |  |

(continued)

Table 2. Continued.

| No. | Anti-angiogenic drug | IUPAC Name | CID/ N° | M.W. (g/mol) | Molecular Formula | Structure |
|-----|----------------------|---|----------|--------------|-----------------------------------|-----------|
| 14 | Ledol | (1aR,4R,4aS,7R,7aS,7bS)-1,1,4,7-tetramethyl-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulen-4-ol | 92812 | 222.37 | C ₁₅ H ₂₆ O | |
| 15 | Zingibereol | 1-methyl-4-(6-methylhept-5-en-2-yl)cyclohex-2-en-1-ol | 13213649 | 222.37 | C ₁₅ H ₂₆ O | |
| 16 | τ-Muurolol | (1S,4S,4aR,8aS)-1,6-dimethyl-4-propan-2-yl-3,4,4a,7,8,8a-hexahydro-2H-naphthalen-1-ol | 3084331 | 222.37 | C ₁₅ H ₂₆ O | |
| 17 | α-Cadinol | (1R,4S,4aS,8aS)-1,6-dimethyl-4-propan-2-yl-3,4,4a,7,8,8a-hexahydro-2H-naphthalen-1-ol | 12302222 | 222.37 | C ₁₅ H ₂₆ O | |
| 18 | α-Bisabolol | (2R)-6-methyl-2-[(1R)-4-methylcyclohex-3-en-1-yl]hept-5-en-2-ol | 1549992 | 222.37 | C ₁₅ H ₂₆ O | |
| 19 | Farnesylacetone | (5E,9E)-6,10,14-trimethylpentadeca-5,9,13-trien-2-one | 1711945 | 222.37 | C ₁₈ H ₃₀ O | |

stations S1 to S7 with altitudes varying from 5 to 854 m. The chemical composition analysis *I. viscosa* essential oils of 10 stations (Table 3) allowed the identification of 19 compounds, accounting for 90.1%–98.8% of oils.

All components were identified by comparing their mass spectra (EI-MS) and retention indices (RIs) with those of mass spectral library, 10 sesquiterpene hydrocarbons and 9 oxygenated sesquiterpenes were identified (Table 3). The EO Coll of *I. viscosa* showed only the presence of sesquiterpenes compounds (97.2%). The oxygenated sesquiterpenes were the most dominant with a percentage of 87.3%. The major components were α -bisabolol (16.0%), (*E*)-Z-farnesylacetone (13.2%), (*E*)-nerolidol (15.5%), α -cadinol (11.6%), caryophyllene oxide (10.6%) and τ -muurolol (9.8%), while the sesquiterpene hydrocarbons were represented by small amounts of (*E*)- β -farnesene (2.6%), allo-aromadendrene (1.8%) and δ -cadinene (1.5%) (Table 3). When we compare our data, with those in the literature, it appears that the chemical composition of our oil is markedly different from other regions of the world. Indeed, the major components of essential oil of Turkey were borneol (25.2%), isobornylacetate (22.5%) and bornyl acetate (19.5%) (Pérez-Alonso et al., 1996), that of France and Spain was fokienol (21.1% and 38.8%, respectively) (Blanc et al., 2006; Camacho et al., 2000), while that of Jordan were fokienol (20.9%) and (*E*)-nerolidol (19.8%) (Al-Qudah et al., 2010). While Eudesma-3,11(13)-dien-12-oic acid was detected as main constituent in *I. viscosa* essential oil from the East

Algeria (56.8%) and southern Italy (62.4%) (De Laurentis et al., 2002; Haoui et al., 2015), on the other hand, δ -terpinène (35.9%) and α -pinène (18.9%) were the major components of essential oil of Sidi Bel Abbes (Algeria) (Benchohra et al., 2011). 3-methoxy cumylnisobutyrate (12%) and α -cadinol (6.3%) dominate the composition of Portugal *I. viscosa* essential oils. The composition of the Tunisian *I. viscosa* leaves essential oil was characterized by high oxygenated sesquiterpenes (92.7%) dominated by isocostic acid (70.8%) (Aissa et al., 2019). Various studies on the essential oil of *I. viscosa* reported the presence of globulol (26, 15.0%), chamazulene (27, 49.6%) and 1,4-dimethylazulene (28, 32.1%) in high percentage (Chiarlo, 1968). On the other hand, the leaves contained the eucalyptol (Lauro & Rolih, 1990).

4.2. Chemical variability of essential oils

However, quantitative differences were greatly observed in the major essential oil constituents of different stations (S1–S10) due their geographic location. Indeed, the cluster analysis according to (CA) (Figure 1) the main compounds (N° 11,12, 16–19 of Table 3) showed significant differences. The dendrogram (CA) was obtained using the nearest neighbor method; it suggests that there were two main groups of *I. viscosa* oils (Figure 1). The first group (I) included oil samples from five localities (S1–S5). The second group (II) constituted of samples from five localities (S6–S10). The second

Table 3. Chemical composition of essential oils of the aerial parts of *I. viscosa* collected in 10 stations in the North West of Algeria.

| No. ^a | Components | RI ^b | RI ^c | RI _p ^d | EO Coll | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | Identification ^e |
|----------------------------|--------------------------------------|-----------------|-----------------|------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-----------------------------|
| 1 | cis- α -Bergamotene | 1411 | 1409 | 1560 | 0.9 | 1.2 | 1.2 | 0.6 | 0.2 | 0.1 | 1.2 | 1.1 | 0.1 | 1.5 | 1.6 | RI, MS |
| 2 | (<i>E</i>)- β -Caryophyllene | 1421 | 1418 | 1590 | 0.3 | 0.3 | 0.2 | 0.6 | 1.5 | 1.8 | 0.3 | 0.2 | 0.6 | 1.2 | 0.3 | RI, MS |
| 3 | β -Copaene | 1431 | 1430 | 1579 | 0.8 | 0.4 | 0.5 | 0.1 | 0.6 | 0.3 | 0.1 | 0.1 | 0.4 | 0.3 | 0.1 | RI, MS |
| 4 | (<i>E</i>)- β -Farnesene | 1448 | 1444 | 1660 | 2.6 | 1.6 | 3.2 | 4.8 | 5.6 | 2.6 | 0.3 | 0.5 | 0.6 | 0.3 | 0.5 | RI, MS |
| 5 | allo-Aromadendrene | 1462 | 1459 | 1637 | 1.8 | 1.3 | 0.8 | 2.3 | 3.3 | 0.3 | 0.5 | 0.6 | 1.3 | 1.1 | 0.9 | RI, MS |
| 6 | Germacrene-D | 1480 | 1477 | 1700 | 0.5 | 0.8 | 1.5 | 1.1 | 0.6 | 0.9 | 0.5 | 0.3 | 0.1 | 0.1 | 0.1 | RI, MS |
| 7 | Zingibrene | 1489 | 1486 | 1715 | 0.1 | 0.4 | 0.5 | 0.1 | 0.1 | 0.3 | 0.8 | 0.2 | 0.1 | 0.1 | 0.1 | RI, MS |
| 8 | Bicyclgermacrene | 1494 | 1492 | 1720 | 0.5 | 0.3 | 0.6 | 0.2 | 0.1 | 0.5 | 1.3 | 0.2 | 0.3 | 0.2 | 0.1 | RI, MS |
| 9 | γ -Cadinene | 1507 | 1509 | 1752 | 0.9 | 1.1 | 0.9 | 2.1 | 0.1 | 0.6 | 1.4 | 0.2 | 0.1 | 0.8 | 0.2 | RI, MS |
| 10 | δ -Cadinene | 1516 | 1522 | 1785 | 1.5 | 0.8 | 0.2 | 0.6 | 0.3 | 1.3 | 6.3 | 0.3 | 0.1 | 0.7 | 0.1 | RI, MS |
| 11 | (<i>E</i>)-Nerolidol | 1546 | 1551 | 2036 | 15.5 | 30.2 | 20.5 | 19.5 | 20.5 | 18.3 | 5.3 | 4.2 | 4.8 | 3.2 | 5.2 | RI, MS |
| 12 | Caryophyllene oxide | 1576 | 1569 | 1985 | 10.6 | 13.6 | 12.3 | 15.6 | 18.1 | 11.8 | 5.8 | 5.1 | 6.5 | 3.5 | 7.3 | RI, MS |
| 13 | Globulol | 1589 | 1581 | 2066 | 2.9 | 2.3 | 1.3 | 2.3 | 2.9 | 5.3 | 5.3 | 6.9 | 7.2 | 9.1 | 7.3 | RI, MS |
| 14 | Ledol | 1600 | 1605 | 2023 | 4.5 | 2.5 | 7.6 | 7.5 | 1.5 | 3.9 | 3.5 | 3.1 | 5.4 | 3.7 | 0.7 | RI, MS |
| 15 | Zingiberenol | 1613 | 1612 | 2169 | 3.2 | 7.3 | 1.8 | 2.6 | 2.3 | 6.5 | 1.6 | 8.5 | 4.3 | 3.5 | 0.5 | RI, MS |
| 16 | τ -Muurolol | 1634 | 1631 | 2142 | 9.8 | 0.3 | 5.7 | 0.6 | 0.2 | 0.1 | 10.5 | 14.5 | 25.3 | 29.5 | 33.2 | RI, MS |
| 17 | α -Cadinol | 1645 | 1641 | 2108 | 11.6 | 8.1 | 9.2 | 7.5 | 5.5 | 10.3 | 25.3 | 26.3 | 19.5 | 20.1 | 18.6 | RI, MS |
| 18 | α -Bisabolol | 1672 | 1671 | 2216 | 16.0 | 3.1 | 5.6 | 4.1 | 1.6 | 6.6 | 26.2 | 22.3 | 15.3 | 17.3 | 16.3 | RI, MS |
| 19 | (<i>E</i>)-Z-Farnesylacetone | 1871 | 1879 | 2331 | 13.2 | 23.2 | 19.6 | 18.5 | 25.1 | 21.6 | 2.6 | 2.2 | 1.1 | 0.6 | 2.3 | RI, MS |
| % Identification | | | | | 97.2 | 98.8 | 93.2 | 90.7 | 90.1 | 93.1 | 98.8 | 96.8 | 93.1 | 96.8 | 95.4 | |
| Sesquiterpene hydrocarbons | | | | | 9.9 | 8.2 | 9.6 | 12.5 | 12.4 | 8.7 | 12.7 | 3.7 | 3.7 | 6.3 | 4.0 | |
| Oxygenated sesquiterpenes | | | | | 87.3 | 90.6 | 83.6 | 78.2 | 77.7 | 84.4 | 86.1 | 93.1 | 89.4 | 90.5 | 91.4 | |

^aOrder of elution is given on apolar column (Rtx-1).

^bRetention indices of literature on the apolar column (RILit).

^cRetention indices on the apolar Rtx-1 column (RIa).

^dRetention indices on the polar Rtx-wax column (RIp).

^eRI: Retention Indices; MS: Mass Spectra in EI mode.

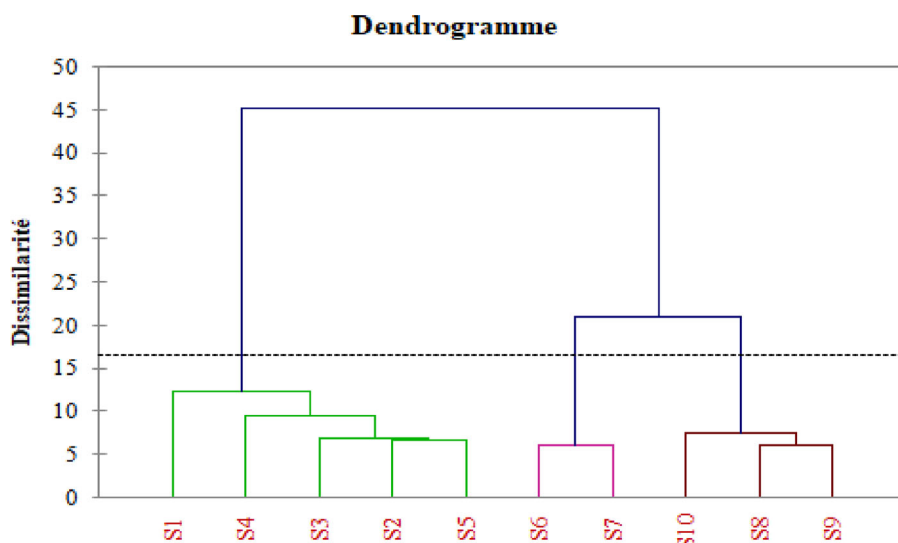


Figure 1. Cluster analysis (CA) of chemical compositions of essential oil of *I. viscosa* from the North West of Algeria.

group was characterized by two subgroups. The subgroup (I1) contained the stations S6 and S7 and the subgroup (I2), the stations S8–S10.

Principle component analysis (PCA) (Figure 2) shows the relationships between family of compounds and geographic location. The first two PCA axes accounted for 82.96% and 8.5% of the total variance, respectively.

The results of PCA highly confirmed the existence of two main groups. Group I (S1–S5) at low altitude was mainly discriminated by the contents of (*E*)-nerolidol (15.5%–20.2%), caryophyllene oxide (10.6%–18.1%), (*E*)-Z-farnesylacetone (13.2%–25.1%) and (*E*)- β -farnesene (1.5%–5.6%). On the other hand, stations S6 and S7 (subgroup I1) were characterized by

the presence of a higher percentage of α -bisabolol (25.3% and 26.3%, respectively) and α -cadinol (25.3% and 26.3%, respectively), compared to other stations, while the subgroup I2 (S8–S10) with higher altitudes (1148–1600 m) was richer by τ -muurolol (25.3%–33.2%) and globulol (7.2%–9.1%) (Figure 2, Table 3). However, the observed differences in the chemical composition of essential oils can be justified by many factors such as abiotic stresses (Belabbes et al., 2017), the cultivation area, collected material, altitude and age of the plant (Ma et al., 2019). Sesquiterpenes were the most distinct group in terms of the structure of the terpenoids, most of which exert biological activities (Hou et al., 2014; Khana et al., 2008) and have been reported to be active against the

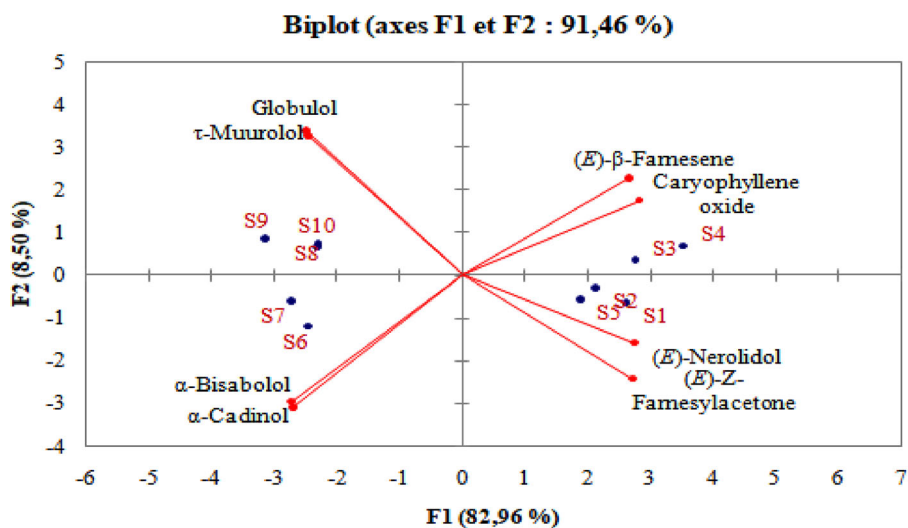


Figure 2. PCA of chemical compositions of essential oils of *I. viscosa*. Distribution of variables.

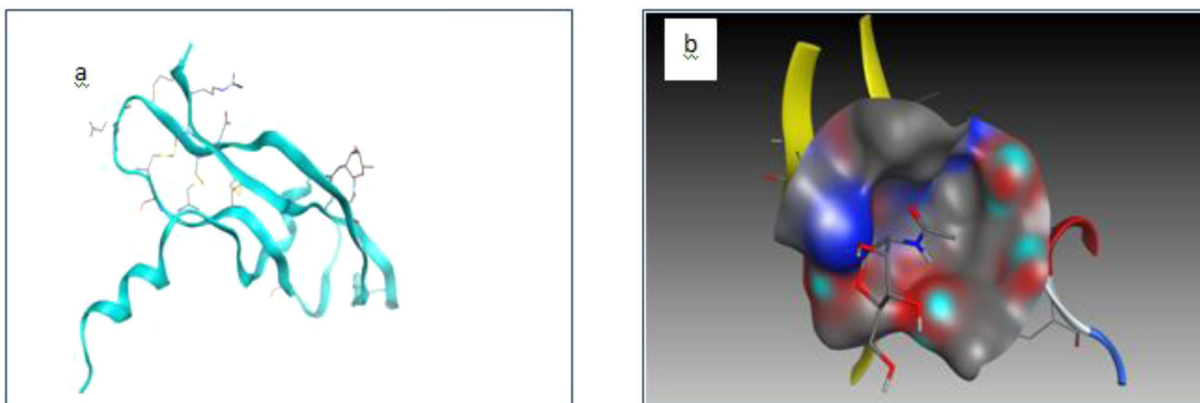


Figure 3. (a) Simplified model of (VEGF). (b) The active site of the isolated VEGF.

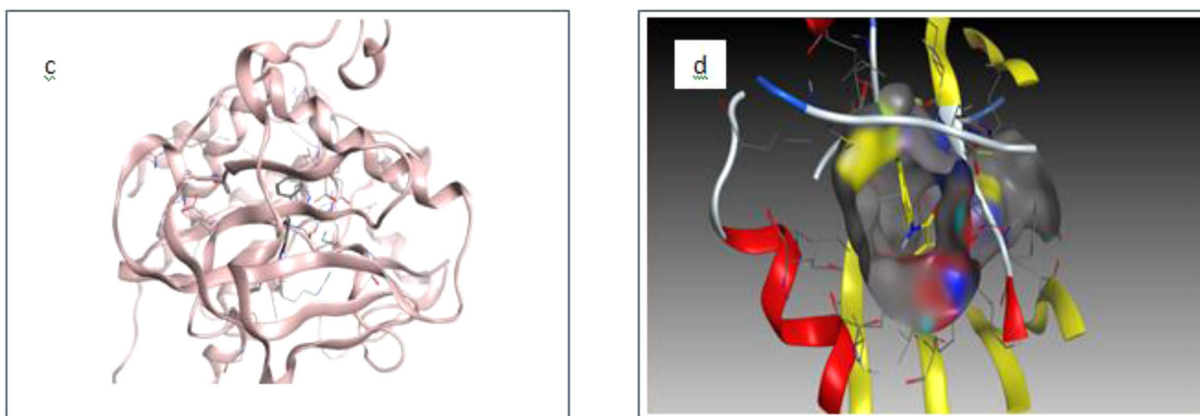


Figure 4. (c) Simplified model of (VEGFR-1 receptor). (d) The active site of the isolated VEGFR-1.

oxidative stress (Su et al., 2015), β -caryophyllene, τ -muurolol, α -cadinol and (2Z,6E)-farnesol exhibit cytotoxic activity against human colon, liver and lung cancer cells (Cavalieri et al., 2004). α -bisabolol was found to have a strong time- and dose-dependent cytotoxic effect on human and rat glioma cells (Cavalieri et al., 2004).

4.2.1. Theoretical

The enzyme's active sites with co-crystallization molecule are shown in Figures 3–5.

The ligands of essential oils of the aerial parts of *I. viscosa* minimized toxicity, and energy obtained by MOE software is shown in Table 4.

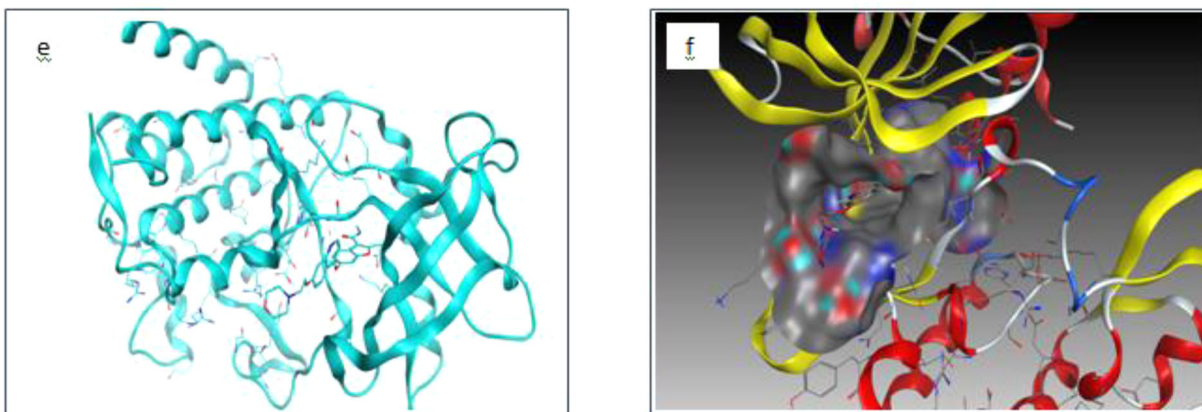


Figure 5. (e) Simplified model of (VEGFR-2 receptor). (f) The active site of the isolated VEGFR-2.

Table 4. Minimization energy of molecules natural for anti-angiogenic drug (kcal/mol).

| Ligand | Molecules | Energies(Kcal/mol) | LogP | LogS | Toxicity |
|--------|--------------------------------------|--------------------|------|-------|----------|
| 1 | cis- α -Bergamotene | 3.91656e + 001 | 4.73 | -5.29 | No |
| 2 | (<i>E</i>)- β -Caryophyllene | 4.00404e + 001 | 3.70 | -3.07 | No |
| 3 | β -Copaene | 4.34100e + 001 | 4.27 | -5.91 | No |
| 4 | (<i>E</i>)- β -Farnesene | 2.18401e + 001 | 5.20 | -6.01 | No |
| 5 | allo-Aromadendrene | 5.36129e + 001 | 4.27 | -6.41 | No |
| 6 | Germacrene-D | 3.17611e + 001 | 4.89 | -4.74 | No |
| 7 | Zingibrene | 2.59488e + 001 | 4.89 | -4.87 | No |
| 8 | Bicyclogermacrene | 4.80650e + 001 | 4.73 | -4.67 | No |
| 9 | γ -Cadinene | 2.61312e + 001 | 4.73 | -4.80 | No |
| 10 | δ -Cadinene | 2.95608e + 001 | 4.58 | -5.17 | No |
| 11 | (<i>E</i>)-Nerolidol | 2.19630e + 001 | 4.40 | -3.93 | No |
| 12 | Caryophyllene oxide | 4.51752e + 001 | 3.94 | -4.39 | Yes |
| 13 | Globulol | 5.49887e + 001 | 3.47 | -4.79 | No |
| 14 | Ledol | 5.91711e + 001 | 3.47 | -4.79 | No |
| 15 | Zingibereol | 2.38235e + 001 | 4.09 | -4.37 | No |
| 16 | τ -Muurolol | 3.54879e + 001 | 3.78 | -3.54 | No |
| 17 | α -Cadinol | 3.89123e + 001 | 3.78 | -3.54 | No |
| 18 | α -Bisabolol | 2.58813e + 001 | 4.23 | -2.92 | No |
| 19 | (<i>E</i>)-Z-Farnesylacetone | 2.17939e + 001 | 5.77 | -5.18 | No |

These ligands are capable of providing crucial biological activities in accordance with the principle of Lipinski et al. (1997) (Pettersson et al., 1988).

As stated in the table above, we find that the molecules L19 and L4 have a high value of Log P and Log S compared to other molecules and also, the results obtained show that these ligands (L19 and L4) have a high value of torsion angle relative to other compounds. This shows that these compounds are more flexible. In addition, it is noted that the growth of the torsion angle depends on the binding number of the molecule. The information of all compounds was obtained from MOE software (Molecular Operating Environment (MOE), 2013.08, 2016).

4.3. Molecular docking

4.3.1. Natural inhibitor approach

4.3.1.1. VEGF. We note that the result obtained (Table 5), out of the best compounds studied, Farnesylacetone (Ligand 19) (Figure 6) was predicted to be the strongest VEGF receptor binder that forms a complex with the most stability with the lowest energy -4.52469969 Kcal/mol. The ligands that interacted with VEGFR-1 were as follows: Ligand L2 interacted with two amino acids (GLU 93 and GLU 93) at a distance of

2.51, 2.82 Å strong with energy of 1.2 and -1.0 , respectively, and ligand L11 interacts with one amino acid GLU 38H – donor at a distance of 2.58 Å strong and energy binding of -1.4 ; similarly, the ligand L12 interacted with one amino acid LEU 97H-acceptor at a distance of 2.96 Å. It is noted that the interactions between the residue of the active site of 5t89 and Farnesylacetone ligand formed a stable complex.

The second best binder was (*E*)- β -Farnesene (Ligand 4) (Figure 7) with the energy of -4.01963854 Kcal/mol. This suggests that (*E*)- β -Farnesene can inhibit VEGF receptors.

4.3.1.2. VEGFR-1. We note that Farnesyl acetone (Ligand 19) (Figure 8) was predicted to be the strongest VEGF receptor binder that formed a complex with the most stability and the lowest energy (-4.52469969 Kcal/mol) that interacted with two amino acids (ARG 1021 and ARG 1021) H-acceptor at a distance of interaction of 3.00 and 2.94 Å, respectively, with the existence of eight electric forces (GLU910, GLU 878, CYS 912, VAL 891, LEU 882, ASP1040, LYS861 and ARG1021). The existence of electric force suggests that Farnesylacetone can inhibit VEGF receptors. It is noted that the interactions between the residues of the active site of 3HNG and the Farnesylacetone ligand form a stable complex with a strong interaction.

The second best binder was (*E*)- β -Farnesene(Ligand 4) (Figure 9) with the energy of -7.55429745 Kcal/mol. The ligands that interacted with VEGFR-1 were as follows: Ligand L3 interacted with a one amino acid PHE 1041 H-pi at a distance of 4.08 Å, low interaction with energy binding of -0.7 , and then, Ligand L14 interacted with two amino acids GLU910 and CYS912 H-donor and H-acceptor, respectively, with energy of 1.8 and 1.7, respectively. Lastly, the ligand L19 interacted with same amino acids ARG 1021 H-acceptor with energy between receptor and amino acids were -4.5 and -1.6 , respectively (See supplementary Figures 16–18).

4.3.1.3. VEGFR-2. We note that Farnesylacetone (Ligand 19) (Figure 10) was predicted to be the strongest VEGF receptor binder that formed a complex with the most stability with the lowest energy -8.10823059 Kcal/mol) with the existence of four electric force (GLU 917, CYS 919, ASP 1024 and LEU 840). This suggests that Farnesylacetone can inhibit VEGF receptors. It is noted that the interactions between the

Table 5. Energy balance of complexes formed with anti-angiogenic drug without water molecules (Kcal/mol).

| Mol | Pose | Score | Rmsd-refine | E-Conf | E- PLACE | E-REFINE | RMSD |
|-----------------------|-------------|--------------|--------------------|---------------|----------------|-----------------|-------------|
| Lref1 (Native) | 10 | -3.78999758 | 3.80355144 | 53.460392 | -42.1670952 | -9.73435974 | 1.428 |
| VEGF | | | | | | | |
| L1 | 3 | -3.49325585 | 1.4171623 | 49.4205856 | -9.02826786 | -9.28911781 | 1.385 |
| L2 | 6 | -3.54622912 | 4.25287771 | 45.9673691 | -22.3249168 | -9.67628956 | 0.394 |
| L3 | 2 | -3.38240385 | 1.97325587 | 49.2433662 | -18.0179806 | -7.8158865 | 0.298 |
| L4 | 5 | -4.01963854 | 1.95341456 | 34.3210564 | -15.5851097 | -9.65795422 | 1.447 |
| L5 | 5 | -3.38378692 | 2.55735159 | 71.8157883 | -24.9921379 | -8.56387329 | 0.111 |
| L6 | 7 | -3.18338823 | 1.66500854 | 40.1790848 | -25.3914356 | -7.22984791 | 0.570 |
| L7 | 2 | -3.73947215 | 1.08847892 | 37.5174332 | -37.0030251 | -10.5696306 | 0.197 |
| L8 | 3 | -3.52705669 | 2.08294535 | 61.8202896 | -16.5464706 | -9.45939064 | 0.198 |
| L9 | 4 | -3.69257712 | 2.31939769 | 43.0718536 | -25.7758541 | -9.50582314 | 0.343 |
| L10 | 4 | -3.45229697 | 3.45120263 | 11.3685236 | -11.34288752 | -8.6183157 | 0.079 |
| L11 | 8 | -3.98458982 | 1.67662919 | 32.5299606 | -34.1233177 | -12.5704956 | 0.211 |
| L12 | 5 | -3.59765863 | 3.55976343 | 13.6160984 | 5.93009233 | -9.25728226 | 0.215 |
| L13 | 8 | -3.80335522 | 6.73355961 | 13.5810099 | -22.7992706 | -10.0778494 | 0.263 |
| L14 | 8 | -3.75419545 | 2.29156828 | 62.2090683 | -20.9349194 | -9.60580826 | 0.139 |
| L15 | 6 | -3.60531759 | 1.56402004 | 22.7498188 | -24.3484097 | -8.58475876 | 0.081 |
| L16 | 7 | -3.23660111 | 3.0124259 | 38.370285 | -12.1734447 | -7.70761824 | 0.197 |
| L17 | 8 | -3.55800462 | 2.01988792 | 40.3355331 | -39.8503456 | -9.25317478 | 0.087 |
| L18 | 6 | -3.71103525 | 1.1156019 | 32.8649559 | -28.2323246 | -10.1320171 | 0.320 |
| L19 | 9 | -4.52469969 | 1.46585608 | 38.5425644 | -34.7141495 | -12.7509823 | 0.053 |
| Mol | Pose | Score | Rmsd-refine | E-Conf | E-PLACE | E-REFINE | RMSD |
| Lref2 | 6 | -10.2159939 | 1.63261998 | 47.5232811 | -84.9253769 | -33.8748169 | 0.659 |
| RESPTOR1/ VEGFR1 | | | | | | | |
| L1 | 5 | -5.67572975 | 0.738956094 | 55.8443031 | -58.5605888 | -3.56594133 | 0.814 |
| L2 | 9 | -5.79094362 | 1.84575272 | 49.6417389 | -62.4633102 | -16.9630489 | 0.673 |
| L3 | 8 | -5.31909132 | 2.69288158 | 50.999691 | -52.446312 | -14.2888889 | 0.242 |
| L4 | 8 | -7.55429745 | 1.32448125 | 39.0443153 | -66.0409241 | -20.4006729 | 1.143 |
| L5 | 8 | -5.51163673 | 3.15417051 | 72.1863174 | -57.3297005 | -16.5133209 | 0.115 |
| L6 | 7 | -5.51596737 | 1.31669843 | 42.5609818 | -58.9852829 | -12.8108568 | 0.250 |
| L7 | 10 | -6.2743659 | 1.10227025 | 36.787323 | -54.5825882 | -19.3177948 | 0.035 |
| L8 | 4 | -4.31459522 | 1.23091698 | 66.5092773 | -45.724987 | 0.929653227 | 0.485 |
| L9 | 7 | -5.35120869 | 2.12681007 | 46.9883537 | -54.7862587 | -10.063139 | 0.433 |
| L10 | 9 | -5.29777861 | 0.688894331 | 15.5641155 | -51.1675949 | -10.7363739 | 0.420 |
| L11 | 10 | -6.78250837 | 1.8873719 | 41.0823135 | -52.1711159 | -13.4837017 | 0.502 |
| L12 | 7 | -5.14908934 | 1.49792802 | 16.2355289 | -53.7675209 | -10.9783001 | 0.356 |
| L13 | 8 | -4.79661131 | 1.32344747 | 15.2820148 | -59.1631584 | -10.7241364 | 0.366 |
| L14 | 7 | -5.34053659 | 2.14731693 | 63.0932159 | -51.9754829 | -14.3296251 | 0.268 |
| L15 | 9 | -5.08465052 | 2.31756425 | 24.4453144 | -60.2406654 | -15.4020233 | 0.444 |
| L16 | 9 | -5.04594994 | 0.91354239 | 39.0615311 | -49.6845665 | -12.8063755 | 0.023 |
| L17 | 10 | -4.74857521 | 1.52684665 | -52.9785118 | -52.9785118 | -12.808341 | 0.416 |
| L18 | 10 | -5.1971302 | 2.11682534 | 30.7944088 | -69.2757645 | -15.7095633 | 0.413 |
| L19 | 10 | -7.96668291 | 2.71927118 | 39.4914207 | -58.6933594 | -22.9831047 | 0.492 |
| RECEPTOR2/ VEGFR2 | | | | | | | |
| Mol | Pose | Score | Rmsd-refine | E-Conf | E-PLACE | E-REFINE | RMSD |
| Lref3 | 10 | -10.4227104 | 2.93172359 | 35.6387901 | -67.0485001 | -16.9801006 | 0.891 |
| L1 | 4 | -4.71330452 | 1.21814144 | 59.5516663 | -50.5476265 | 3.48999476 | 0.354 |
| L2 | 8 | -5.82724428 | 1.41441953 | 61.5446854 | -58.9625435 | -10.2989044 | 0.326 |
| L3 | 9 | -5.65643024 | 2.08355451 | 50.0475235 | -51.9137001 | -15.2227755 | 0.261 |
| L4 | 5 | -7.39465475 | 1.14210582 | 44.2986488 | -63.8447723 | -14.1900234 | 1.425 |
| L5 | 7 | -4.11897755 | 1.77988875 | 84.4658127 | -46.6783981 | 8.42493057 | 0.232 |
| L6 | 9 | -5.33562517 | 3.33458853 | 42.0842743 | -51.0991707 | -11.4472246 | 0.160 |
| L7 | 10 | -6.07355309 | 1.99330485 | 43.4474983 | -53.7330627 | -7.46748018 | 0.489 |
| L8 | 8 | -5.7500782 | 1.74556887 | 64.2186813 | -48.4922371 | -14.2425623 | 0.360 |
| L9 | 10 | -5.50285721 | 1.36944818 | 45.387619 | -46.9052887 | -14.3059397 | 0.400 |
| L10 | 8 | -5.40128326 | 2.46785975 | 19.8633728 | -55.3035316 | -9.68788242 | 0.261 |
| L11 | 9 | -6.50306749 | 1.21159434 | 37.4934464 | -41.4966698 | -4.83200741 | 0.433 |
| L12 | 7 | -5.50666094 | 1.6465497 | 15.9660406 | -45.2692986 | 15.9660406 | 0.506 |
| L13 | 9 | -5.69354916 | 1.88015425 | 14.4716063 | -55.745636 | -11.8592186 | 0.264 |
| L14 | 6 | -5.68339872 | 2.26530838 | 62.3576508 | -59.3398705 | -16.6589127 | 0.240 |
| L15 | 10 | -5.73431635 | 1.14088261 | 39.3782997 | -51.5355682 | -5.62705517 | 0.429 |
| L16 | 9 | -5.5116353 | 1.2355634 | -55.0190964 | -55.0190964 | -16.762455 | 0.278 |
| L17 | 6 | 1.13416386 | 1.97636449 | 73.7940826 | -59.6460381 | 65.2220993 | 0.434 |
| L18 | 8 | -6.22930002 | 0.602132857 | 43.9919434 | -65.4302063 | 5.66625738 | 0.395 |
| L19 | 8 | -8.10823059 | 1.78886366 | 47.4458733 | -87.4332504 | -21.52174 | 0.557 |

residue of the active site of 2XIR and the Farnesylacetone ligand form a stable complex with a strong interaction.

The second best binder was (*E*)- β -Farnesene (Ligand 4) (Figure 11) with the energy of -7.39465475 Kcal/mol (Table 5), with the existence of four electric force (GLU 917, CYS 919, LEU

840 and ASP 1046). This suggests that (*E*)- β -Farnesene can inhibit VEGF receptors. The ligands that interact with VEGFR-2 were as follows: Ligand 1 interacts with one amino acid PHE 1041 H-pi at a distance of 4.44 Å, low interaction with energy binding of -0.7. Then, Ligand L16 interacts with two amino

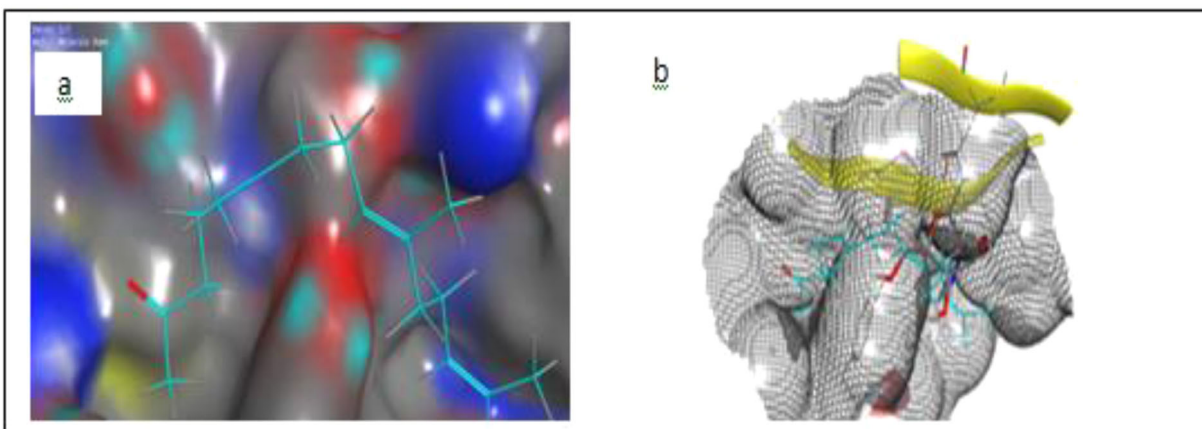


Figure 6. (a) The top scoring compound. (b) A novel inhibitor L-19 identified by molecular docking Farnesylacetone is shown in the active site.

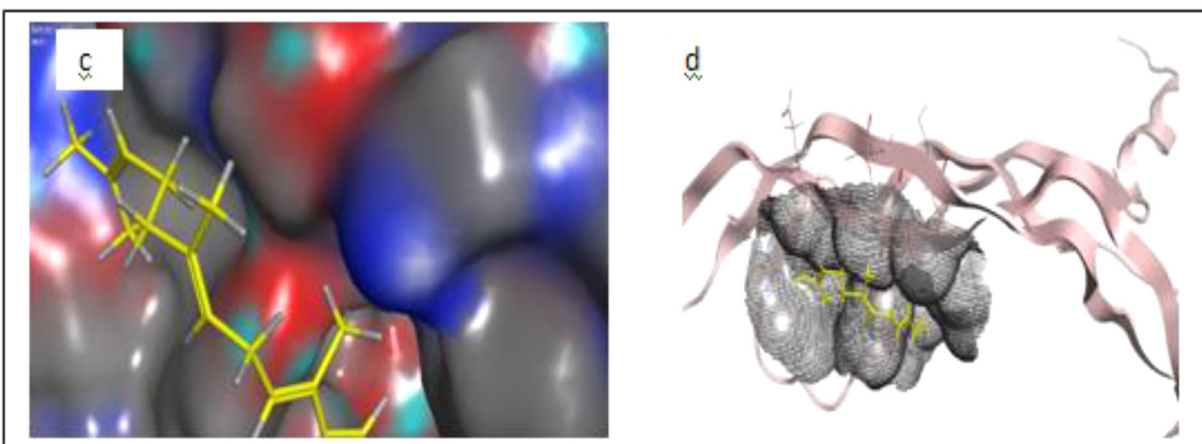


Figure 7. (c) The top scoring compound. (d) A novel inhibitor L-4 identified by molecular docking (E)-β-Farnesene is shown in the active site.

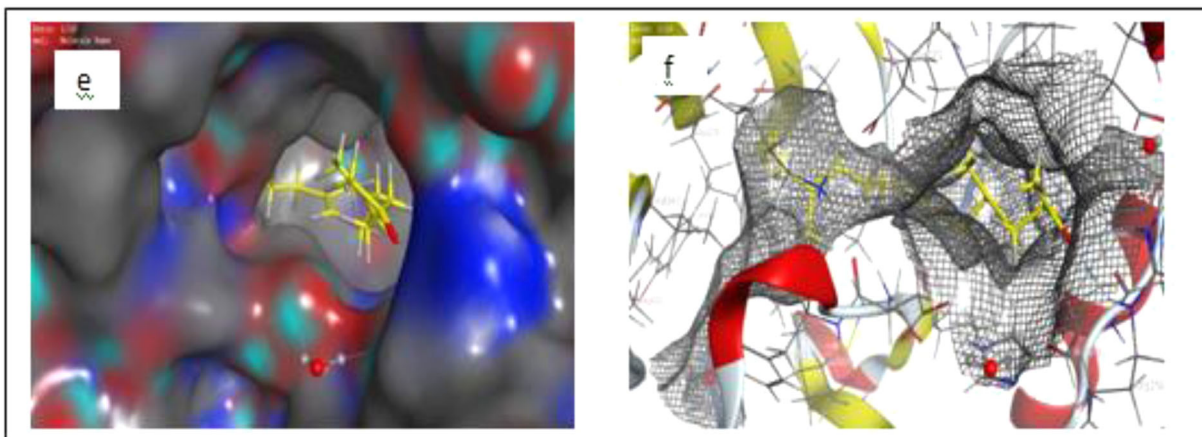


Figure 8. (e) The top scoring compound. (f) A novel inhibitor L-19 identified by molecular docking Farnesylacetone is shown in the active site.

acids HOH 3159 and ASN 923H-donor and H-acceptor, respectively, with energy of 0.5 and -0.5 at distance of 2.51 and 3.26, respectively. Also quote, Ligand L11 defined by strong interaction at distance of 2.95 Å and interaction binding energy of -0.8 with one amino acid ASP 1046 H-donor. Lastly, the Ligand L18 interacts with one amino acid PHE 1047H-pi with energy between receptor and amino acids is -0.6 . Results of 19 compound bonds between atoms of compounds and residues of the active site are given in Table 6.

4.3.2. VEGF-VEGFR interaction

The two VEGF monomers participate in the interaction with the d2 domain of VEGFR1 (Figure 12). The results of docking energies of VEGF/VEGFR inhibitors are shown in Table 7.

Treatments targeting VEGF can have direct effects on the tumor cell (strain). The ligand is designated as the best inhibitor and forms a stable complex. The ligand (E)-Z-Farnesylacetone L19 was able to replace ATP, thereby preventing phosphorylation activity. We can conclude that for

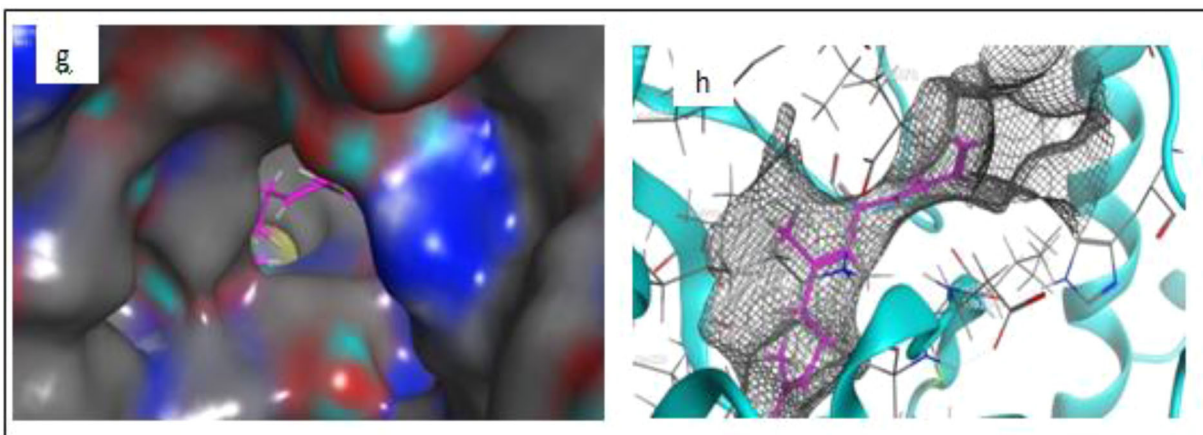


Figure 9. (g) The top scoring compound. (h) A novel inhibitor L-4 identified by molecular docking (*E*- β -Farnesene) is shown in the active site.

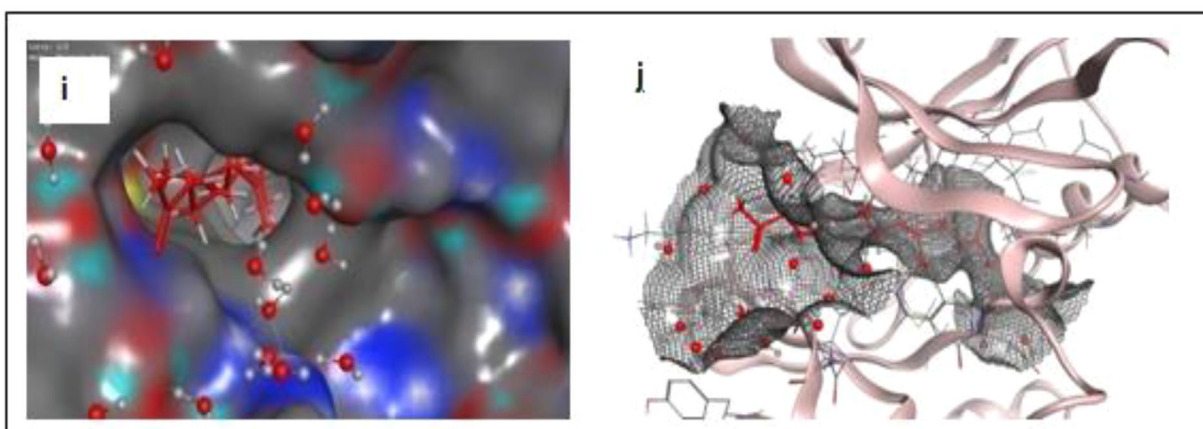


Figure 10. (i) The top scoring compound. (j) A novel inhibitor L-19 identified by molecular docking Farnesylacetone is shown in the active site.

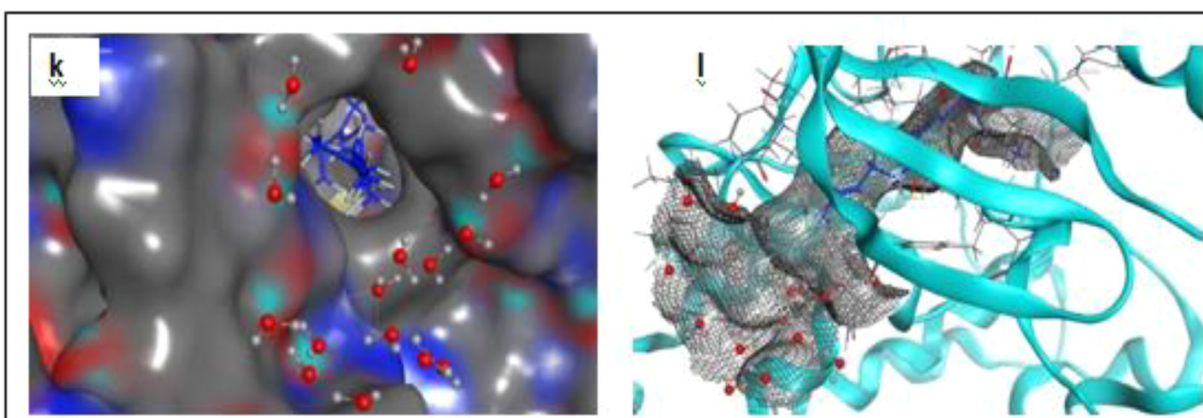


Figure 11. (k) The top scoring compound. (l) A novel inhibitor L4 identified by molecular docking (*E*- β -Farnesene) is shown in the active site.

the ligand L19, the amino acid residues NE and NH₂ at the N-terminal level of the α 1 helix of VEGF were strongly involved in the interaction with the d2 domain of VEGFR1 (see Table 7).

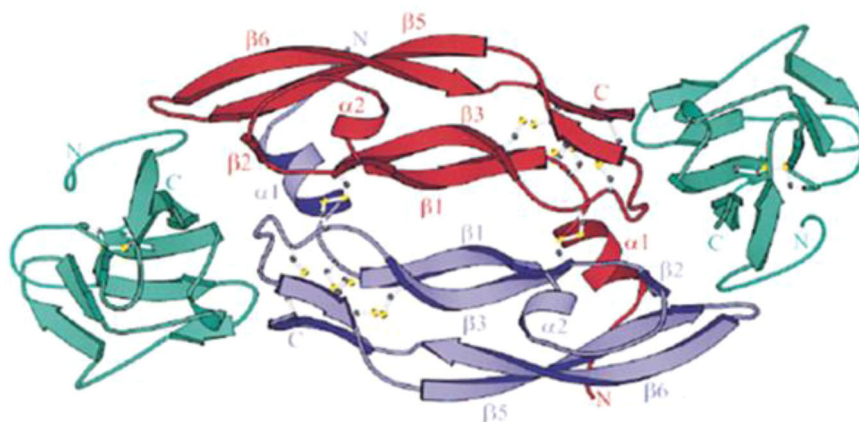
4.4. MD

Using the MD simulation approach, we have studied the evolution thermodynamic properties of the ligand of complex 19 in NVT ensemble (Table 8).

For the ligand L19 in the VEGF enzyme and the VEGFR1 receptor, the kinetic energies of translation and the internal energy were low compared to the VEGFR2 receptors and the fluctuation in pressure for the VEGFR2 receptor was significant. In contrast to the complex formed by L19 for the VEGF enzyme, the VEGFR1 receptor was low. Canonical ensemble (NVT): moles (N), volume (V) and temperature (T) are conserved in simulation by molecular dynamic. Therefore, L19 was predicted to be the most interactive system. These results are in total agreement with the Docking prediction results (see Tables 5–7). We have shown the

Table 6. Results of bonds without water between atoms of best compounds and residues of the active site.

| Compounds | S-score (kcal/mol) | Bonds between atoms of compounds and residues of the active site | | | | | Distances (Å) | Energies (kcal/mol) |
|--------------|--------------------|--|-------------------------|----------------------------|------------------------------------|------------------------------|---------------|---------------------|
| | | Atom of compound | Involved Receptor Atoms | Involved Receptor residues | Type of interaction bond | | | |
| VEGF | | | | | | | | |
| Lref1 | -3.78999758 | O3 21 | ND2 | ASN 75 | H-acceptor | 2.95 | -0.7 | |
| L2 | -3.54622912 | O1 1 | O | GLU 93 GLU 93 | H-donor | 2.51 | 1.2 | |
| | | O1 1 | N | | H-acceptor | 2.82 | -1.0 | |
| L11 | -3.98458982 | O1 1 | O | GLU 38 | H-donor | 2.58 | -1.4 | |
| L12 | -3.59765863 | O1 1 | N | LEU 97 | H-acceptor | 2.96 | -1.3 | |
| VEGFR1 | | | | | | | | |
| Lref2 | -10.2159939 | N9 12 | OE2 | GLU 878 | H-donor H-acceptor H-acceptor pi-H | 2.88 2.79 2.81 3.86 | -4.3 | |
| | | O8 15 | N | ASP 1040 | | | -3.9 | |
| | | N22 36 | N | CYS 912 LYS 861 | | | -4.9 | |
| | | 6-ring | CE | | | | -0.8 | |
| L3 | -5.31909132 | C10 10 | 6-ring | PHE 1041 | H-pi | 4.08 | -0.7 | |
| L14 | -5.34053659 | O1 1 | O | GLU 910 CYS 912 | H-donor | 2.49 | 1.8 | |
| | | | | | H-acceptor | 2.49 | | |
| L19 | -7.96668291 | O1 1 | N | | | | 1.7 | |
| | | O1 1 | NE | ARG 1021 | H-acceptor | 3.00 | -4.5 | |
| | | O1 1 | NH2 | ARG 1021 | H-acceptor | 2.94 | -1.6 | |
| VEGFR2 | | | | | | | | |
| Lref3 | -10.4227104 | N28 3 | OE2 | GLU 885 HOH | H-donor H-donor | 2.74 | -4.2 | |
| | | N29 50 | | 3334 ASP 1046 | | 2.62 | | |
| | | O30 | | | | 2.66 | | |
| | | N27 35 | O | CYS 919 | H-acceptor | 3.24 | -6.1 | |
| | | | N | | H-acceptor | | -1.9 | |
| | | | N | | | | -3.3 | |
| L1 | -4.71330452 | C2 2 | 6-ring | PHE 1047 | H-pi | 4.44 | -0.7 | |
| L16 | -5.5116353 | O1 1 | O | HOH | H-donor | 2.51 | 0.5 | |
| | | | | 3159ASN 923 | H-acceptor | 3.26 | | |
| L11 | -6.22930002 | O1 1 | N | | | | -0.5 | |
| L18 | -6.22930002 | O1 1 | O | ASP 1046 | H-donor | 2.95 | -0.8 | |
| | | C6 6 | 6-ring | PHE 1047 | H-pi | 4.44 | -0.6 | |

**Figure 12.** Structure of the VEGF / VEGFR1-d2 complex determined by X-ray crystallography. The VEGF dimer is represented in red and blue and the two VEGFR1-d2 domains in green (Ma et al., 2019).

detailed analysis of MD simulation results of only compound L19 with target VEGF receptors (Figures 13–15).

4.5. In silico assessment of the ADME

A computational study of two top scoring lead compounds was performed for the assessment of ADME properties and the obtained value is depicted in Table 9.

The results presented in Table 9 revealed that compound L19 has high absorption but compound L4 has low absorption. In addition, we can note that these compounds comply with Lipinski's rule of 5, Veber's rule and Egan's rule (Wiesmann et al., 1997), where $\log P$ values ranged between 4.50 and 4.84 (<5), MW range 204.35 – 262.43 (<500), HBA range 0–0 (≤ 10) and HBD range 0–0 (<5), suggesting that these compounds would

Table 7. The docking energies of VEGF/VEGFR inhibitors.

| Compound | Receptor | DE (kcal/mol) | ETOR (kT) | EVDW(kcal/mol) | EIE (kcal/mol) |
|-----------------------------|----------|---------------|-----------|----------------|----------------|
| Lref1 (Native) | VEGF | -3.78999758 | 446.407 | 1047540 | -1737.84 |
| Lref2 (Native) | VEGFR-1 | -10.2159939 | 1368.097 | 2080.669 | -5387.31 |
| Lref3 (Native) | VEGFR-2 | -10.4227104 | 1390.209 | 3828.302 | -12968.3 |
| cis- α -Bergamotene | VEGF | -3.49325585 | 1335.02 | 3562.36 | -5425.32 |
| | VEGFR-1 | -5.67572975 | 1393.038 | 4147.532 | -7697.59 |
| | VEGFR-2 | -4.71330452 | 1432.478 | 7939.513 | -16668.8 |
| (E)- β -Caryophyllene | VEGF | -3.54622912 | 433.400 | 1139.926 | -2146.11 |
| | VEGFR-1 | -5.79094362 | 1385.513 | 4149.818 | -7881.64 |
| | VEGFR-2 | -5.82724428 | 1410.691 | 7362.473 | -16633.5 |
| β -Copaene | VEGF | -3.38240385 | 427.554 | 1158.022 | -2143.96 |
| | VEGFR-1 | -5.31909132 | 1389.996 | 5010.046 | -7878.19 |
| | VEGFR-2 | -5.65643024 | 1407.077 | 532379.7 | -16614.5 |
| (E)- β -Farnesene | VEGF | -4.01963854 | 414.741 | 2090.109 | -2145.24 |
| | VEGFR-1 | -7.5542974 | 1359.000 | 4145.776 | -7902.41 |
| | VEGFR-2 | -7.3946547 | 1392.285 | 6897.438 | -16714.3 |
| allo-Aromadendrene | VEGF | -3.38378692 | 430.065 | 1145.375 | -2153.00 |
| | VEGFR-1 | -5.51163673 | 1371.339 | 231553.0 | -7920.27 |
| | VEGFR-2 | -4.1189775 | 1443.131 | 6965.071 | -16707.4 |
| Germacrene-D | VEGF | -3.18338823 | 422.577 | 1307.654 | -2170.70 |
| | VEGFR-1 | -5.51596737 | 1351.486 | 876706.2 | -8017.91 |
| | VEGFR-2 | -5.33562517 | 1413.735 | 6276.327 | -16765.2 |
| Zingibrene | VEGF | -3.73947215 | 419.629 | 1557.475 | -2169.54 |
| | VEGFR-1 | -6.2743659 | 1361.151 | 3623.325 | -7950.19 |
| | VEGFR-2 | -6.0735530 | 1418.436 | 6344.492 | -16807.0 |
| Bicyclogermacrene | VEGF | -3.52705669 | 435.838 | 1161.354 | -2167.69 |
| | VEGFR-1 | -4.31459522 | 1425.992 | 4574.672 | -8036.23 |
| | VEGFR-2 | -5.7500782 | 1433.365 | 6378.849 | -16813.3 |
| γ -Cadinene | VEGF | -3.69257712 | 418.943 | 1173.223 | -2176.87 |
| | VEGFR-1 | -5.35120869 | 1346.747 | 3704.720 | -8035.35 |
| | VEGFR-2 | -5.50285721 | 1418.794 | 6395.554 | -16875.6 |
| δ -Cadinene | VEGF | -3.45229697 | 417.317 | 1176.686 | -2181.88 |
| | VEGFR-1 | -5.29777861 | 1361.461 | 3664.779 | -8029.96 |
| | VEGFR-2 | -5.40128326 | 1439.985 | 7144.735 | -16887.5 |
| (E)-Nerolidol | VEGF | -3.98458982 | 422.134 | 1164.999 | -2186.37 |
| | VEGFR-1 | -6.78250837 | 1344.803 | 3657.513 | -8052.21 |
| | VEGFR-2 | -6.5030674 | 1415.265 | 6451.999 | -16925.8 |
| Caryophyllene oxide | VEGF | -3.59765863 | 440.442 | 1168.931 | -2181.25 |
| | VEGFR-1 | -5.14908934 | 1367.995 | 3668.767 | -8032.72 |
| | VEGFR-2 | -5.5066609 | 1432.530 | 6516.935 | -17038.0 |
| Globulol | VEGF | -3.80335522 | 434.962 | 1154.406 | -2180.96 |
| | VEGFR-1 | -4.79661131 | 1372.607 | 3671.807 | -8054.30 |
| | VEGFR-2 | -5.6935491 | 1419.371 | 6517.878 | -17007.6 |
| Ledol | VEGF | -3.75419545 | 447.435 | 1172.155 | -2193.51 |
| | VEGFR-1 | -5.34053659 | 1370.180 | 3672.611 | -8060.27 |
| | VEGFR-2 | -5.68339872 | 1426.335 | 6473.761 | -16958.5 |
| Zingiberol | VEGF | -3.60531759 | 422.277 | 120516.4 | -2201.03 |
| | VEGFR-1 | -5.08465052 | 1355.302 | 3664.348 | -8048.25 |
| | VEGFR-2 | -5.73431635 | 1420.350 | 7133.076 | -17031.6 |
| τ -Muurolol | VEGF | -3.23660111 | 429.610 | 1175.279 | -2180.06 |
| | VEGFR-1 | -5.04594994 | 1364.183 | 3653.479 | -8038.35 |
| | VEGFR-2 | -5.5116353 | 1384.837 | 6176.431 | -16669.6 |
| α -Cadinol | VEGF | -3.55800462 | 428.868 | 1497.248 | -2185.07 |
| | VEGFR-1 | -4.74857521 | 1360.631 | 3667.001 | -8025.35 |
| | VEGFR-2 | -1.13416386 | 1403.009 | 6226.658 | -16646.7 |
| α -Bisabolol | VEGF | -3.71103525 | 424.113 | 1168.157 | -2185.81 |
| | VEGFR-1 | -5.1971302 | 1350.979 | 3664.608 | -8020.31 |
| | VEGFR-2 | -6.22930002 | 1389.838 | 6244.659 | -16740.6 |
| cis- α -Bergamotene | VEGF | -4.52469969 | 413.838 | 1568.082 | -2164.04 |
| | VEGFR-1 | -7.96668291 | 1353.882 | 4156.540 | -8033.24 |
| | VEGFR-2 | -8.10823059 | 1386.549 | 6252.439 | -16605.2 |

DE: docking energy; ETOR: torsion energy; VDW: Van der Waals; EIE: electrostatic interaction energy.

not be expected to cause problems with oral bioavailability and thus showing possible utility of both compounds for developing the compound with good drug-like properties and in the meantime, we propose Ligand L19 Farnesylacetone present in essential oils of the aerial parts of *I. viscosa* with its proven activity score (-4.52469969, -7.96668291, -8.10823059), respectively, for VEGF, VEGFR-1, VEGFR-2 as a *new oral ligand* despite obeying Lipinski's rule.

4.6. Pharmacokinetics and medicinal chemistry properties

The results of Medicinal Chemistry and Pharmacokinetics showed that compound L19 has high GI absorptions but compound L4 has low GI absorptions. We notice that there is a correlation between our results for assessment of ADME properties (Table 9) and the predicted results in medicinal chemistry and pharmacokinetics (Table 10).

Table 8. Thermodynamic properties calculated in real units. Pressure $P = P^* \epsilon/\sigma^{-3}$, energy of configuration $U = U^* N\epsilon$, translation kinetic energy $EKT = EKT^* N\epsilon$ and enthalpy $H = H^* N\epsilon$.

| SP _i | Method | H | U | EKT | P | V | T |
|-----------------|-----------------|--------------|--------------|------------|-------------|-------------|------------|
| SP ₁ | VEGF-Lig-19 | -96.0353775 | 1507.44727 | 1388.18652 | 160.447647 | 12775.3398 | 357.959808 |
| | VEGR1-Lig-19 | -292.459259 | 3246.96533 | 4352.19336 | -36.4663124 | 37559.7031 | 370.825592 |
| | VEGR2-Lig-19 | -346.652924 | -1199.86816 | 4999.32471 | -55.1730194 | 44492.7852 | 363.23175 |
| | VEGR-Lig-19 | -0.186085999 | 937.379517 | 1097.74744 | -40.0676231 | 12775.3398 | 283.066742 |
| | VEGR1-Lig-19 | -7.79605532 | 2488.10181 | 4052.91699 | 183.276642 | 37559.7031 | 345.325989 |
| | VEGR2-Lig-19 | -0.443735003 | -2920.92236 | 4110.30371 | -254.836838 | 44492.7852 | 298.638916 |
| | VEGR-Lig-19 | 0.175413504 | 959.181213 | 1135.01062 | -58.5749931 | 12775.3398 | 292.675476 |
| | VEGR1-Lig-19 | 0.186976507 | 1493.63403 | 3423.13843 | 93.5603485 | 37559.7031 | 291.666138 |
| | VEGR2-Lig-19 | 1.34591353 | -3326.87671 | 4023.22656 | 86.2301178 | 44492.7852 | 292.312225 |
| | SP ₂ | VEGR-Lig-19 | 0.323196739 | 926.048157 | 1186.59265 | 167.378677 | 12775.3398 |
| VEGR1-Lig-19 | | 0.186976507 | 1493.63403 | 3423.13843 | 93.5603485 | 37559.7031 | 291.666138 |
| VEGR2-Lig-19 | | 1.34591353 | -3326.87671 | 4023.22656 | 86.2301178 | 44492.7852 | 292.312225 |
| VEGR-Lig-19 | | -0.609911978 | 803.180115 | 1162.90198 | -276.769501 | 12775.3398 | 299.867584 |
| VEGR1-Lig-19 | | -0.533955097 | -0.805478334 | 3475.16797 | 39.4337997 | 37559.7031 | 296.099274 |
| VEGR2-Lig-19 | | -0.431310326 | -3345.53491 | 4122.03809 | 43.8750153 | 44492.7852 | 299.491516 |
| VEGR-Lig-19 | | 0.588058352 | 808.85286 | 1155.84644 | 132.805405 | 37559.7031 | 298.048248 |
| VEGR1-Lig-19 | | -0.527443051 | 1390.62939 | 3412.94312 | -188.243103 | 37559.7031 | 290.797455 |
| VEGR2-Lig-19 | | 1.69389367 | -3485.01563 | 4041.8418 | -1.55086923 | 44492.7852 | 293.664734 |
| SP ₃ | | VEGR-Lig-19 | -0.239414528 | 832.668152 | 1137.39722 | -119.197212 | 12775.3398 |
| | VEGR1-Lig-19 | 1.1400882 | 1405.06104 | 3494.96069 | 41.3016739 | 37559.7031 | 297.785706 |
| | VEGR2-Lig-19 | 1.69389367 | -3485.01563 | 4041.8418 | -1.55086923 | 44492.7852 | 293.664734 |
| | VEGR-Lig-19 | 0.697540104 | 853.860718 | 1097.72119 | 168.965363 | 12775.3398 | 283.059998 |
| | VEGR1-Lig-19 | 1.57997549 | 1337.56262 | 3379.97607 | 117.144455 | 37559.7031 | 287.988525 |
| | VEGR2-Lig-19 | -1.35737085 | -3514.72388 | 4062.38794 | -46.1831398 | 44492.7852 | 295.157532 |
| | VEGR-Lig-19 | -0.016821704 | 1133.17383 | 1133.17383 | 124.116997 | 12775.3398 | 292.201874 |
| | VEGR1-Lig-19 | 3.1954596 | 1367.98035 | 3419.4978 | -95.8944092 | 37559.7031 | 291.355927 |
| | VEGR2-Lig-19 | 1.8799262 | -3460.46631 | 3998.78369 | -149.61528 | 44492.7852 | 290.536285 |

Table 9. ADME properties for two top scoring lead compounds.

| Entry | ABS | TPSA (Å ²) | n-ROTB | MW | MLog P | n-ON acceptors | n-OHND donors | Lipinski's violations | Veber violations | Egan violations |
|-------|------|------------------------|--------|--------|--------|----------------|---------------|-----------------------|------------------|-----------------|
| Rule | - | - | - | <500 | ≤5 | <10 | <5 | ≤1 | ≤1 | ≤1 |
| L4 | Low | 00.00 | 6 | 204.35 | 4.84 | 0 | 0 | 1 | 1 | 1 |
| L19 | High | 17.07 | 9 | 262.43 | 4.50 | 1 | 0 | 1 | 1 | 1 |

ABS: absorption, TPSA: topological polar surface area, n-ROTB: number of rotatable bonds, MW: molecular weight, MLogP: logarithm of partition coefficient of compound between n-octanol and water, n-ON acceptors: number of hydrogen bond acceptors, n-OHND donors: number of hydrogen bonds donors.

Table 10. Pharmacokinetics and medicinal chemistry properties for molecule scoring lead compounds.

| N | Compounds | Pharmacokinetics | | Medicinal chemistry | | |
|----|-----------------------------|------------------|--------------------------------------|--|--|-------------------------|
| | | GI absorption | Log K _p (skin permeation) | Lipophilicity Log P _{o/w} (MLOGP) | Lead-likeness | Synthetic accessibility |
| 1 | cis- α -Bergamotene | Low | -2.97 cm/s | 4.63 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 5.07 |
| 2 | (E)- β -Caryophyllene | High | 5.53 cm/s | 3.56 | No; 1 violation: MW < 250 | 4.48 |
| 3 | β -Copaene | Low | 5.65 cm/s | -4.37 cm/s | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 4.62 |
| 4 | (E)- β -Farnesene | Low | -3.20 cm/s | 4.84 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 3.72 |
| 5 | allo-Aromadendrene | Low | -4.20 cm/s | 5.65 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 3.70 |
| 6 | Germacrene-D | Low | -4.18 cm/s | 4.53 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 4.55 |
| 7 | Zingibrene | Low | -3.88 cm/s | 4.53 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 4.81 |
| 8 | Bicyclogermacrene | Low | -4.61 cm/s | 4.63 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 4.34 |
| 9 | γ -Cadinene | Low | -4.85 cm/s | 4.63 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 4.14 |
| 10 | δ -Cadinene | Low | -4.49 cm/s | 4.63 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 4.35 |
| 11 | (E)-Nerolidol | High | -4.23 cm/s | 3.86 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 3.53 |
| 12 | Caryophyllene oxide | High | -5.12 cm/s | 3.67 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 4.35 |
| 13 | Globulol | High | -5.00 cm/s | 3.81 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 3.58 |
| 14 | Ledol | High | -5.00 cm/s | 3.81 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 3.58 |
| 15 | Zingibereol | High | -4.63 cm/s | 3.56 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 4.15 |
| 16 | τ -Muurolool | High | -5.29 cm/s | 3.67 | No; 1 violation: MW < 250 | 4.29 |
| 17 | α -Cadinol | High | -5.29 cm/s | 3.67 | No; 1 violation: MW < 250 | 4.29 |
| 18 | α -Bisabolol | High | -4.97 cm/s | 3.56 | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 3.95 |
| 19 | (E)-Z-Farnesylacetone | High | -3.95 cm/s | 4.50 | No; 2 violations: Rotors > 7, XLOGP3 > 3.5 MW < 350 | 3.47 |

(E)-Z-Farnesylacetone essential oils of the aerial parts of *I. viscosa* (oxygenated sesquiterpenes) (Ligand 19) was predicted to be characterized by a high lipophilicity and high coefficient of skin permeability log K_p by providing (E)-

β -Farnesene (Ligand 4). We can resolve that the more negative the log K_p (with K_p in cm/s), the less the molecule is absorptive to the skin (Kacprzyk & Pedrycz, 2015), which explains the reliability of our results. We cite the works that

Table 11. Energy balance of complexes formed with VEGF under other experiments and our results for essential oils of *I. viscosa*.

| Majority Molecule | Score | Reference |
|--|----------------------------|--|
| VEGF was received in the PDB database https://www.rcsb.org/ PDB ID: 5t89 | | |
| Lref (Native) | -3.78999758 | |
| Other experiments | | |
| (E)-nerolidol(19.8%) Jordan | -3.98458982 | (Al-Qudah et al., 2010; Parikesit et al., 2015). |
| L-Bornéol (25.2%) Bornylacetate(19.5%) Turkey | -3.23326421 -3.47815251 | (Berendsen et al., 1984; Pérez-Alonso & Velasco-Negueruela, 1996). |
| Fokienol (21.1% et 38.8%, respectively) France and Spain | -4.01069689 | (Blanc et al., 2006; Camacho et al., 2000; Parikesit et al., 2015; Pérez-Alonso & Velasco-Negueruela, 1996). |
| Acid Eudesma-3,11 (13) -dien-12-oïque (56.8%) and (62.4%, respectively) Eastern Algeria and southern Italy | -3.50546718 | (Al-Qudah et al., 2010; Blanc et al., 2006; De Laurentis et al., 2002; Haoui et al., 2015). |
| δ -terpinene(35.9%) and α -pinene (18.9%) Sidi Bel Abbes (Algeria) | -3.20504832 -3.30435085 | (Benchohra et al., 2011). |
| Acide isocostique (70.8%) Tunisian | -3.62129688 | (Aissa et al., 2019; Benchohra et al., 2011). |
| isobutyrate de 3-méthoxy cuminyle(12%) Portugal | -4.26027393 | (Mesli et al., 2019; Miguel et al., 2008). |
| Our Results | | |
| (E)-Z-Farnesylacetone L19 (13.2%) Algeria | -4.52469969 | (Oxygenated sesquiterpenes) |
| (E)- β -Farnesene L4 (2.6%) Algeria | -4.01963854 | (Hydrocarbonsesquiterpene) |

have proved the stability of complexes and their affinities by MOE software (Mesli et al., 2019; Mesli & Bouchentouf, 2018). $\text{Log } P_{o/w}L19 > \text{Log } P_{o/w}L4 > \text{Log } P_{o/w}L11$.

So Ligand L19 represents high affinity with VEGF receptors. Synthetic accessibility (SA) was a major factor to take into account in this selection process an acceptable value between 3.27 and 3.47 for the ligands L19 and L4, respectively, and these are more promising molecules that can be synthesized or subjected to bioassays or other experiments. Our previous research has shown that oils from our region have better biological activities (Benyoucef et al., 2020; Miguel et al., 2008). Validation of our results, for essential oils of *I. viscosa*, in different region is mentioned in Table 11.

Our molecular docking results coincide with our experimental results; the oxygenated sesquiterpenes were the most dominant with a percentage of 87.3%.

Our ligand (E) -Z-Farnesylacetone (13.2%) better stabilizes the system with its energy of -4.52469969 Kcal/mol we compare with the components of other regions of the world (see Table 11). The latter allows good stabilization and complementarity of the complex. It is validated as a major ligand against cell cancer. The present molecular docking analysis MD simulations used to investigate new oxygenated sesquiterpene compound inhibitor of VEGF receptors. Previous studies have shown that (2Z,6E)-farnesol exhibited cytotoxic activity against human colon, liver and lung cancer cells (Cavalieri et al., 2004).

The ligands (E)-Z-Farnesylacetone inhibitor 19 and (E)- β -Farnesene (Ligand 4) we found are from the same family as (2Z,6E)-farnesol. The latter has good affinities to the VEGF receptors, which brings us back to the conclusion that the family oxygenated sesquiterpene was effective VEGF anti-angiogenic drugs.

In vitro, many studies were focused on the inhibitory effect of *I. viscosa* and nanobodies, on key enzymes linked to cancer therapy, VEGF receptors. Anti-VEGF NB strongly inhibits the migration of human endothelial cells ($p=0.045$) (Kazemi-Lomedasht et al., 2017). Anti-VEGF NB significantly inhibits tumor growth in tumor-bearing mice ($p=0.001$). Results indicate that NBs that are a novel class of antibodies derived from the camel can develop as a promising candidate for cancer drugs. The cross-reactive cross-linked NB showed high specificity and binding affinity in the nanomolar range for both human and mouse VEGF. In the case of anticancer activity, the American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer products for future bioguided studies if IC_{50} value is lower than $30 \mu\text{g/mL}$ (Seca et al., 2014). According to Merghoub et al. (2009), the IC_{50} value greater than $54 \mu\text{g/mL}$ 'identifies a tumor effect'. For the same anticancer activity, IC_{50} values greater than $200 \mu\text{g/mL}$ (Mazzio & Soliman, 2009) are unacceptable. Talib and Mahasneh (2010) and Ferrara et al. (2004) found that *I. viscosa* flower extracts present low toxicity toward normal human cells (Vero cell line IC_{50} $202.43 \pm 73.70 \mu\text{g/mL}$). For *Inulaviscosa*, the IC_{50} values

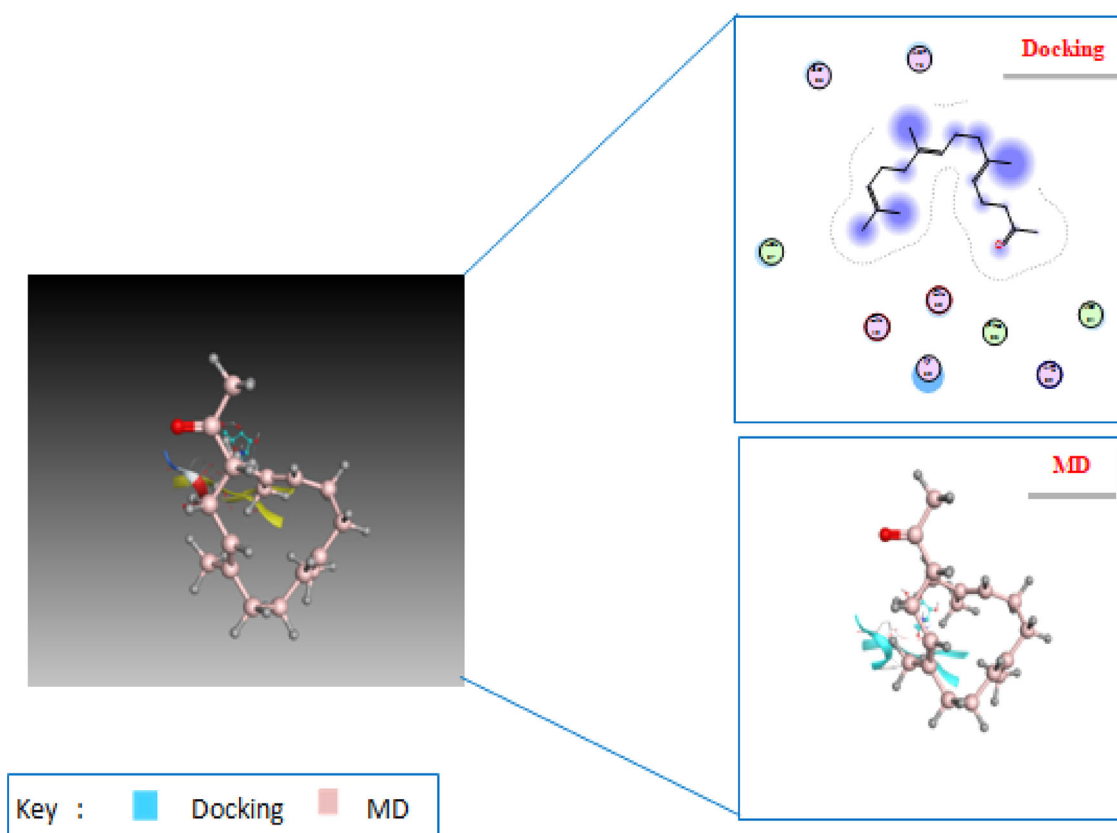


Figure 13. The compound – 19 Farnesylacetone is docked without water well into the binding site of VEGF and has the highest dock score; there is also a clear difference between the final ligand pose and the docking pose after a molecular dynamics (MD) simulation.

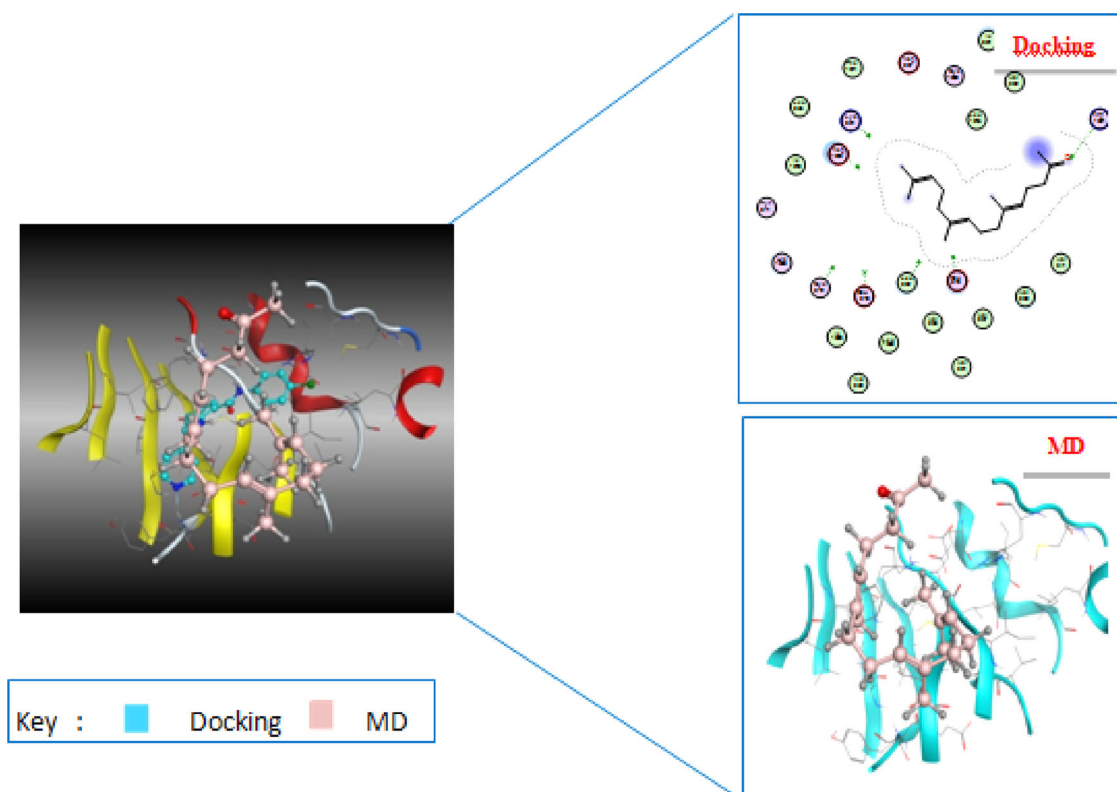


Figure 14. The compound – 19 Farnesylacetone is docked without water well into the binding site of VEGFR-1 and has the highest dock score; there is also a clear difference between the final ligand pose and the docking pose after a molecular dynamics (MD) simulation.

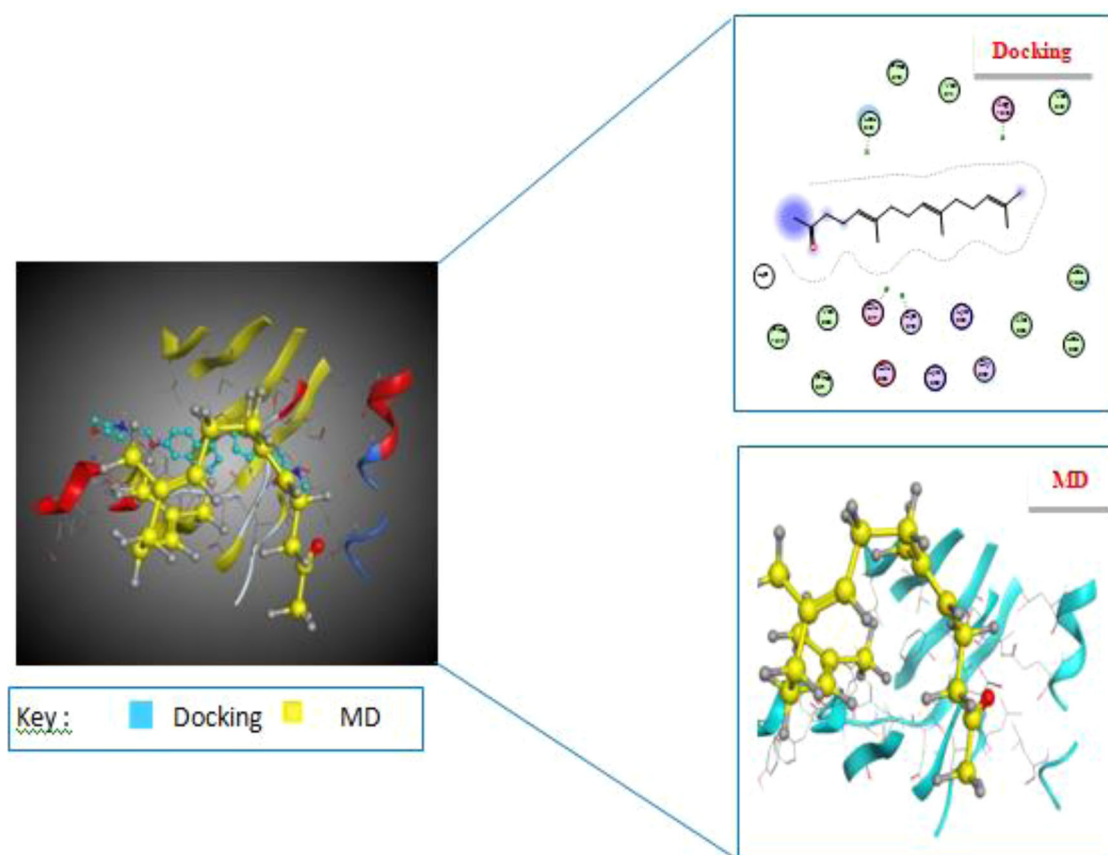


Figure 15. The compound – 19 Farnesylacetone is docked without water well into the binding site of VEGF-2 and has the highest dock score; there is also a clear difference between the final ligand pose and the docking pose after a molecular dynamics (MD) simulation.

recorded were in most cases 30 $\mu\text{g}/\text{mL}$. In addition, this sesquiterpene lactone from *Inulaviscosa* has anti-inflammatory activity according to several researchers (Hernández et al., 2001; Máñez et al., 2007;). In our case, the software package (MOE) does not identify any trace of the hydrophobic interactions between (*E*-Z-Farnesylacetone and both the VEGF receptors, which may be related to the large size of this ligand and the high number of torsion angles (flexibility). The results are identified to have inhibitory activities against novel VEGF receptors. Of these compounds, (*E*-Z-Farnesylacetone has a stronger bond and high affinity with VEGF. Therefore, the results obtained in this research honor ancestral know-how and provide real scientific support for the use of these plants by herbalists and traditional healers, while offering an imminent starting point for several studies to come.

5. Conclusion

The essential oil yield of *I. viscosa* showed a significant variability. Results showed the positive correlations between essential oil oxygenated sesquiterpene components and geographical locations. These compounds have been widely studied as VEGF inhibitors, which is of potential alternative drugs for the treatment of cancerous cells. Molecular docking used to study interaction between new compounds and VEGF receptors with score energy investigation and druglikeness properties experiments, ADME/T tests, Molecular

dynamics simulation have been performed to verify in silico the drug properties of the top ligand (of essential oils of the aerial parts of the *I. viscosa*). The best ligand (*E*-Z-Farnesylacetone which is the major component, in of essential oils of the aerial parts of the *I. viscosa*) has high binding affinity (Score) and good substitution for ATP, thus preventing phosphorylation activity. The natural inhibitor – (*E*-Z-Farnesylacetone – established different interactions between H- π and H-acceptor with key residues for active site of targets. These results allow us to propose (*E*-Z-Farnesylacetone natural and reliable treatment during the first stage of cancerous cells. Further *in vivo* and clinical studies regarding oxygenated sesquiterpenes to use as a useful supplementary agent in the pre-treatment of cancer are highly recommended.

Disclosure statement

The authors declare no conflict of interest.

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Statistical analysis/theoretical investigations of novel vascular endothelial growth factor of Davanoide from *Scolymus grandifloras* Desf as potent anti-angiogenic drug properties

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ABSTRACT

Many pro-angiogenic factors acting directly or indirectly on the proliferation and differentiation of endothelial cells have been highlighted, in particular: VEGF ('Vascular Endothelial Growth Factor'), FGF ('Fibroblast Growth Factor'), PDGF ('Platelet-Derived Growth Factor'), VEGF exerts its pro-angiogenic activity by binding to the surface of receptors with tyrosine kinase activity (VEGFR). The first objective of this study was to elucidate the composition of the essential oil of the roots of *Scolymus grandifloras* Desf. The second aim was to describe the intra-species variation in essential oil composition in natural populations of 21 oil samples from different Algerian locations using statistical analysis and bioinformatical study of VEGFR inhibition. The essential oil isolated from the root parts, was a really source of Davanoide compounds. The results of the docking simulation revealed that davanone (Ligand 13) has an affinity to interact with cDNA, VEGF and its receptors. The ADMET properties and BOILED-Egg plot validate the compound 13 pass the brain barrier and have high absorption in the intestines with good bioavailability. The findings of this study contribute to the pharmacological knowledge and the therapeutic efficacy of davanone and can initiate the development of new anti-angiogenic drugs. Results showed that essential oil of *Scolymus grandiflorus* presented a large level of percentage of davanone, davanol D1 and 2-hydroxy davanone. These components may be a new source of nontoxic anticancer agents. However, an additional *in vitro* and/or *in vivo* experimental study should make it possible to verify the theoretical results obtained *in silico*.

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1. Introduction

Cancer has been a disease described since Antiquity. Biologically, cancer results from the occurrence of a dysfunction in certain cells of the body. These start to multiply in an anarchic way and to proliferate, first locally, then in the surrounding tissue, then, at a distance where they form metastases. For decades, scientists could not detect a safe way (or drug) to treat cancer and its connected complications (Chakraborty & Rahman, 2012; Mehrabi et al., 2017). Consequently, try to find or search for new effective anti-cancer agents is a critical strategy in any cancer therapy program. Cancer is never the result of a single cause, but there are a number of factors, external and internal, have been identified. External factors are linked to the environment (radiation, viruses, industrial products, etc.) or lifestyle (tobacco, alcohol, food, etc.). Internal factors are linked to age and inheritance. Without forgetting the genetic predisposition to cancer (special case of inherited mutations) the best-known concern the BRCA1 and BRCA2 genes; they

generate a significant risk of breast and ovarian cancer. Medicinal plants possess a characteristic that helps in food security and treating diseases by improving people's health conditions. These plants produce substances that are capable to altering the synthetic compounds already existed, so they become an excellent source to research a new compound for therapeutic use. With an area of over 2.3 million km², Algeria is the largest country in the Mediterranean basin. It presents a great plant biodiversity which amounts to more than 1600 species. The genus *Scolymus* belonging to Asteraceae family including three species: *Scolymus hispanicus* L., *Scolymus maculatus* L. and *Scolymus grandifloras* Desf (Quezel et al., 1962). In Sicily, these wild species are commonly consumed in salads (Guarrera & Savo, 2016), they are distributed in the Mediterranean, Macaronesia and Near-Eastern zones (Vázquez, 2000). To our knowledge, there are no chemical studies on the species *S. grandiflorus*. The most studied species belonging to this genus is *S. hispanicus* commonly called 'Golden thistle', locally called 'Ghernina'. This

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| Codes | Stations | Latitude | Longitude | Altitudes |
|-------|------------------|------------|-----------|-----------|
| Y1 | El Ourit | 34°52'41"N | 1°15'05"W | 818 m |
| Y2 | Sebdou | 34°42'05"N | 1°18'46"W | 1100 m |
| Y3 | Ain fezza centre | 34°51'53"N | 1°13'22"W | 900 m |
| Y4 | Temi ben hadiel | 34°48'24"N | 1°23'21"W | 1163 m |
| Y5 | Maaziz | 34°56'24"N | 1°48'14"W | 880 m |
| Y6 | Oum el' Alou | 34°55'05"N | 1°11'24"W | 858 m |
| Y7 | Tizi | 34°54'24"N | 1°10'00"W | 800 m |
| Y8 | Sabra | 34°48'57"N | 1°31'44"W | 610 m |
| Y9 | Ouled Mimoun | 34°55'16"N | 1°03'30"W | 620 m |
| Y10 | Beni snous | 34°40'56"N | 1°30'34"W | 680 m |
| Y11 | Bouhlou | 34°47'36"N | 1°35'02"W | 531 m |
| Y12 | Azayza | 34°59'08"N | 1°10'41"W | 600 m |
| Y13 | Maghnia 2 | 34°54'04"N | 1°44'39"W | 440 m |
| Y14 | Sidi Abdeli | 35°03'11"N | 1°08'59"W | 510 m |
| Y15 | Ain El Hout | 34°57'13"N | 1°19'15"W | 360 m |
| Y16 | Sidi Medjahed | 34°46'48"N | 1°40'04"W | 434 m |
| Y17 | Bensekrane | 35°03'58"N | 1°13'45"W | 300 m |
| Y18 | Fellaoucen | 35°03'47"N | 1°32'51"W | 170 m |
| Y19 | Zenata | 35°01'14"N | 1°27'32"W | 275 m |
| Y20 | Maghnia | 34°49'33"N | 1°41'22"W | 380 m |
| Y21 | Nedroma | 35°03'10"N | 1°45'29"W | 185 m |

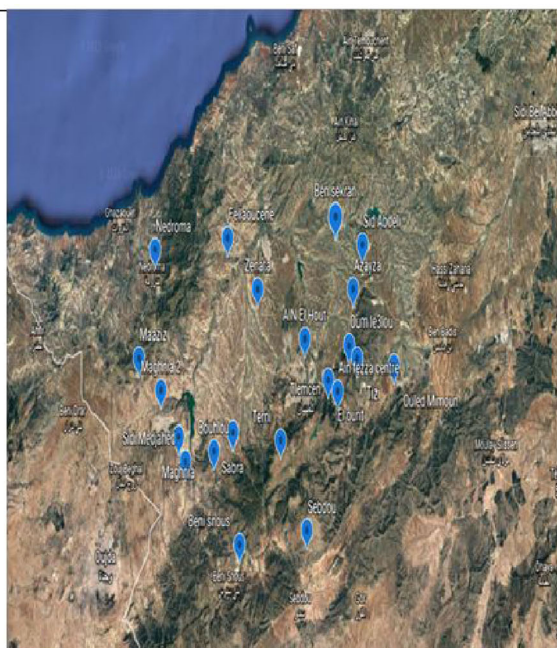


Figure 1. Geographical distribution of *S. grandiflorus* from western Algeria.

species is one of the most appreciated-consumed wild vegetables in Mediterranean countries, recognized for their medicinal properties such as diuretic, depurative, digestive, choleric and lithiuretic (Polo et al., 2009).

Our study was about *S. grandiflorus* originally of north-Africa (Martin & Ruperté, 1979) described for the first time by Desfontaines (1800). Several new molecules, targeting cell proliferation and/or angiogenesis have been recently tested, whose modest effectiveness nevertheless allows us to foresee a new global approach to this pathology. Indeed, these new treatments go beyond the classical chemo- or radio-therapeutic perspective of blocking cell replication at the level of DNA and its machinery, by targeting intracellular signaling mechanisms, intercellular paracrine connections or even the tumor micro-environment. The main interest was to develop unique potential inhibitors of the VEGF (Vascular Endothelial Growth Factor)/VEGFR interaction, the earlier a cancer is treated, the less the treatments are heavy and the better the chances of cure. The previous works of Ravi and Krishnan (2016) proved that *N*-hexadecanoic acid extracted from *Kigelia pinnata* leaves has high affinity interaction with DNA topoisomerase-I and the research of Hosseinzadeh et al. (2019) proved that hydroperoxide of davanone a potential antitumor agent.

Furthermore, modeling and simulation have become standard practices in many scientific and technical fields and in particular in Chemistry. They are often necessary when the real experience is too difficult, too dangerous and too expensive. Digital chemistry subsidizes a better understanding of the action of medicinal plants against diseases and offers high-level training, focused on the study of living things at the molecular level. In addition, represents a bridge between theory and experience. It makes it possible to represent, interpret and predict biomolecular structures and functions (Mesli et al., 2019). However, there are no reports on

chemical composition and biological activities of *S. grandiflorus*. Therefore, this work was aimed to study for the first time, the chemical composition from root parts and the intraspecies variations of essential oils from 21 locations using statistical analysis and the second study was to try to tested the molecules of this oil for their anticancer activity by interested at the interaction between VEGF and its receptors (VEGFR). Knowing that VEGF seems to be one of the main players in tumor angiogenesis. It exerts its pro-angiogenic activity by binding to the surface of receptors with tyrosine kinase activity (Walker, 1996). The essential oils of *S. grandiflorus* roots inhibitors were the subject of our investigation. In order to block tumor growth and we target receptors (VEGFR). These receptors have different affinities for VEGF and induce different cellular and biological effects. The inhibition of VEGF receptors was theoretically investigated by two methods of computational chemistry: molecular docking analyzes and molecular dynamics (MD) simulations. In this contribution, a combined of three theoretical approaches by using drug likeness, pharmacokinetics, medicinal Chemistry and ADME Properties to explore potential inhibitors among compounds of essential oils of *S. grandiflorus* roots against three enzymes: VEGF, VEGFR1 and VEGFR2.

2. Material and methods

2.1. Plant material and isolation of the essential oil

Roots of *S. grandiflorus* were collected at the flowering stage in May 2018 from 21 locations (Y1–Y21) widespread in two areas of western Algeria (Tlemcen), (Y1–Y7) from Tell mountain and (Y8–Y21) from littoral (Figure 1).

The plant material was botanically identified by Prof. Noury Benabadji (Laboratory of Ecology and Ecosystem Management of University of Tlemcen Algeria) (Mejdoub et al., 2020). Voucher specimens were installed with the Herbarium of the University of

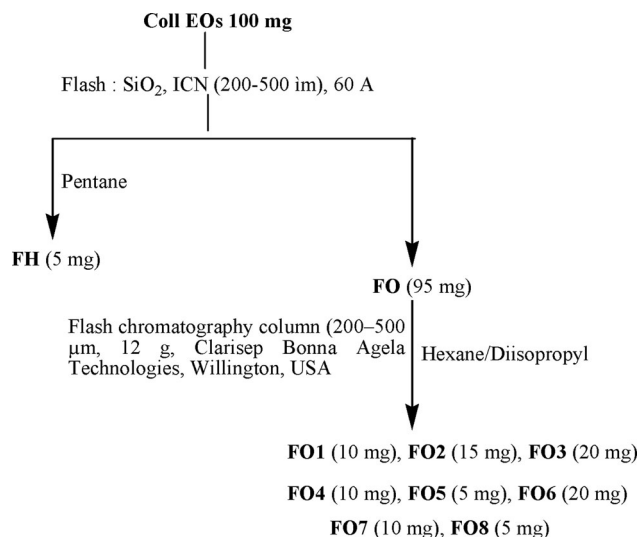


Figure 2. Fractionation of Coll EOs from roots *S. grandiflorus*.

Tlemcen. The root parts were air-dried at room temperature. The plant material from each population was submitted to hydro-distillation for 5 h using a Clevenger apparatus according to the procedure described in the European Pharmacopeia (Conseil de l'Europe, 1996). The isolated essential oils were dried over anhydrous sodium sulfate Na_2SO_4 , filtered and then the essential oil mass was determined.

2.2. Fractionation of collective essential oil

One (1) g of Coll EOs was subjected to fractionation flash chromatography (SiO_2 , ICN 200–500 mm, 60 A). By elution with pentane, an FH fraction containing the hydrocarbon compounds (5 mg) was obtained; elution with diethyl ether then leads to an FO fraction containing the oxygenated compounds (95 mg). The oxygenated fraction (95 mg) were submitted to fractionation on silica flash chromatography column (200–500 μm , 12 g, ClarisepBonna-Agela Technologies, Wellington, USA) using an Automated Combi Flash apparatus (Teledyne ISCO, Lincoln, USA), equipped with automatic fraction collector monitored by an UV detector, eluted with a gradient of hexane (A) and di-isopropyl ether (B) from: (A: 100%; B: 0%) to (A: 0%; B: 100%). eight fractions were obtained and submitted to GC–FID, GC–MS and nuclear magnetic resonance (NMR) analyses. The fractionation is shown schematically in (Figure 2).

FO1 (5,5-Dimethyl-2(5H)-furanone):

^1H NMR (400 MHz, CDCl_3) δ 1.49 (6H, s), 5.98–5.99 (1H, d, $J = 5.6$ Hz), 7.39–7.40 (1H, d, $J = 5.6$ Hz).

^{13}C NMR (101 MHz, CDCl_3) δ 25.4, 86.6, 119.9, 161.2, 172.5.

HRMS calcd for $\text{C}_6\text{H}_8\text{O}_2$:112.13, found: 112; LRMS (EI +) m/z ; 97 (100), 69 (80),43 (80), 26 (30).

FO3 (Davana furan):

^1H NMR (400 MHz, CDCl_3) δ 1.20 – 1.21 (d, 3H), 1.29 (s, 3H), 1.75 (m, 2H), 1.87 (m, 2H), 2.25(s, 3H), 3.02 (m, $J = 7.3$, 6.9 Hz, 1H), 4.29–4.20 (q, 1H), 4.97 (dd, $J = 10.7$, 6.8, 1.6 Hz, 1H), 5.22–5.17 (dd, 1H), 5.88 (s, 1H), 5.90 (s, 1H), 5.99–5.92 (m, 3H).

^{13}C NMR (101 MHz, CDCl_3) δ 13.6, 14.2, 26.0, 28.1, 37.8, 37.9, 81.2, 82.7, 105.6, 105.8, 111.4, 144.5, 150.2, 156.1.

HRMS calcd for $\text{C}_{14}\text{H}_{20}\text{O}_2 \sim 220.1478$, found: 220; LRMS (EI +) m/z ; 220(20), 135(20), 111(30), 109(100), 93(30), 55(30), 43(40).

FO5 (Davanone):

^1H NMR (CDCl_3 , 400 MHz) δ 0.96–1.04 (3H, d, $J = 6.9$ Hz), 1.23–1.28 (3H, s), 1.52–1.65 (1H, m), 1.62 (3H, s), 1.63 (3H, s, $J = 3.8$ Hz), 1.68–1.80 (1H, m), 1.84–1.95 (1H, m), 1.95–2.07 (1H, dddd, $J = 3.6$, 5.8, 7.2, 11.9 Hz), 2.65–2.76 (1H, dq, $J = 6.9$, 8.8 Hz), 3.15–3.37 (2H, m), 4.04–4.14 (1H, td, $J = 5.8$, 8.7 Hz), 4.94–5.03 (1H, dd, $J = 1.7$, 10.7 Hz), 5.15–5.26 (1H, dd, $J = 1.7$, 17.2 Hz), 5.29–5.39 (1H, tdq, $J = 1.5$, 2.9, 7.2 Hz), 5.84–5.96 (1H, dd, $J = 10.7$, 17.3 Hz)

^{13}C NMR (101 MHz, CDCl_3) δ 13.2, 18.1, 25.7, 26.6, 29.9, 37.6, 42.7, 51.3, 81.0, 83.0, 111.4, 116.1, 135.4, 144.7, 212.1.

HRMS calcd for $\text{C}_{15}\text{H}_{24}\text{O}_2 \sim 236.18$, found: 236; LRMS (EI +) m/z 180 (20), 125 (20), 111 (100), 93 (90), 69 (80), 55 (70), 41 (60).

FO7 (2-Hydroxy davanone):

^1H NMR (CDCl_3 , 400 MHz) δ 1.01–1.08 (3H, d, $J = 7.0$ Hz), 1.22–1.31 (3H, d, $J = 2.6$ Hz), 1.35–1.41 (6H, d, $J = 1.7$ Hz), 1.60–1.70 (1H, ddt, $J = 8.0$, 9.2, 11.9 Hz), 1.72–1.80 (1H, ddd, $J = 7.1$, 9.2, 12.0 Hz), 1.85–1.96 (1H, ddd, $J = 3.9$, 7.8, 11.7 Hz), 1.96–2.07 (1H, m), 2.15–2.20 (2H, s), 2.90–2.98 (1H, dd, $J = 7.0$, 8.3 Hz), 4.18–4.28 (1H, td, $J = 6.0$, 8.3 Hz), 4.93–5.01 (1H, dd, $J = 1.7$, 10.7 Hz), 5.13–5.22 (1H, dd, $J = 1.6$, 17.2 Hz), 5.82–5.96 (1H, m), 6.38–6.46 (1H, d, $J = 15.7$ Hz), 6.88–6.96 (1H, d, $J = 15.7$ Hz).

^{13}C NMR (101 MHz, CDCl_3) δ 13.1, 25.4, 26.5, 29.3, 29.4, 29.4, 37.6, 49.9, 71.9, 80.5, 83.0, 111.5, 125.2, 144.6, 152.5, 202.9.

HRMS calcd for $\text{C}_{15}\text{H}_{24}\text{O}_3$: 252.17, found ([M+] $^-$ - H_2O) : 237 ; LRMS (EI +) m/z 166 (20), 138 (20), 125 (30), 113 (80), 111 (80), 93 (90), 85 (60), 67 (40), 55 (70), 43 (100).

2.3. Identification of the oil components

2.3.1. Gas chromatography

The gas chromatography (GC) analysis was carried out using Clarus 500 Perkin-Elmer Auto system apparatus equipped by two flame ionization detectors (FID), with a fused capillary columns (50 m \times 0.22 mm I.D; film thickness 0.25 μm), BP-1 (polymethyl-siloxane) and BP-20 (polyethylene glycol); carrier gas, helium; linear velocity, 0.8 mL/min (Bekhechi et al., 2010). The oven temperature was fixed from 60 $^\circ\text{C}$ to 220 $^\circ\text{C}$ at 2 $^\circ\text{C}/\text{min}$ and then held isothermal (20 min). Injector temperature was 250 $^\circ\text{C}$ (injection mode: split 1/60); detector temperature 250 $^\circ\text{C}$. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization, without using correction factors, as described previously (Medbouhi et al., 2018).

2.3.2. Gas chromatography/mass spectrometry

Essential oils were analyzed with a PerkinElmer Turbo-Mass quadrupole analyzer, coupled to a PerkinElmer Autosystem XL, equipped with two fused-silica capillary columns and

operated with the same GC conditions described above, except for a split of 1/80. EI mass spectra were acquired under the following conditions: Ion source temp. 150 °C, energy ionization 70 eV, mass range 35–350 Da (scan time: 1 s) (Tabet Zatlá et al., 2017).

2.3.3. Nuclear magnetic resonance

NMR spectroscopy experiments on the fractions were performed on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.13 MHz (¹³C), equipped with a 5 mm probe, in deuterated chloroform (CDCl₃), with all shifts specified to internal tetramethyl silane (TMS). Spectra were recorded with the following parameters: pulse width (PW), 4 μs (flip angle 45°); acquisition time, 2.7 s for 128 K data table with a spectral width (SW) of 24,000 Hz (240 ppm); CPD mode decoupling; digital resolution 0.183 Hz/pt (Esselin et al., 2017). The number of accumulated scans ranged was 3000 for each sample (50–60 mg in 0.5 mL of CDCl₃) (Bouzabata et al., 2010).

2.4. Theoretical background and computational details

2.4.1. Targets and compounds preparations

In this study, the interactions of essential oils of *S. grandiflorus* roots from compounds were investigated. The structures of inhibitors were downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>). The PDB database (<https://www.rcsb.org/>) were used to obtain the complete structure of vascular endothelial growth factor receptors (VEGFR-1) (PDB ID: 3HNG (Tresaugues et al., 2013)), VEGFR-2 (PDB ID: 2XIR (Marrone et al., 2007)), VEGF (PDB ID: 5T89 (Markovic-Mueller et al., 2017)) was obtained by X-ray diffraction method).

2.4.2. Molecular docking

In this research, specific molecular operating environment (MOE) was used to study the molecular interaction between essential oils of *S. grandiflorus* roots compounds and the vascular endothelial growth factor receptors (VEGFR-1, VEGFR-2 and VEGF) enzymes. During this molecular docking study, the number of interactions was 10, the cut-off for coulomb interaction and van der Waal interaction was (about 30 angstrom) with the ability to study the hydrogen-electrostatic in the total active site of the enzyme was validated and the results were discussed. The present research aimed at indicating the binding mode of essential oils of *S. grandiflorus* roots compounds into the three targets VEGFR-1, VEGFR-2 and VEGF worming molecular docking. The energy of the enzyme was minimized and geometry was conducted using Hamiltonian implanted in MOE software and then isolation of the active site of the target. The most stable geometry of each compound was minimized by the same semi-empirical method (AM1) (Stewart, 2007). All simulations were run by using all explicit salvation models using TIP3P water. After that, the binding energy between ligands and targets was calculated and based on molecular mechanics (Halgren, 1996, 1999).

2.4.3. MD simulation

The favorite conformer of Vascular Endothelial Growth Factor receptors with compounds was subjected to MDs Simulations MD was achieved for both the complex (3HNG, 2XIR, 5T89) adopting the MOE software (Al-Haderet et al., 1993). Dynamics simulation needs the Nose–Poincaré–Andersen (NPA) equations of motion (Bond et al., 1999; Sturgeon, & Laird, 2000). The coordinates were saved every 0.2 ps to get an accurate view of molecular movement. MDs simulations require the Berendsen thermostat to rescale the velocities of particles (Berendsen et al., 1984). In all simulations the van der Waals cut-out distance was set to 8 Å. Energy minimization process was activated by using MMFF94x force field (Parikesit et al., 2015). We have shown the detailed analysis of (MD) simulation results of only compound L25 with target VEGF receptors because these compounds show superior binding affinity for both VEGF receptors. At last and according to (MD) simulation analysis among these two compounds the most effective molecules were L25 and L13 (Graphical MD for ligand 13 [see Supporting Information Figures 15–17]) in VEGF receptors. The MOE software was used for our study because it has proven its performance in several recent studies; we can cite some example of work: Naz et al. (2020), Stitou et al. (2020), Daoud et al. (2018), Ghufraan et al. (2019) and Mesli and Ghalem (2017).

3. Results and discussion

3.1. Composition of the Coll EO and yields

The essential oil *S. grandiflorus* roots collected in Tlemcen from Western Algeria, afforded pale yellow oil with average yield varied of 0.07% to 0.08% (w/w) based on the dry mass of the plant. Preliminary analysis of the essential oils of *S. grandiflorus* obtained from the roots of 21 sites (Table 1) was identified only 10 compounds by comparing their mass spectra (EI-MS) and retention indices (RIs) with those of mass-spectral library (Arome) and by comparison of their mass spectra and RIs with those listed in commercial mass-spectral libraries (Table 1). The compounds identified in the Coll EO were Lavender lactone (1.5%), cis-Arbusculon (2.1%), trans-Arbusculon (0.9%), cis-Linalool oxide (0.4%), E-β-Elemene (0.5%), iso davanone (1.6%), davanol D1 (6.5%), Eudesma-11-en-4α-ol, (2.1%) Tetradecanoic acid (0.8%) and hexadecanoic acid (0.8%; Table 1). All individual oil samples were pooled to produce a 'collective essential oil' (EO Coll.) that was used to perform detailed analysis using successive column chromatography (CC), GC (RI), GC/MS and NMR analysis (Table 1). Flash Chromatography of oxygenated fraction (FO) afforded eight fractions of the total Coll Eos, in order of their elution (Figure 2). The analysis by GC-MS and NMR of the oxygenated fractions by comparison with the different fragments (GC-MS) and chemical shifts (NMR) with those of the literature (Alwahibi et al., 2016; Naegeli & Weber, 1970; Thomas et al., 1974; Wan et al., 2013) (see material and methods) showed that fraction **FO1** contained the component of 5,5-dimethyl-2(5H)-furanone with percentage of 89.7%, **FO3** contained four stereoisomers of Davana furan (5.9% (1), 11.3%

Table 1. Percentage of compounds identified in the essential oils of *S. grandiflorus* isolated from various localities.

| No | Compounds | ¹ RiA | ² RiA | ³ RiP | Id. Methods | Col.EO | Stations | | | | | | | | | | | | | | | | | | | | | | | |
|-----------------------------------|------------------------|------------------|------------------|------------------|-----------------------------|--------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------|------|------|
| | | | | | | | Y1 | Y2 | Y3 | Y4 | Y5 | Y6 | Y7 | Y8 | Y9 | Y10 | Y11 | Y12 | Y13 | Y14 | Y15 | Y16 | Y17 | Y18 | Y19 | Y20 | Y21 | | | |
| 1 | 5,5-dimethyl Furanone | 914 | 919 | 1594 | IR, MS, ¹³ C RMN | 2.9 | 5.5 | 5.5 | 6.3 | 6.6 | 5.9 | 7.5 | 9.0 | 3.4 | 2.5 | 2.8 | 2.1 | 2.2 | 1.2 | 1.5 | 1.1 | 0.6 | 0.6 | 0.9 | 1.1 | 1.6 | 1.0 | | | |
| 2 | Lavender lactone | 1002 | 996 | 1647 | IR, MS, ref | 1.5 | 2.2 | 3.9 | 3.1 | 2.9 | 1.9 | 2.2 | 3.2 | 1.0 | 0.5 | 1.1 | 0.4 | 0.7 | 0.3 | 0.6 | 0.4 | 0.3 | 0.2 | 0.3 | 0.8 | 0.6 | 0.3 | | | |
| 3 | cis-Arbusculon | 1052 | 1032 | 1442 | IR, MS, ref | 2.1 | 1.9 | 0.5 | 1.6 | 0.7 | 2.7 | 4.9 | 5.6 | 2.1 | 1.7 | 1.7 | 1.2 | 1.9 | 0.6 | 1.1 | 0.8 | 0.8 | 0.3 | 0.6 | 1.1 | 1.1 | 0.8 | | | |
| 4 | trans-Arbusculon | 1071 | 1048 | 1481 | IR, MS, ref | 0.9 | 1.5 | 0.7 | 1.3 | 0.6 | 1.9 | 3.7 | 3.4 | 1.4 | 0.9 | 1.1 | 0.8 | 1.2 | 0.7 | 0.8 | 0.6 | 0.4 | 0.3 | 0.4 | 0.7 | 0.7 | 0.4 | | | |
| 5 | cis-Linalool oxide | 1065 | 1055 | 1493 | IR, MS, ref | 0.4 | 0.2 | 0.5 | 0.3 | 0.4 | 0.3 | 0.3 | 0.2 | 0.1 | 0.1 | 0.3 | 0.4 | 0.1 | 0.3 | 0.4 | 0.3 | 0.6 | 0.2 | 0.2 | 0.5 | 0.4 | 0.3 | | | |
| 6 | Davanafuran (1) | / | 1371 | 1702 | IR, MS | 0.2 | 0.3 | 0.2 | 0.4 | 0.3 | 0.4 | 0.2 | 0.4 | 0.3 | 0.2 | 0.1 | 0.2 | 0.5 | 0.5 | 0.4 | 0.3 | 0.2 | 0.2 | 0.2 | 0.4 | 0.4 | 0.3 | | | |
| 7 | Davanafuran (2) | / | 1379 | 1722 | IR, MS | 0.7 | 1.1 | 0.2 | 0.7 | 0.5 | 1.1 | 0.7 | 0.5 | 0.3 | 0.4 | 0.5 | 0.4 | 0.3 | 0.2 | 0.9 | 0.1 | 0.3 | 0.6 | 0.4 | 0.5 | 0.7 | 0.2 | | | |
| 8 | Davanafuran (3) | / | 1383 | 1729 | IR, MS | 0.4 | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 | 0.5 | 0.4 | 0.2 | 0.7 | 0.2 | 0.2 | 0.3 | 0.6 | 0.6 | 0.4 | 0.4 | 0.2 | tr | 0.1 | 0.2 | 0.6 | | | |
| 9 | E-β-Elementene | 1387 | 1389 | 1593 | IR, MS, ref | 0.5 | 0.9 | 0.4 | 0.1 | 0.6 | 1.3 | 0.2 | 0.4 | 0.3 | 0.4 | 0.4 | 0.4 | 0.4 | 0.3 | 0.1 | 0.7 | 0.7 | 0.7 | 0.6 | 0.3 | 0.3 | 0.6 | | | |
| 10 | Davanafuran (4) | 1393 | 1394 | 1766 | IR, MS, ¹³ C RMN | 2.6 | 2.0 | 2.5 | 1.3 | 3.5 | 2.3 | 3.0 | 2.3 | 2.0 | 2.7 | 2.3 | 3.9 | 2.2 | 2.2 | 3.2 | 2.6 | 3.2 | 3.2 | 0.9 | 1.7 | 1.4 | 3.6 | 3.4 | | |
| 11 | Davana ether (1) | / | 1466 | 1890 | IR, MS, ref | 0.8 | 0.2 | 0.7 | tr | 0.6 | 0.1 | 0.2 | 0.3 | 0.2 | 0.8 | 0.3 | 0.4 | 0.7 | 0.5 | 1.2 | 1.2 | 1.3 | 0.2 | 0.1 | 0.3 | 1.7 | 1.2 | | | |
| 12 | Davana ether (2) | / | 1474 | 1908 | IR, MS, ref | 2.1 | 0.5 | 0.5 | 0.5 | 0.7 | 0.6 | 0.5 | 0.7 | 0.3 | 0.4 | 0.9 | 0.6 | 0.4 | 1.1 | 1.4 | 2.4 | 1.6 | 1.9 | 1.3 | 1.2 | 0.3 | 3.2 | | | |
| 13 | Davanone (1) | / | 1534 | 1955 | IR, MS | 1.1 | 1.0 | 0.2 | 0.3 | 0.3 | 1.4 | 0.7 | 0.3 | 0.4 | 0.9 | 0.6 | 0.4 | 1.1 | 1.4 | 2.4 | 1.6 | 1.9 | 1.3 | 1.2 | 0.3 | 2.8 | 1.4 | | | |
| 14 | Davanone (2) | / | 1540 | 1973 | IR, MS | 1.1 | 0.7 | 0.3 | 0.5 | 0.2 | 0.9 | 0.7 | 0.7 | 0.7 | 0.9 | 1.9 | 1.1 | 1.0 | 1.7 | 0.4 | 1.4 | 1.6 | 1.5 | 1.8 | 1.5 | 0.8 | 1.3 | | | |
| 15 | Iso davanone | 1562 | 1537 | / | IR, MS, ref | 1.6 | 0.7 | 0.9 | 0.5 | 1.2 | 0.5 | 0.9 | 1.1 | 1.1 | 2.5 | 1.3 | 1.3 | 2.0 | 1.7 | 1.9 | 2.1 | 1.5 | 2.2 | 2.4 | 1.1 | 1.9 | 1.7 | | | |
| 16 | Davanone (3) | / | 1546 | 1994 | IR, MS | 1.8 | 1.4 | 0.4 | 1.1 | 0.7 | 1.1 | 1.5 | 1.3 | 1.4 | 2.7 | 1.6 | 1.7 | 2.2 | 2.9 | 2.7 | 2.0 | 1.1 | 1.8 | 2.2 | 1.9 | 1.7 | 2.0 | | | |
| 17 | Davanone (4) | 1569 | 1570 | 2035 | IR, MS, ¹³ C RMN | 45.3 | 24.4 | 19.9 | 26.4 | 23.0 | 27.8 | 30.6 | 35.0 | 51.9 | 45.0 | 51.0 | 45.8 | 49.6 | 53.7 | 41.3 | 52.7 | 50.4 | 73.2 | 68.4 | 61.5 | 48.4 | 54.5 | | | |
| 18 | Davanol D1 | 1591 | 1602 | 2090 | IR, MS | 6.5 | 2.4 | 4.9 | 4.0 | 6.2 | 3.6 | 3.5 | 2.0 | 2.9 | 5.8 | 3.5 | 3.2 | 6.7 | 2.1 | 3.1 | 4.9 | 2.7 | 2.8 | 3.3 | 2.5 | 2.3 | 5.3 | | | |
| 19 | Eudesma-11-en-4α-ol | 1642 | 1638 | 2233 | IR, MS | 2.1 | 1.8 | 4.0 | 3.7 | 2.9 | 1.9 | 1.1 | 0.8 | 2.3 | 1.4 | 1.9 | 2.7 | 1.8 | 1.8 | 2.2 | 1.7 | 1.1 | 1.7 | 2.1 | 1.8 | 1.0 | 1.4 | | | |
| 20 | 2-Hydroxy davanone (1) | / | 1644 | 2496 | IR, MS | 0.1 | 0.5 | 0.1 | 0.2 | 0.2 | 0.3 | 0.4 | 0.4 | 0.3 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.3 | 0.4 | 0.9 | 0.8 | 0.1 | 0.2 | 0.2 | | | |
| 21 | 2-Hydroxy davanone (2) | / | 1657 | 2513 | IR, MS | 0.4 | 1.4 | 0.9 | 0.9 | 1.3 | 1.7 | 1.3 | 0.8 | 0.4 | 0.5 | 0.3 | 0.2 | 0.6 | 0.4 | 0.5 | 0.2 | 0.2 | 0.3 | 0.3 | 0.1 | 0.3 | 0.1 | | | |
| 22 | 2-Hydroxy davanone (3) | / | 1665 | 2531 | IR, MS | 5.3 | 4.3 | 3.2 | 5.2 | 4.2 | 3.9 | 2.1 | 1.7 | 1.3 | 1.3 | 1.2 | 0.9 | 1.2 | 0.8 | 0.9 | 0.6 | 0.6 | 0.7 | 1.0 | 0.2 | 0.5 | 0.7 | | | |
| 23 | 2-Hydroxy davanone (4) | 1668 | 1682 | 2566 | IR, MS, ¹³ C RMN | 11.2 | 26.1 | 33.2 | 23.1 | 29.8 | 23.9 | 19.7 | 14.0 | 10.8 | 8.7 | 5.7 | 5.1 | 6.1 | 1.8 | 2.4 | 2.5 | 2.2 | 1.8 | 2.7 | 3.1 | 2.3 | 2.8 | | | |
| 24 | Tetradecanoic acid | 1748 | 1745 | 2682 | IR, MS | 0.8 | 0.3 | 0.2 | 0.2 | 0.4 | 0.2 | 0.3 | 0.2 | 0.4 | 0.3 | 0.2 | 0.3 | 0.4 | 0.7 | 0.6 | 0.6 | 0.3 | 1.3 | 1.0 | 0.7 | tr | 0.5 | | | |
| 25 | Hexadecanoic acid | 1942 | 1942 | 2628 | IR, MS | 0.8 | 0.5 | 0.3 | 0.2 | 0.6 | 0.3 | 0.7 | 0.4 | 9.5 | 8.5 | 10.2 | 12.5 | 9.8 | 13.1 | 11.5 | 8.6 | 15.2 | 3.2 | 3.8 | 8.9 | 16.8 | 10.3 | | | |
| % Total identification | | | | | | | 93.2 | 82.2 | 84.4 | 82.2 | 88.7 | 86.4 | 87.5 | 84.9 | 95.6 | 92.8 | 86.3 | 96.0 | 92.0 | 86.7 | 91.0 | 92.1 | 99.7 | 98.3 | 91.3 | 96.1 | 95.4 | | | |
| Hydrocarbon sesquiterpenes | | | | | | | 0.5 | 0.9 | 0.4 | 0.1 | 0.6 | 1.3 | 0.2 | 0.4 | 0.3 | 0.4 | 0.4 | 0.4 | 0.3 | 0.4 | 0.3 | 0.1 | 0.7 | 0.7 | 0.6 | 0.3 | 0.3 | 0.6 | | |
| Oxygenated monoterpenes | | | | | | | 0.4 | 0.2 | 0.5 | 0.3 | 0.4 | 0.3 | 0.3 | 0.2 | 0.1 | 0.1 | 0.3 | 0.4 | 0.1 | 0.3 | 0.4 | 0.3 | 0.4 | 0.3 | 0.6 | 0.2 | 0.5 | 0.4 | 0.3 | |
| Oxygenated sesquiterpenes | | | | | | | 79.4 | 65.4 | 69.2 | 64.0 | 71.3 | 67.8 | 63.3 | 58.9 | 74.6 | 73.9 | 69.6 | 63.5 | 76.0 | 71.3 | 65.0 | 74.5 | 69.0 | 91.0 | 88.2 | 74.8 | 69.7 | 76.7 | 76.7 | 67.7 |
| Non-terpenic oxygenated compounds | | | | | | | 12.9 | 15.7 | 14.3 | 15.4 | 16.4 | 17.0 | 23.7 | 25.4 | 20.6 | 18.4 | 20.2 | 22.0 | 19.5 | 20.1 | 21.2 | 15.5 | 21.8 | 7.8 | 9.3 | 15.7 | 25.7 | 17.8 | 17.8 | 17.8 |

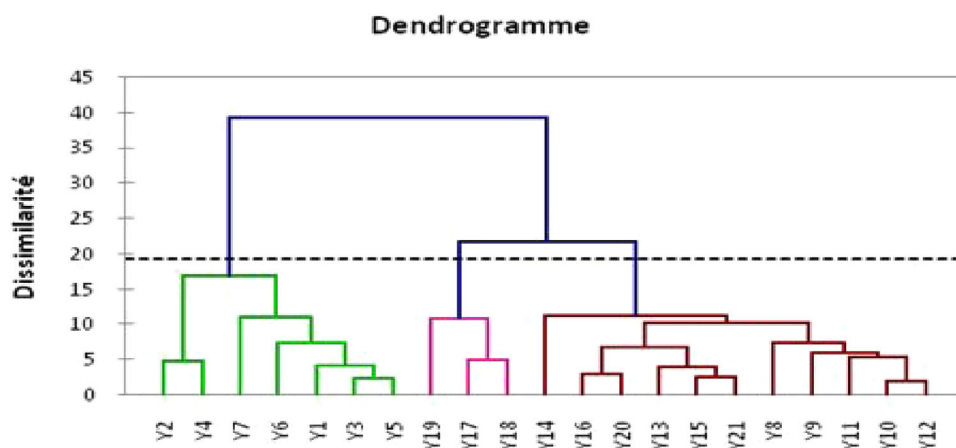


Figure 3. Cluster analysis (CA) of chemical compositions of *S. grandiflorus* from Algeria.

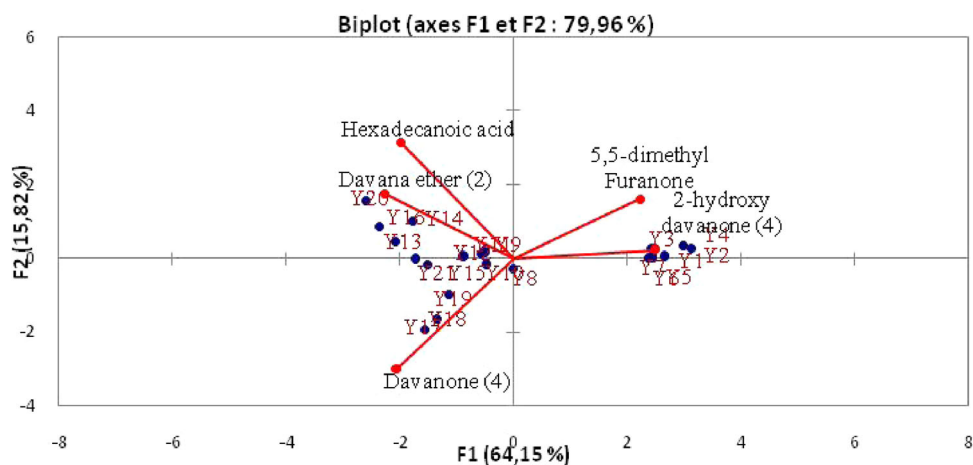


Figure 4. Principal component analysis (PCA) of chemical compositions of *S. grandiflorus*.

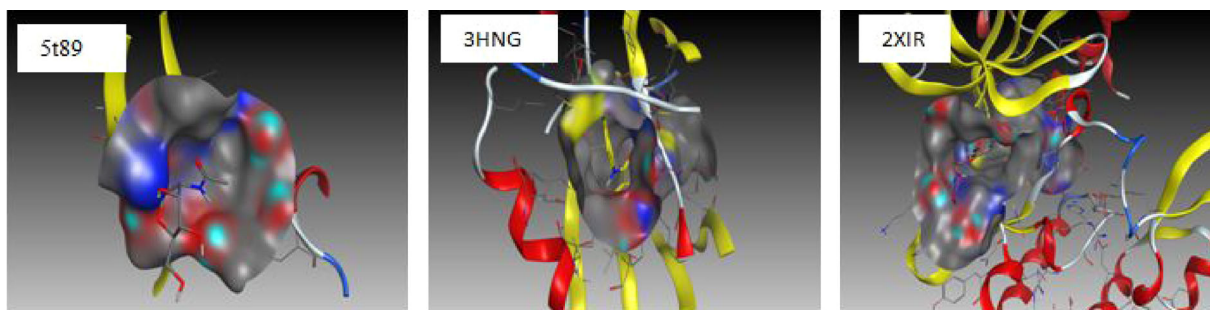


Figure 5. Enzymatic cavity with the residues of the active site for the targets: 5T89, 3HNG and 2XIR.

(2), 2.6% (3) and 66.5% (4)), **FO4** contained davanol (D) (88.2%) and Fraction **FO5** contained four stereoisomers of davanone (6.2% (01), 7.5% (2), 7.2% (3) and 75.7% (4)) and Fraction **FO7** contained four stereoisomers of 2-hydroxy davanone (0.8% (1), 6.2% (2), 10.4% (3) and 63.3% (4)) (Table 1). The most abundant davanoid was cis-davanone, Natural (+)-davanone, a sesquiterpenoid ketone that was first isolated from *Artemisia pallens* (Sipma & Van der Wal, 2010). Davanone was the main constituent of several species of *Artemisia* species of the Asteraceae family. The major component of essential oils of aerial parts of *Artemisia ciniformis* (Rustaiyan et al., 2007). *Artemisia kermanensis*, *Artemisia kopetdaghensis* and *Artemisia khorassanica* of Iran (Ramezani

et al., 2005; Rustaiyan et al., 2009) and also identified in the leaves and flowers of *Lantana camara* L (El Baroty et al., 2014; Saikia & Sahoo, 2011).

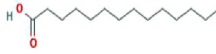
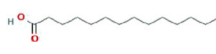
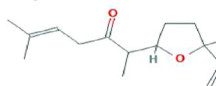
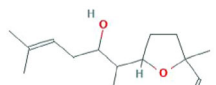


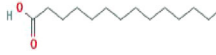
3.2. Chemical variability of *S. grandiflorus* essential oils

Twenty-one wild populations (Y1 to Y21) of *S. grandiflorus* were collected during flowering with different altitudes. Seven samples (S1–S7) at high altitude (600–1100 m) with an important precipitation, located in Tlemcen mountains and fourteen stations (S8–S21) at low altitude (170 m to 500 m) in littoral of Tlemcen with low precipitation (Table 1, Figure 1).

Table 2. Some properties of all compounds for anti-angiogenic drug.

| Ligand Compound | Toxic | LogP | Energies (Kcal/mol) | LogS | Hdon + Hacc | Flexibility |
|---------------------------|-------|------|---------------------|-------|--------------|-------------|
| L01 5.5-dimethyl Furanone | No | 1.70 | 1.82361 e+ 001 | -1.18 | don:0; acc:2 | 3 out 3 |
| L02 Lavender lactone | No | 1.27 | 8.81161 e+ 000 | -1.05 | don:0; acc:1 | 1 out 1 |
| L03 Cis-Arbusculon | No | 1.70 | 2.65666 e+ 001 | -1.45 | don:0; acc:2 | 2 out 2 |
| L04 Trans-Arbusculon | No | 1.70 | 2.73648 e+ 001 | -1.45 | don:0; acc:2 | 2 out 2 |
| L05 cis-Linalool oxide | No | 1.88 | 3.73575 e+ 001 | -1.56 | don:1; acc:2 | 2 out 2 |
| L06 Davanafuran | No | 3.51 | 2.49849 e+ 001 | -2.89 | don:0; acc:0 | 2 out 2 |
| L09 (E)-β-Elemene | No | 4.75 | 4.43553 e+ 001 | -6.04 | don:0; acc:0 | 3 out 3 |
| L11 Davana ether | No | 3.75 | 2.97817 e+ 001 | -3.03 | don:0; acc:2 | 2 out 2 |
| L13 Davanone | No | 3.67 | 3.22538 e+ 001 | -2.88 | don:0; acc:2 | 5 out 5 |
| L15 Iso davanone | No | 3.67 | 3.10733 e+ 001 | -2.88 | don:0; acc:2 | 5 out 5 |
| L18 Davanol D1 | No | 3.46 | 3.72150 e+ 001 | -2.66 | don:1; acc:2 | 5 out 5 |
| L19 Eudesma-11-en-4α-ol | No | 3.92 | 4.83154 e+ 001 | -4.36 | don:1; acc:1 | 1 out 1 |
| L20 2-Hydroxy davanone | No | 2.64 | 3.23785 e+ 001 | -2.48 | don:1; acc:3 | 5 out 5 |
| L24 Tetradecanoic acid | No | 4.77 | -1.4216 e+ 001 | -5.46 | don:1 acc:2 | 12 out 12 |
| L25 Hexadecanoic acid | No | 5.55 | -1.4606 e+ 001 | -6.49 | don:1; acc:2 | 14 out 14 |

Table 3. Results of bonds between atoms of best compounds and active site residues of three targets.

| No | Chemical structure | S-score (Kcal/mol) | Bonds between atoms of compounds and residues of the active site | | | | Distances (Å) | Energies (Kcal/mol) |
|------|---|--------------------|--|-------------------------|----------------------------|--------------------------|----------------|---------------------|
| | | | Atom of compound | Involved receptor atoms | Involved receptor residues | Type of interaction bond | | |
| L24 |  | -3.809 | O1 1 | N | LEU 97 | H-acceptor | 2.82 | -3.4 |
| | | | O1 1 | NH2 | ARG 56 | ionic | 3.99 | -0.5 |
| L25 |  | -4.003 | O1 1 | N | LEU 97 | H-acceptor | 2.90 | -2.8 |
| L13 |  | -6.529 | O1 1 | N | ASP 1040 | H-acceptor | 2.93 | -3.4 |
| L18 |  | -7.345 | C6 6 | 6-ring | PHE 1041 | H-pi | 4.27 | -0.8 |
| L24 |  | -7.470 | O1 1 O1 1 | NE | ARG 1021 ARG | H-acceptor H- | 2.87 | -5.8 |
| | | | O2 2 | NH2 | 1021 ARG 1021 | acepr | 2.99 | -4.0 |
| | | | O2 2 O1 1 O1 1 | NH2 | HOH 3024 ARG | H-accor | 3.26 | -3.7 |
| | | | 1 O2 2 | O | 1021 ARG 1021 | -acceptor ionic | 3.24 | -1.3 |
| | | | | NE | ARG 1021 | ionic | 2.87 | -5.4 |
| L25 |  | -8.504 | | NH2 | | | 2.99 | -4.6 |
| | | | | NH2 | | | 3.26 | -3.0 |
| | | | O1 1 O1 1 | NE | ARG 1021 ARG | H-acceptor H- | 3.03 2.83 3.03 | -5.3 |
| | | | O2 2 O2 2 O1 1 | NH2 | 1021 ARG 1021 | acceptor ionic | 3.45 3.93 2.83 | -8.1 |
| | | | 1 | NE | ARG 1021 ARG | ionic | | -4.3 |
| | | | O1 1 | NH2 | 1021 ARG 1021 | ionic | | -2.1 |
| O2 2 | NE | | ionic | | -0.6 | | | |
| | NH2 | | | | | -5.7 | | |
| L24 |  | -6.753 | O2 2 O1 1 | N | ASP 1046 | H-acceptor | 3.41 4.00 | -2.5 |
| | | | | NZ | LYS 868 | ionic | | -0.5 |

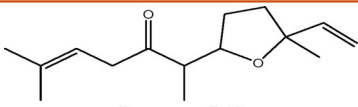
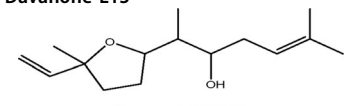
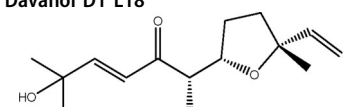
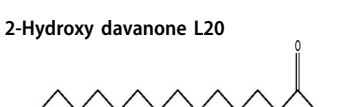
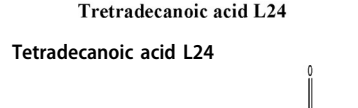
Analysis of 21 samples showed that the GC chromatograms of all samples from the same species were qualitatively similar but differ by abundances of their major components. CA Performed from four discriminant compounds suggest the existence of two main clusters of *S. grandiflorus* essential oils (Figure 3).

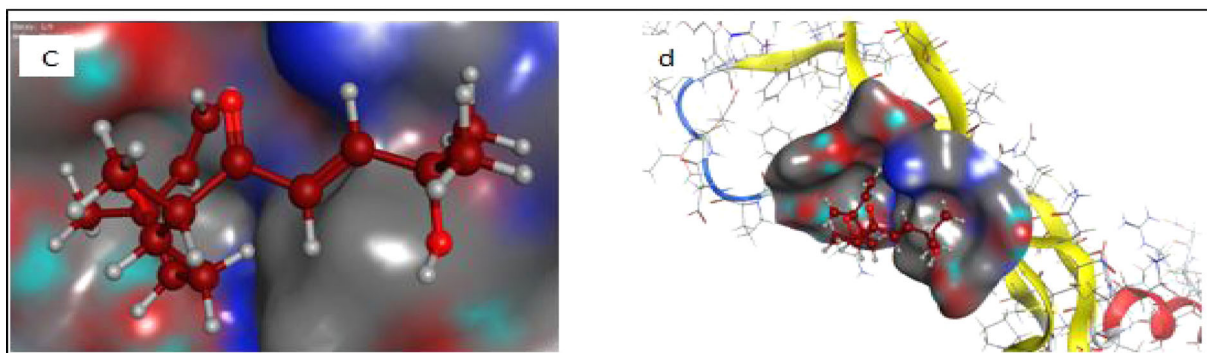
Group I (Y1–7) consisted mainly of oil samples dominated by 2-hydroxy davanone (4) (14.0–33.2%) and 5.5-dimethyl Furanone (5.5–9%). Whereas. Group II (Y8–Y21) includes oil samples

characterized by davanone (4) (41.3–73.2%), Davana ether (2) (0.7–5.3%) and hexadecanoic acid (3.2–16.8%; Figure 4, Table 1).

Cis-davanone has never been identified as up to 50% in essential oil of all species studied. The oils from stem parts of *Artemisia ciniformis* and *Artemisia kopetdaghensis* was the high percentage in all these species 40.1% and 47.9%, respectively. Davanoides are reported to have antifungal, antispasmodic and antibacterial properties. Indeed, the most compelling target among these was cis-hydroxy davanone,

Table 4. Results of energy balance of best complexes formed with anti-angiogenic drug molecules.

| Targets | Chemical structure | Binding energy (Kcal/mole) | Rmsd -refine | Energy-conf | Energy- place | Energy- refine | RMSD |
|----------------------------|---|-----------------------------|--------------------------|-------------------------------|--------------------------------|------------------------------|-------------------------|
| VEGF VEGFR-1 VEGFR-2 |  Davanone L13 | -3.992 -6.529 -4.778 | 1.454 1.338 1.1872 | 37.594 51.148 53.321 | -29.409 -61.149 -54.385 | -10.361 3.690 -8.944 | 1.417 1.237 1.053 |
| VEGF VEGFR-1 VEGFR-2 |  Davanol D1 L18 | -3.811 -6.657 -6.657 | 2.0319 1.275 1.275 | 42.106 56.214 56.214 | -28.2838 -65.602 -65.602 | -9.492 -2.615 -2.615 | 1.397 1.278 1.278 |
| VEGF VEGFR-1 VEGFR-2 |  2-Hydroxy davanone L20 | -4.066 -6.161 -3.391 | 1.343 1.716 1.533 | 17.758 21.885 35.967 | -24.060 -52.651 -39.906 | -9.659 -16.814 26.020 | 1.395 1.116 1.070 |
| VEGF VEGFR-1 VEGFR-2 |  Tetradecanoic acid L24 | -3.809 -7.470 -6.753 | 0.958 1.327 1.187 | -81.019 -76.820 -72.972 | -38.265 -58.290 -66.076 | -7.389 -18.760 -13.576 | 1.517 1.316 1.140 |
| VEGF VEGFR-1 VEGFR-2 |  Hexadecanoic acid L25 | -4.0031 -8.504 -7.318 | 2.502 1.124 1.078 | -79.796 -72.783 -69.892 | -14.401 -58.660 -60.781 | -9.407 -25.356 -8.456 | 1.560 1.219 1.011 |

**Figure 6.** The graphical illustration of interaction between (A) Davanone; (B) iso davanone; (C) The top scoring compound, d) A novel inhibitor L-20 identified by molecular docking 2- Hydroxydavanone was shown in the active site.

whose proved to have cytotoxic property (Hosseinzadeh et al., 2019). To our knowledge, there is only one study on the genus *Scolymus*. The main compounds of *S. hispanicus* were heneicosane (19.4%), hexahydro farnesly acetone (17.0%) and phytol (17.0%). However, no work was performed on chemical composition of essential oil of *S. grandiflorus* and this work is the first one (Servi, 2019).

The results showed important differences between the stations indicating the existence of chemical polymorphism. The observed differences in the chemical composition of

essential oils may be due to ecological factors or many other factors as to soil type's water stress and climatic conditions that influenced the plant (Belabbes et al., 2017).

3.3. Computational and theoretical approach

Enzymatic cavity with the residues of the active site for the three targets is shown in Figure 5. The ligands' of essential oils from the root of *S. grandiflorus* minimized toxicity and energy obtained by MOE software is shown in Table 2.

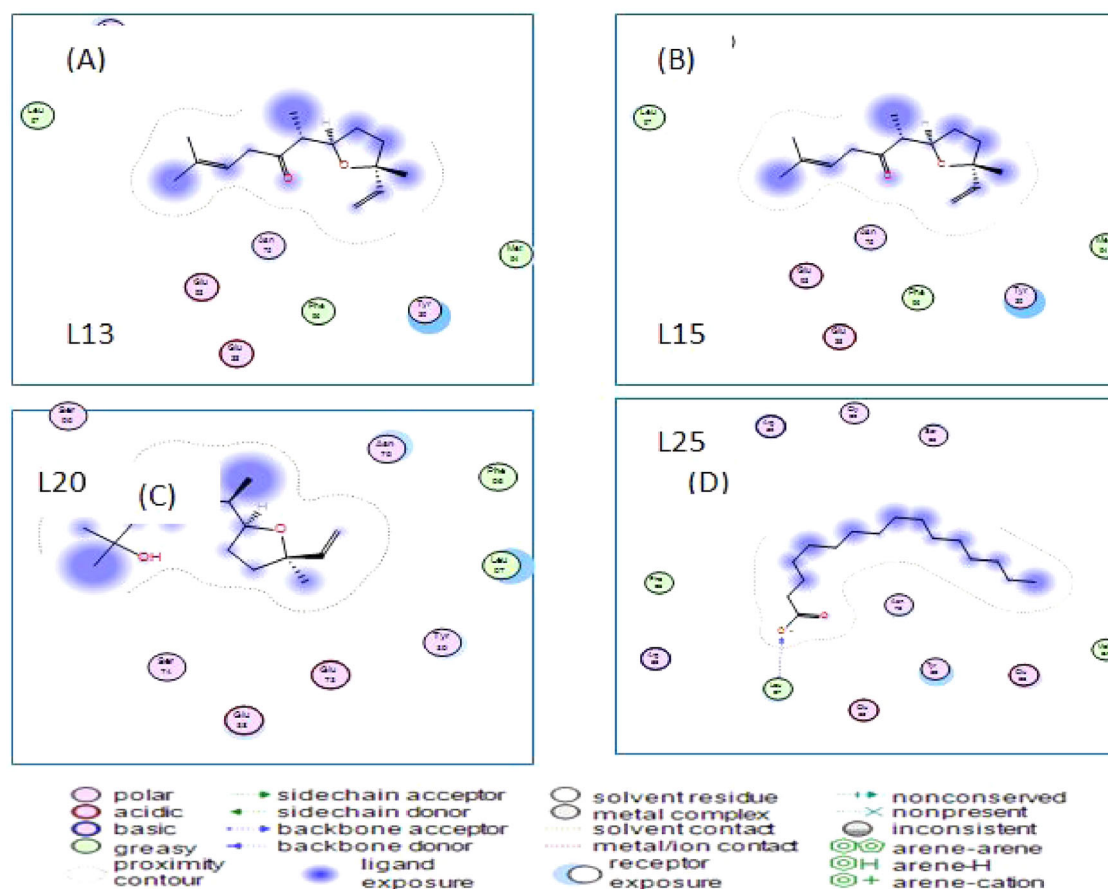


Figure 6 (Continued)

These ligands were capable of providing crucial biological activities in accordance with the principle of Lipinski et al. (1997) (Pettersson et al., 1988). As stated in the table above. We find that the molecules L13, L24 and L25 have a high value of Log P and Log S compared to other molecules and also the results obtained show that these ligands (L24, L13 and L25) have a high value of torsion angle relative to other compounds. This shows that these compounds were more flexible. As well, it is noted that the growth of the torsion angle depends on the binding number of the molecule.

3.4. Affinity of compounds with three targets

Results of docking calculations and bonds between atoms of best compounds and residues of the active site are given in Table 3. The results of bonds of the other compounds are given in Supporting Information Table 3(a).

3.5. Molecular docking analysis

3.5.1. Interaction with VEGF

According to the results of Tables 3 and 4, out of the best compounds studied, 2-hydroxy davanone (Ligand 20; Figure 6) was predicted to be the strongest VEGF receptors binder that forms a complex with the most stability with the lowest energy -4.066 Kcal/mol). The ligands that interact with VEGF were: Ligand L3 interacted with one amino acid LEU 97 at a distance of 2.95 Å strong with energy of -1.2

and ligand L6 interacted with one amino acid GLU 38H-donor at a distance of 3.08 Å strong and energy binding of -2.0 . Similarly, the ligand L24 interacted with two amino acids (LEU 97 and ARG 56) (H-acceptor, ionic) at a distance of 2.82 and 3.99 Å strong and low, respectively.

It is noted that the interactions between the residues of the active site of 5T89 and 2-hydroxy davanone ligand formed a stable complex. The second-best binder was hexadecanoic acid (Ligand 25) with the energy of 4.0039 Kcal/mol) that interacts with one amino acid LEU 97 H-acceptor at a distance of 2.90 Å strong interaction and energy binding of -2.8 Kcal/mol. This suggests that hexadecanoic acid can inhibit VEGF receptors. Best ligand with VEGF is shown in Figure 6(a).

3.5.2. Interaction with VEGFR-1

We note that hexadecanoic acid (Ligand 25; Figure 7) was predicted to be the strongest VEGF receptors binder that formed a complex with the most stability with the lowest energy -8.504 Kcal/mol) that interacts with six amino acids (ARG 1021, ARG 1021) two H-acceptor and four ionic at a distance of 3.00 , 2.83 and 3.03 , 3.45 , 3.93 and 2.83 Å strong, low, average interaction, with the existence of six electric force (GLU 910, GLU 878, CYS 912, LEU 882, ASP 1040 and LYS 861). The existence of electric force, suggesting that hexadecanoic acid can inhibit VEGF receptors. It is noted that the interactions between the residues of the active site of 3HNG and the hexadecanoic acid ligand formed a stable

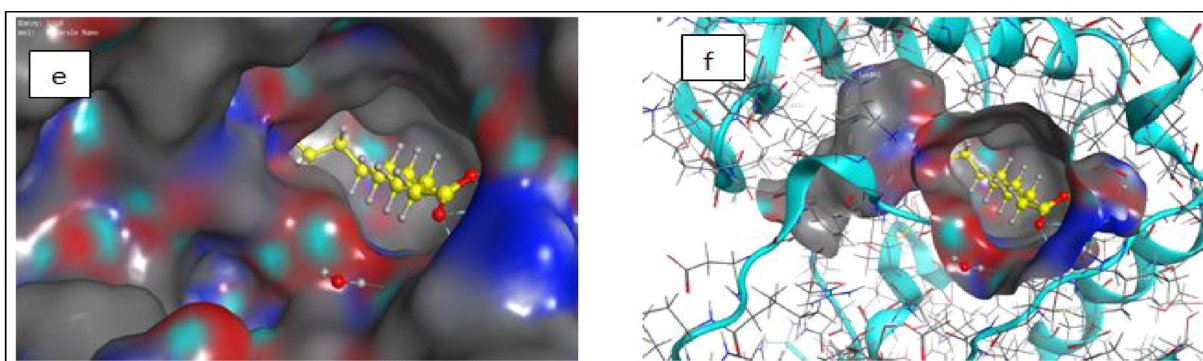


Figure 7. The graphical illustration of interaction between (A) iso davanone; (B) Tetradecanoic acid; (C) Hexadecanoic acid and (D) Davanol D1 with VEGFR-1. e) The top scoring compound f) A novel inhibitor L-25 identified by molecular docking Hexadecanoic acid was shown in the active site.

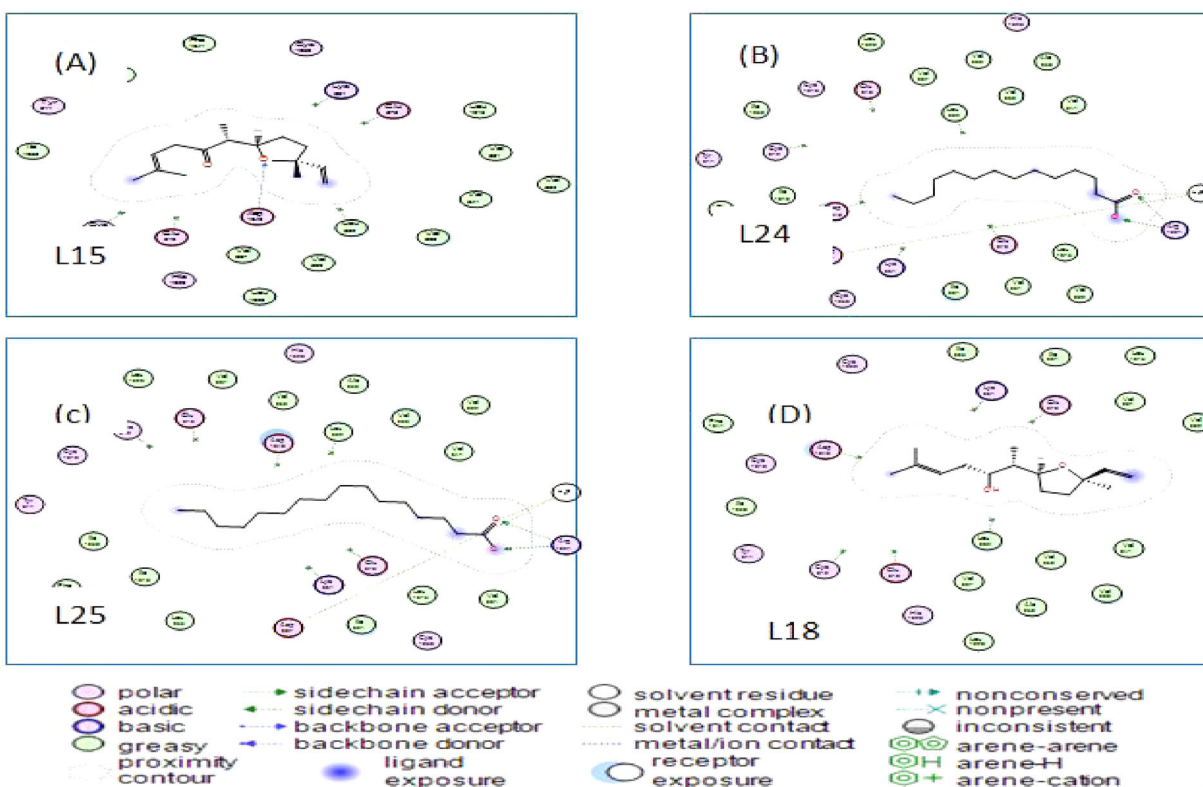


Figure 7 (Continued)

complex with a strong interaction. Best ligand with VEGFR-1 is given in Figure 7(a).

3.5.3. Interaction with VEGFR-2

We note that hexadecanoic acid (Ligand 15; Figure 8) was predicted to be the strongest VEGF receptors binder that formed a complex with the most stability with the lowest energy -7.318 Kcal/mol. The ligands that interact with VEGFR-2 were: Ligands L3, L5 and L6 interacted with one amino acid ASP 1046 and GLU 885 with H-acceptor and H-donor, respectively, at a distance of 2.98 and 2.58 Å low interaction with energy binding of -3.7 and -2.8 Kcal/mol. It is noted that the interactions between the residue of the active site of 2XIR and the hexadecanoic acid ligand formed a stable complex with a strong interaction.

The second best binder was tetradecanoic acid (Ligand 24) with the energy of -6.753 Kcal/mol (Table 3), with the existence of two amino acids ASP 1046 and LYS 868 with H-acceptor and ionic at distance of 3.41 and 4.00, respectively, with energy binding of -2.5 and -0.5 Kcal/mol, respectively. Results of energy balance of best complexes formed with three targets are given in (Table 4). Energy for other compounds see best complexes formed Supporting Information Table 4(a). Best ligand with VEGFR-2 is given in Figure 8(a).

3.6. Interaction with ctDNA

Circulating tumor DNA (ctDNA) is a small fragment of DNA that found in the bloodstream of cancerous patients. Studies proposed that this tumor-based DNA fragment can act as a

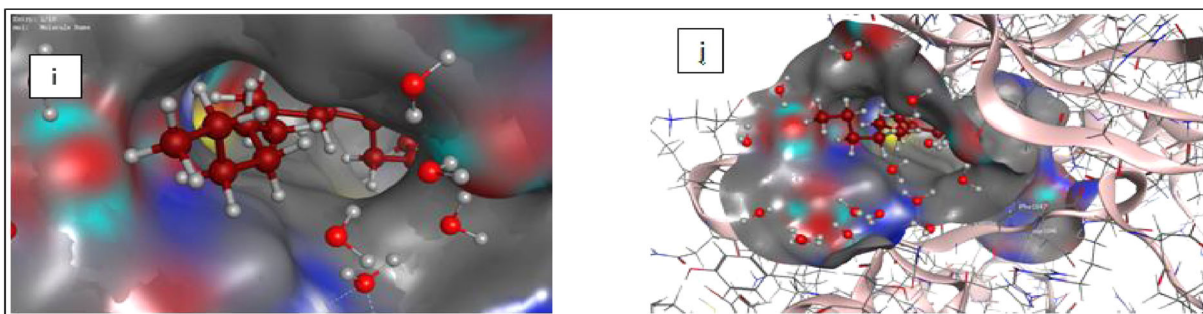


Figure 8. i) The top scoring compound, j) A novel inhibitor L-15 identified by molecular docking was Hexadecanoic acid was shown in the active site.

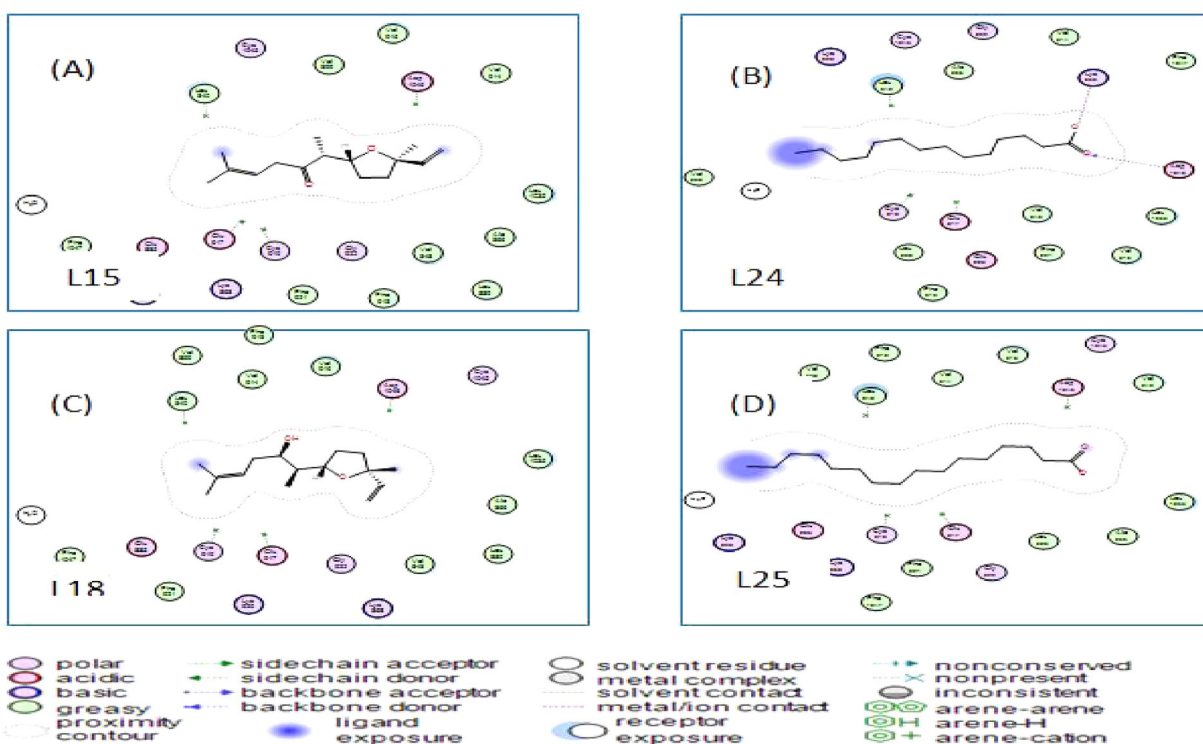


Figure 8 (Continued)

leader for the migration of tumor cells to other. Recently, this small fragment of DNA was considered as detectable biomarker in early- and late Stage human malignancies (Drach et al., 1996). A bioinformatic molecular docking study was carried out on ctDNA and is shown in (Table 5).

Our results demonstrated that the four compounds: hexadecanoic acid (Ligand 25), tetradecanoic acid (Ligand 24), davanone (Ligand 13) and davanol D1 (Ligand 18) were the best interacting compounds (Figure 9). The calculated docking energies for these compounds were, respectively, -7.210 , -6.984 , -6.223 and -6.171 Kcal/mol. With the exception of Lavender lactone, other compounds were located in the small cDNA groove. The docking simulation showed that Lavender lactone existed in the main groove region. The calculated docking energy for this compound was -4.191 Kcal/mol (Table 4). From these results, it should be noted that several factors, notably the variability of the ligand structures and the capacity to build covalent and/or

noncovalent bonds, could affect their binding affinity to the small cDNA groove. There are even other factors such as the distribution of electrostatic charges with DNA and also the base pairs A: T (Neidle, 2001). Studies have suggested that minor groove binding ligands carry a cationic charge, complementing the potential in A: T regions. Our results are fully consistent with previous reports and the compounds studied interacted with A: T base pairs.

Figure 9 is replaced by another, from which we have assembled only the best ligands.

3.7. Interaction with VEGF-VEGFR

The two VEGF monomers participate in the interaction with the d2 domain of VEGFR1 (Figure 10). The results of docking energies of VEGF/VEGFR best inhibitors are shown in

(Table 6). The results of other compounds are shown in Supporting Information Table 6(a).

Numerous studies have shown that the VEGF gene is a key mediator of angiogenesis in cancer (Carmeliet, 2005). The VEGF protein is a homodimeric glycoprotein. Molecular docking results revealed that hexadecanoic acid, 2-Hydroxy davanone, davanone and iso davanone were the best compounds interacting with the suspected binding residues at the active VEGF site (see Supporting Information Figure 18).

Table 5. The docking energies of ctDNA inhibitors.

| Compound | DE ^a (Kcal/mol) | ETOR (kT) | VDW (kT) | EIE (kT) |
|------------------------------|----------------------------|-----------|----------|----------|
| 5,5-dimethyl Furanone | -5.053 | 278.718 | 336.166 | -1550.53 |
| Lavender lactone | -4.191 | 276.077 | 337.637 | -1574.26 |
| Cis-Arbusculon | -4.746 | 278.032 | 348.588 | -1575.77 |
| Trans-Arbusculon | -4.576 | 278.901 | 344.688 | -1555.81 |
| cis-Linalool oxide | -4.611 | 286.323 | 357.123 | -1576.79 |
| Davanafuran | -5.247 | 288.118 | 358.120 | -1573.26 |
| (E)- β -Elemene | -5.022 | 281.461 | 363.974 | -1578.91 |
| Davana ether | -5.009 | 281.490 | 363.947 | -1578.89 |
| Davanone | -6.223 | 280.120 | 356.716 | -1571.56 |
| Iso davanone | -5.843 | 278.929 | 361.956 | -1562.72 |
| Davanol D1 | -6.171 | 283.362 | 360.574 | -1568.81 |
| Eudesma-11-en-4 α -ol | -4.393 | 282.663 | 377.026 | -1567.59 |
| 2-Hydroxy davanone | -5.884 | 280.596 | 335.328 | -1550.61 |
| Tetradecanoic acid | -6.984 | 257.680 | 349.569 | -1562.28 |
| Hexadecanoic acid | -7.210 | 255.039 | 349.577 | -1564.55 |

^aDE: Docking Energy; ETOR: torsion Energy; VDW: van der Waals; EIE: electrostatic interaction Energy.

The calculated docking energies for these molecules were, respectively, -4.003 , -4.066 , -3.992 and -3.984 Kcal/mol. Lavender lactone was the weakest interacting compound with this receptor. The calculated docking energy calculated for this compound was -3.251 Kcal/mol, respectively. Also, among the anchored compounds, hexadecanoic acid, tetradecanoic acid, iso davanone and davanol D1 were the excellent compounds interacting with VEGFR-1 (see Supporting Information Figure 19). The calculated docking energies calculated for these compounds were, respectively, -8.504 , -7.470 , -6.534 and -7.345 Kcal/mol. Davana furan was the weakest compound with this receptor. The calculated docking energy calculated for this compound was -4.671 Kcal/mol. Conforming to our molecular docking results, hexadecanoic acid, tetradecanoic acid, iso davanone and davanol D1 were the best compounds interacting with VEGFR-2 (see Supporting Information Figure 20). The calculated docking energies calculated for these compounds were, respectively, -7.318 , -6.753 , -4.780 and -6.340 Kcal/mol. With the exception of (E)- β -Elemene, other compounds were found in the active site of VEGFR-2. The calculated docking energy observed for this compound was -3.314 Kcal/mol. We observed that hexadecanoic acid showed a binding affinity for interacting with receptors for cDNA, VEGFR-1, VEGFR-2 and VEGF. Tetradecanoic acid interacted with cDNA, VEGFR-1 and VEGFR-2. In addition, davanol D1 has the affinity to interact with the cDNA of VEGFR-1 and VEGFR-2. Davanone

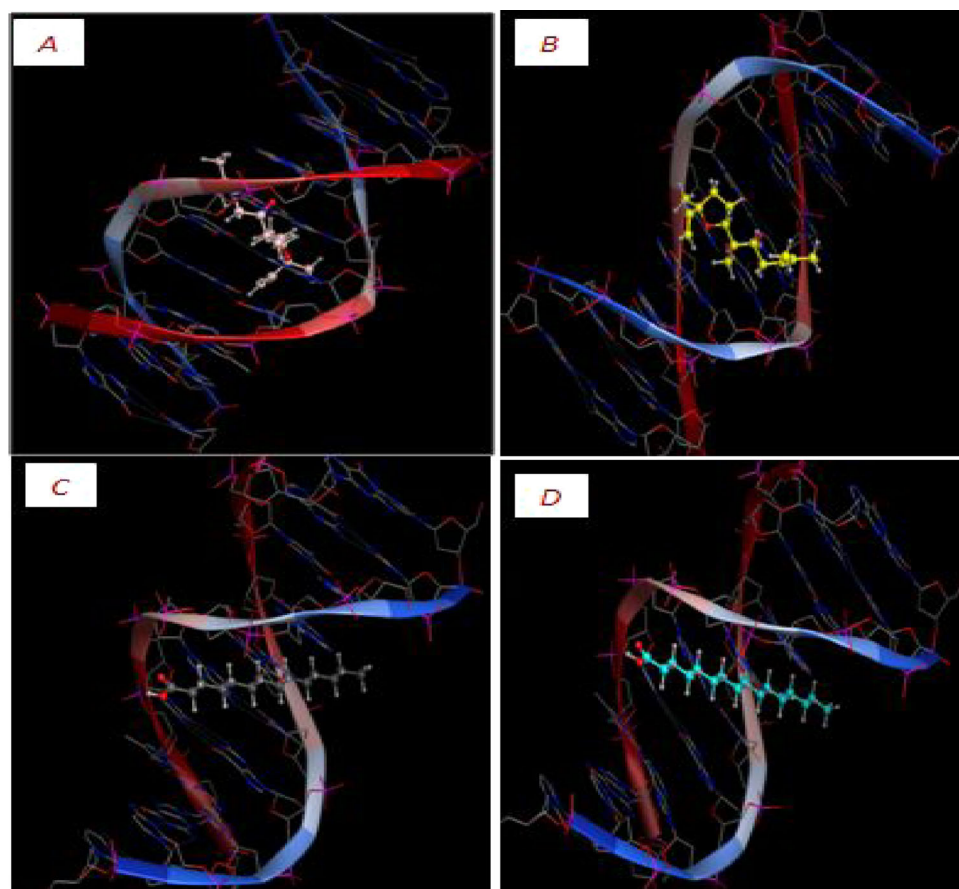


Figure 9. The graphical illustration of interaction between the four top docked compounds (A) davanone L13; (B) davanol D1 L18; (C) tetradecanoic acid L24 and (D) hexadecanoic acid L25 with ctDNA. The graphical illustration of interaction between the top docked compounds (A) Davanone L13; compound (B) hexadecanoic acid L25 with ctDNA.

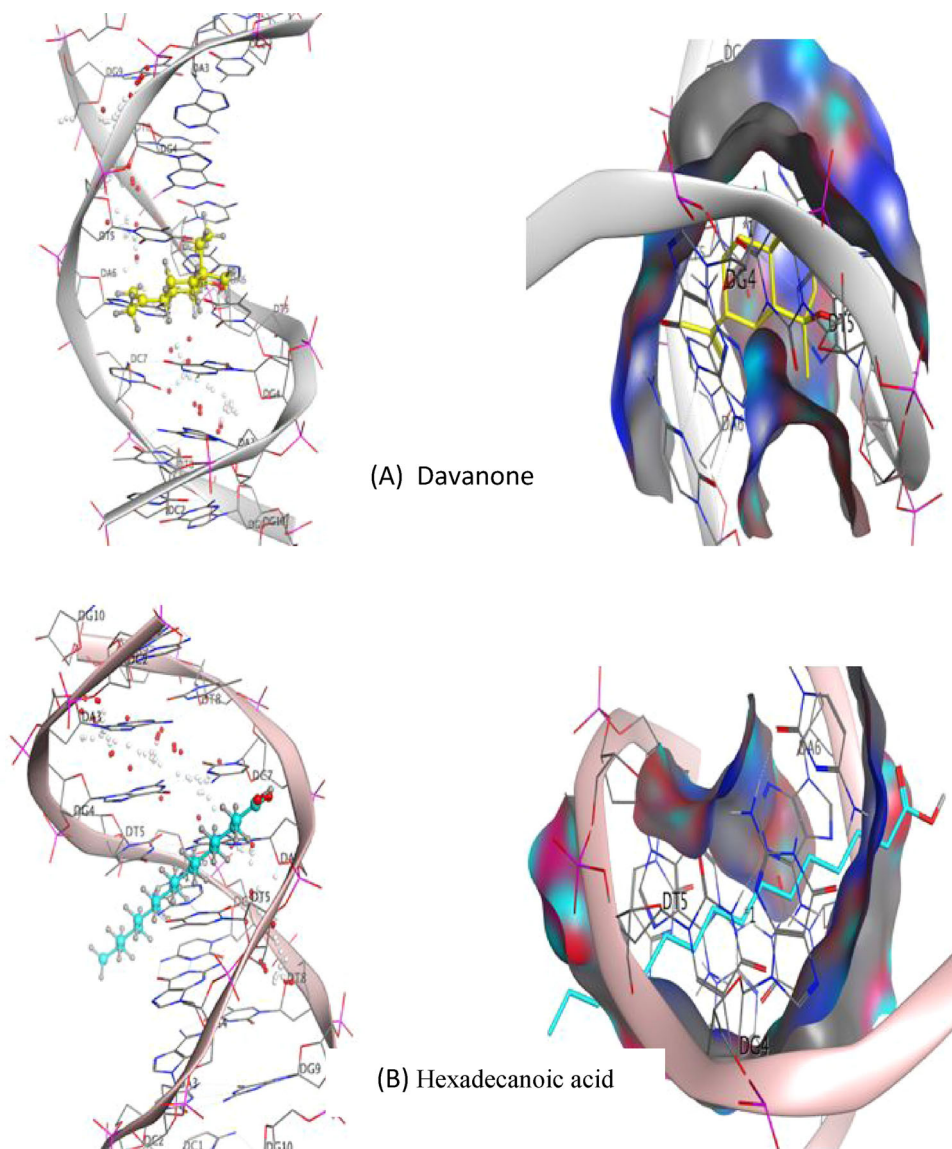


Figure 9. Continued

has the affinity to interact with cDNA and VEGF. Iso davanone has the affinity to interact VEGF of VEGFR-1 and VEGFR-2. 2-hydroxy davanone has the affinity to interact with only VEGF. Inhibiting the interaction between VEGF and VEGFR is an important way to prevent angiogenesis and the development of a malignant tumor in other tissues. The results given, we can conclude that the best inhibition this produced by two molecules hexadecanoic acid (Ligand L25) and davanone (Ligand L13).

3.8. MDs analysis

Thermodynamic properties using the MD simulation approach, we have studied the evolution thermodynamic properties of the ligands of complex 25 and 13 in NVT ensemble (Table 7).

The results represented in Table 7 revealed that the kinetic energies of translation and the internal energy for the L25 and L13 (for L13 see Supporting Information Figures

15–17) in the VEGF enzyme and the VEGFR1 receptor, were low compared to the VEGFR2 receptors, and the fluctuation in pressure for the VEGFR2 receptor was significant. Therefore, L13 and L25 were predicted to be the most interactive system. These results were in total agreement with the docking prediction results (see Tables 4 and 5). We can show the detailed analysis of MD simulation results of only compound L25 with target VEGF receptors to Figures 11–13.

3.9. In silico assessment of the ADME properties and drug-likeness

A computational study of best compounds was performed for the assessment of ADME properties (Table 8).

The ADME analysis of other compounds was shown in Supporting Information Table 8(a). The results exposed in Table 8 revealed that compounds (L13, L24 and L25) have high absorption. Also, we can note that these compounds comply with Lipinski's rule of 5, Veber's rule and Egan's rule.

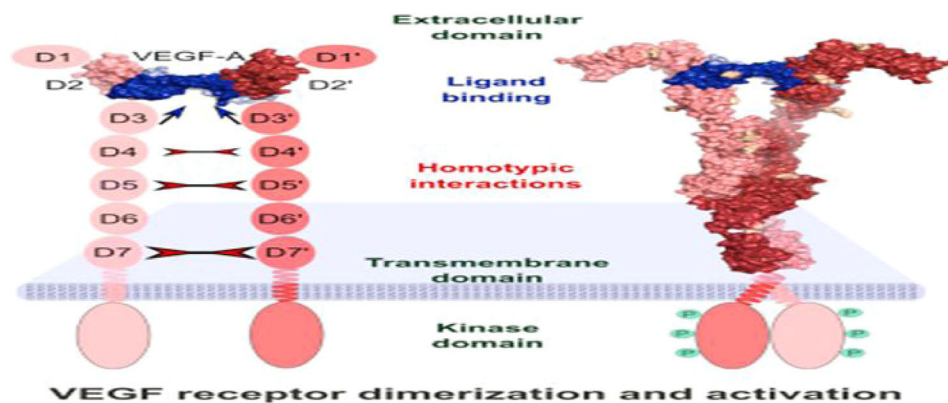


Figure 10. Structure of the VEGF receptor dimerization and activation in extracellular domain.

Table 6. The docking energies of VEGF/VEGFR inhibitors.

| Compound | Receptor | DE | | | |
|------------------------|----------|------------|-----------|-----------|----------|
| | | (Kcal/mol) | ETOR (kT) | EVDW (kT) | EIE (kT) |
| Davanone L13 | VEGF | -3.992 | 435.552 | 1130.180 | -2146.00 |
| | VEGFR -1 | -6.529 | 1348.713 | 4060.459 | -7791.00 |
| | VEGFR-2 | -4.778 | 1408.056 | 6684.242 | -16584.6 |
| Davanol D1 L18 | VEGF | -3.811 | 431.094 | 1113.038 | -2086.24 |
| | VEGFR -1 | -7.345 | 1387.813 | 4085.864 | -7713.85 |
| | VEGFR-2 | -6.340 | 1433.824 | 7013.454 | -16721.3 |
| 2-Hydroxy davanone L20 | VEGF | -4.066 | 432.167 | 1173.493 | -2215.62 |
| | VEGFR-1 | -6.161 | 1384.406 | 3576.242 | -7906.98 |
| | VEGFR-2 | -3.391 | 1410.636 | 7085.165 | -16877.8 |
| Tetradecanoic acid L24 | VEGF | -3.809 | 415.596 | 1189.618 | -2260.86 |
| | VEGFR -1 | -7.470 | 1353.340 | 4062.425 | -7922.66 |
| | VEGFR-2 | -6.753 | 1367.229 | 6873.763 | -16871.6 |
| Hexadecanoic acid L25 | VEGF | -4.003 | 388.611 | 1137.990 | -2116.06 |
| | VEGFR -1 | -8.504 | 1378.607 | 4115.919 | -7973.84 |
| | VEGFR-2 | -7.318 | 1411.548 | 7260.273 | -16765.5 |

DE: docking energy; ETOR: torsion energy; VDW: van der Waals; EIE: electrostatic interaction energy.

Where $\log P$ values ranged between (1.66. 3.69 and 4.19), respectively, (<5), MW range (236.35. 228.37 and 256.42), respectively, (<500), HBA range 3 – 2 and 2 (≤ 10) and HBD range 1–1 and 1 (<5), suggesting that these compounds would not be expected to cause problems with oral bioavailability, and thus, showing possible utility of both compounds for developing the compound with good drug like properties.

About the absorption parameters compounds L13, L20, L24, L18 and L25 present a promising oral availability, due to the optimal Caco-2 cell permeability and HIA (>0.9 and $>90\%$, 197, respectively, Table 9) and skin permeability ($\log K_p < -2.5$ for L24 and L25 (Table 9). Active components: L13-davanone, L20-(2-hydroxy davanone, L18-davanol D1, L24-tetradecanoic acid and L25-hexadecanoic acid. BBB: Blood-brain barrier. ADMET: Absorption. Distribution. Metabolism and Excretion and Toxicity. Minnow toxicity: < -0.3 ; high acute toxicity, VDSS: < -0.15 low, >0.45 high, BBB: >0.3 cross BBB, < -1 poorly distributed to the BBB, CNS: > -2 penetrate CNS, < -3 unable to penetrate CNS, Low skin permeability: > -2.5 , Caco-2 permeability: >0.9 , Human intestinal absorption: >90 . The overall lecture of (Table 9) highlights that compounds L13 could be excellent candidate as drugs. However, lead to further studies and manipulations. All compounds are not substrates of the renal

Table 7. Thermodynamic properties calculated in reels units. Pressure $P = P^* \epsilon / \sigma^3$.

| SP _i | Method | H | U | EKT | P |
|-----------------|--------------|--------|----------|----------|----------|
| SPI | VEGR-Lig-13 | 0.3565 | 856.0253 | 1202.520 | -52.263 |
| | VEGR1-Lig-13 | 0.7526 | 1320.523 | 2542.023 | -653.254 |
| | VEGR2-Lig-13 | 1.0145 | 758.201 | 895.326 | -45.2365 |
| | VEGR-Lig-25 | 0.2105 | 850.2948 | 1141.135 | 346.096 |
| | VEGR1-Lig-25 | 0.8368 | 1331.317 | 3419.039 | -25.890 |
| | VEGR2-Lig-25 | 0.8934 | 827.0451 | 1123.195 | 50.126 |

Energy of configuration $U = U^* N\epsilon$. Translation Kinetic Energy $EKT = EKT^* N\epsilon$ and Enthalpy $H = H^*$.

organic cation transporter 2 (OCT2). All compounds passed the AMES tests. The volume of distribution (VDs) for our two best ligands L13 and L18 were, respectively, 0.199 and 0.033 suggest that the drug will be distributed in plasma since values of the VDss < -0.15 . So, VDss describes the extent of drug distribution and the fraction unbound describes the portion of free drug in plasma that may extravasate. The compounds L13 and L18 may be interesting potential agents as a part of an anticancer therapy to relieve cancer-related pain.

The compounds reveal almost intermediate values of VDss. Molecule L20 was entirely unable to penetrate the central nervous system (CNS). Molecules L24 and L25 were unable to penetrate plasma or tissue and presented Minnow toxicity. The absorption and distribution parameters, respectively, have been graphically represented by the extended and renewed version of the Edan-Egg model named Brain Or Intestinal Estimated (BOILED) permeation predictive model (BOILED-Egg) (Figure 14). The Figure 14 showed that all ligands enter the brain by crossing the blood-cerebrospinal fluid barrier except L9. The BBB is a biological barrier that protects the brain from molecules that are toxic to the CNS (Central nervous system). Permeability of the BBB is employed for drug delivery to the brain (Jain, 2012). The blocking of active efflux transporters likep-glycoprotein (P-gp) is a method worn to get through the BBB.

Knowing that p-glycoprotein (P-gp) is localized in several tissues, such as the intestines, the kidneys, the liver, the immune system at the level of the blood-brain and placental barrier and has a great variability of substrates. The latter plays a role in the immune and hematological systems is still hypothetical. Moreover, it is involved in the transport of

Table 8. Lipinski's rule of five for ADME analysis of best inhibitors (ligands).

| N° | Name | Lipinski's rule of five | | | | No. of rule violations Less than 2 Violations | Drug-likeness Lipinski's rule follows |
|----|--------------------|---|--------------------------------------|-------------------------------------|---|--|--|
| | | Molecularweight (g/mol) Less than 500 Dalton | Lipophilicity (MLogP) Less than 5 | Hydrogen bond donors Less than 5 | Hydrogen bond acceptors Less than 10 | | |
| 13 | Davanone | 236.35 | 2.54 | 0 | 2 | 0 violation | Yes |
| 18 | Davanol D1 | 238.37 | 2.63 | 1 | 2 | 0 violation | Yes |
| 20 | 2-Hydroxy davanone | 252.35 | 1.66 | 1 | 3 | 0 violation | Yes |
| 24 | Tetradecanoic acid | 228.37 | 3.69 | 1 | 2 | 0 violation | Yes |
| 25 | Hexadecanoic acid | 256.42 | 4.19 | 1 | 2 | 1 violation: MLOGP > 4.15 | Yes |

MW: molecular weight; MLogP: logarithm of partition coefficient of the compound between water and n-octanol: n-OHND: number of hydrogen bonds donors; n-ON: number of hydrogen bond acceptors; n-ROTB: number of rotatable bonds.

Table 9. Pharmacokinetic and toxicity evaluated parameters of best compounds.

| | | 13 | 20 | 18 | 24 | 25 |
|--------------|----------------------------------|---------|---------|---------|---------|---------|
| Absorption | Human intestinal | 96.829 | 95.234 | 93.407 | 92.691 | 92.004 |
| | Skin permeability | -2.269 | -3.169 | -2.286 | -2.705 | -2.717 |
| | Caco-2 permeability | 1.366 | 1.337 | 1.638 | 1.56 | 1.558 |
| Distribution | Surface area | 104.738 | 109.533 | 109.533 | 100.439 | 113.169 |
| | VDss (human) | 0.199 | 0.033 | 0.23 | -0.578 | -0.543 |
| | Fraction unbound (human) | 0.385 | 0.476 | 0.401 | 0.171 | 0.101 |
| | BBB permeability | 0.553 | -0.189 | 0.544 | -0.027 | -0.111 |
| | CNS permeability | -2.684 | -3.091 | -2.841 | -1.925 | -1.816 |
| Excretion | Total clearance | 1.512 | 1.432 | 1.494 | 1.693 | 1.763 |
| | Renal organic cation transporter | No | No | No | No | No |
| Toxicity | Oral rat acute toxicity (LD50) | 1.997 | 1.995 | 1.853 | | 1.44 |
| | AMES toxicity | No | No | No | No | No |
| | Tetrahymena Pyriformis toxicity | 1.265 | 0.995 | 1.499 | 0.978 | 0.84 |
| | Min now toxicity | 0.979 | 1.794 | 1.241 | -0.601 | -1.083 |

green = good, yellow = tolerable msmoh, red = bad. tdkhol.

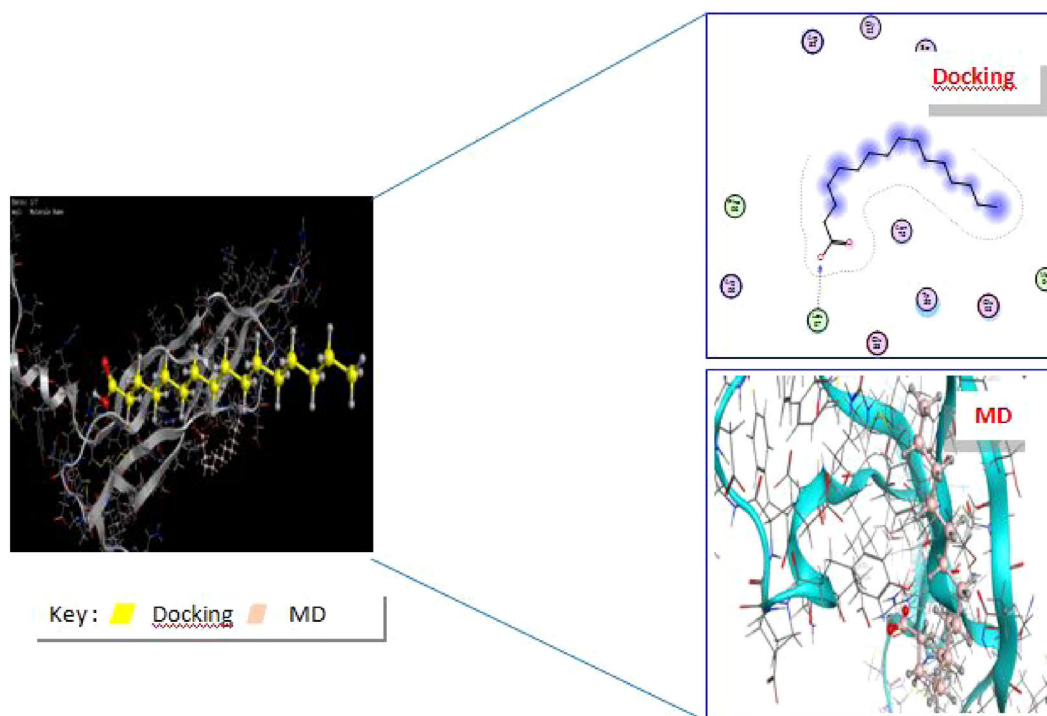


Figure 11. The compound – 25 hexadecanoic acid was docked without water well into the binding site of VEGF and has the highest dock score; there was also a clear difference between the final ligand pose and the docking pose after a molecular dynamics (MD) simulation.

certain interleukins and gamma interferon, as well as in the protection of stem cells against endogenous compounds and xenobiotics (Mesli & Bouchentouf, 2019). So, it is very effective to know their influences on the nervous system since their substrates are probably to change pharmacokinetics.

All molecule (P-gpsubstrate: No) were not predicted to be effluated from central nervous system by g-glycoprotein. So, it is preferable to choose L13 (davanone) to both VEGF (Vascular Endothelial Growth Factor)/VEGFR interaction to other ligands because L13 (davanone) have potent binding

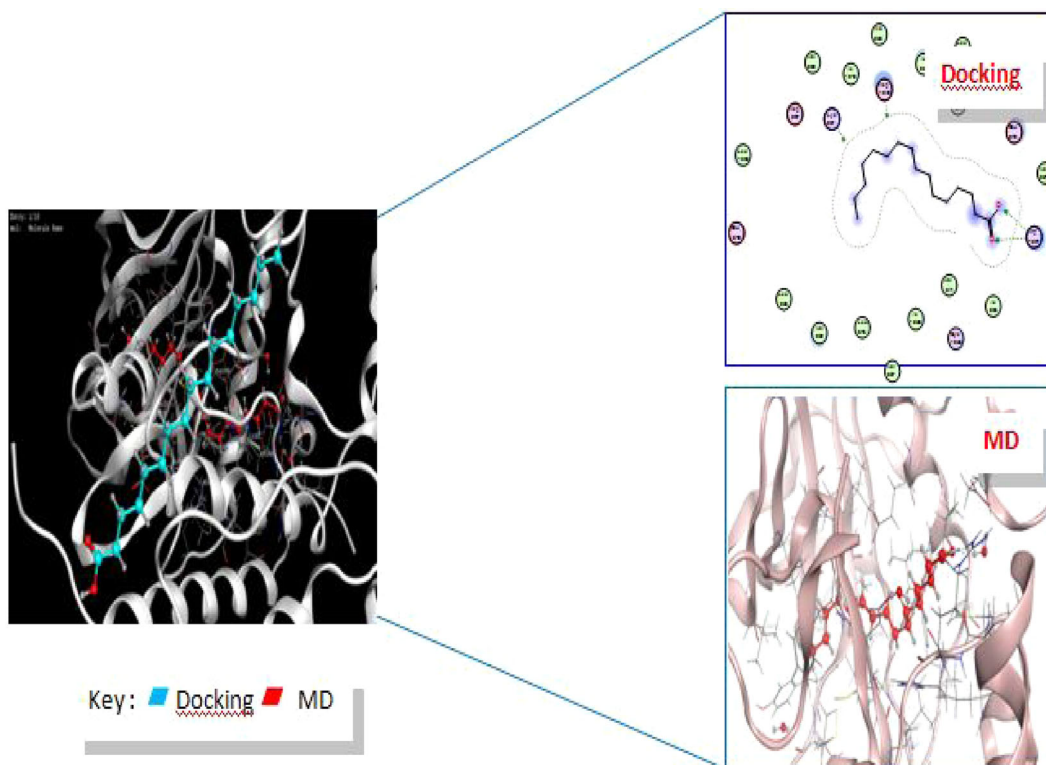


Figure 12. The compound – 25 hexadecanoic acid is docked without water well into the binding site of VEGFR-1 and has the highest dock score; there was also a clear difference between the final ligand pose and the docking pose after a MD simulation.

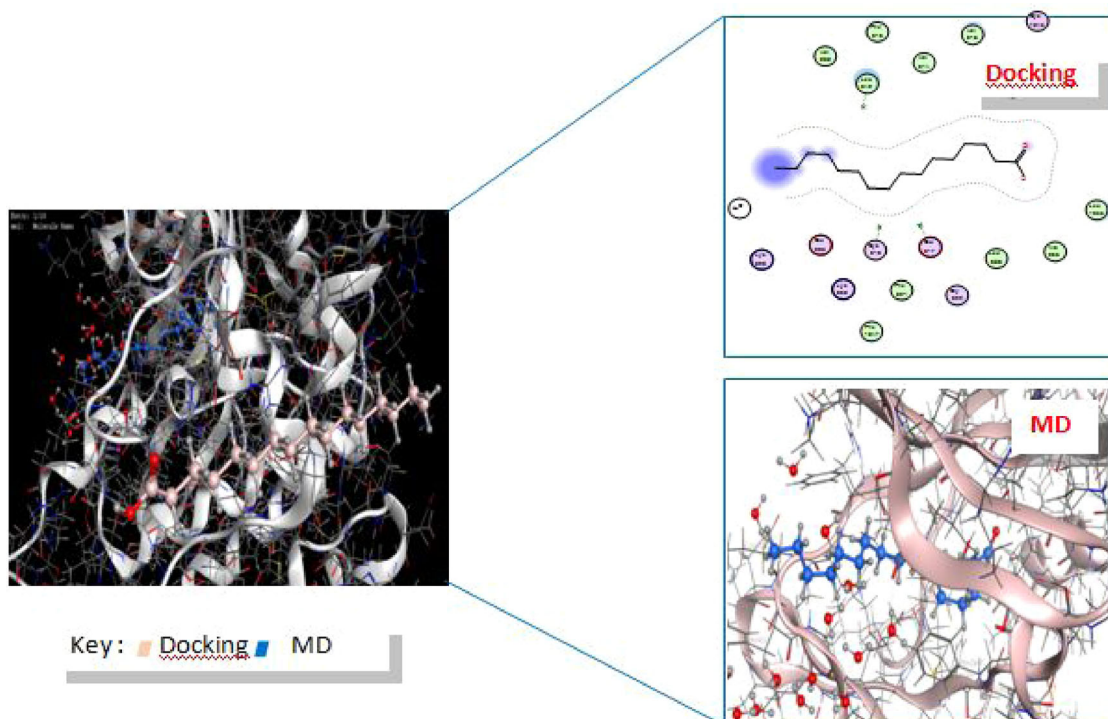


Figure 13. The compound – 25 hexadecanoic acid is docked without water well into the binding site of VEGFR-2 and has the highest dock score; there was also a clear difference between the final ligand pose and the docking pose after a MD simulation.

affinities, (L13 was not predicted to be effluated from central nervous system by g-glycoprotein). The ADMET properties and BOILED-Egg (Figure 14) plot validate the compound 13 and 18 pass the brain barrier and have high absorption in the intestines with good bioavailability. Compound 13

(davanone) has the highest binding affinity among all the inhibitors, it is proposed as a natural orally active drug. By analyzing the drug's score (*S*-value), davanone ligand 13 showed the lowest *S*-value –6.223 Kcal/mol with ctDNA for VEGF (Vascular Endothelial Growth Factor)/VEGFR interaction,

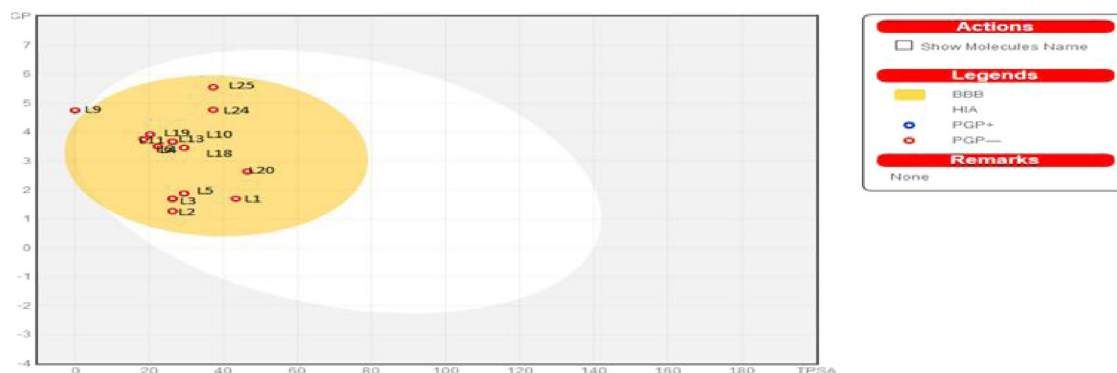


Figure 14. BOILED-Egg plot. Points located in the BOILED-Egg's yolk (yellow) represent the molecules predicted to passively permeate through the BBB. Whereas the ones in the egg white were relative to the molecules predicted to be passively absorbed by the gastrointestinal tract; the blue dots indicate the molecules for which it was expected to be effluated from the central nervous system (CNS) by the P-glycoprotein. Whereas the red ones point-out to the molecules predicted not to be effluated from the CNS by the P-glycoprotein.

Table 10. Drug-likeness, lead-likeness and PAINS parameters of best compounds.

| MNP ID | | L13 | L20 | L18 | L24 | L25 |
|--------------------------|---------------------|-----|-----|-----|-----|-----|
| Drug-likeness | Lipinski violations | 0 | 0 | 0 | 0 | 1 |
| | Ghose violations | 0 | 0 | 0 | 0 | 1 |
| | Veber violations | 0 | 0 | 0 | 1 | 1 |
| | Egan violations | 0 | 0 | 0 | 0 | 1 |
| | Muegge violations | 0 | 0 | 0 | 1 | 2 |
| Lead-likeness violations | 1 | 0 | 2 | 3 | 2 | |
| PAINS alerts | 0 | 0 | 0 | 0 | 0 | |

resulting as the best ligand among our selected ligands to block tumor growth without inducing too many side effects. So, ligand 13 (davanone) was proposed to be a potential therapeutic inhibitor of VEGF/VEGFR interaction. In this study, Ligand 18 (davanol D1) reveals the lowest Score of -6.171 Kcal/mol with ctDNA was predicted as the second-best target inhibitor (with maximum binding affinity for VEGF/VEGFR interaction after L13).

In addition to the Lipinski rule of five, other four drug-likeness rules named Ghose, Egan, Veber and Muegee, have been contemporarily satisfied by compound L13 with the exception of molecule L24 and L25 (Table 10). Instead, the stringent lead-like criteria of Teague have been passed by compounds L13. Since lead-likeness tests were intended to provide leads with high affinity in high-throughput screens that allow for the discovery and exploitation of additional interactions in the lead-optimization phase, molecule L13 was excellent candidate to be investigated based on scaffold hopping approach. Finally, the outcome of the pan assay interference structures (PAINS) model, conceived to exclude small molecules that are likely to show false positives in biological assays, post no alert for the compound L13, concerning the presence of a molecule moiety.

3.10. Pharmacokinetics and medicinal chemistry properties

The results Medicinal Chemistry and Pharmacokinetics revealed that all compounds have High GI absorptions. We notice that there was a complement between our results for assessment of ADME properties (Table 9) and the predicted

results in medicinal chemistry and pharmacokinetics (Table 11).

Davanone of essential oils of the root of the *S. grandiflorus* (Ligand 13) was predicted to be characterized by a high lipophilicity and high coefficient of skin permeability $\log K_p$ by providing hexadecanoic acid (Ligand 25) and 2-hydroxy davanone (Ligand 20). We can explain that the more negative the $\log K_p$ (with K_p in cm/s), the less the molecule was absorptive to the skin, which explains the reliability of our study. We cite the works which have valid the stability of complexes and their affinities by MOE software (Mesli & Bouchentouf, 2019; Mesli et al., 2019). $\log Po/w L25 > \log Po/w L18 > \log Po/w L13$. So, Ligand L13 represents high affinity with VEGF receptors. Synthetic accessibility (SA) was a major factor to take into account in this selection process an acceptable value between 3.96, 4.27 and 2.31 for the ligands (L13, L18 and L25), respectively, these are more encouraging compounds which can be synthesized or subjected to bioassays or other experiments. Our previous research has revealed that oils from our region have better biological activities (Benyoucef et al., 2020; Drach et al., 1996). Validation of our results, for essential oils of the *S. grandiflorus* immunotherapy (Clinic) is mentioned in (Table 12). These four (4) molecules (Afinib, Erlotinib, Gefitinib, Osimertinib) prevent tumor growth by inhibiting the action of EGFR, a protein that sends a division signal to the cell. Unfortunately, they have side effects, and therefore, the main anti-angiogenic agent administered is the Bevacizumab a monoclonal antibody (non-small cell lung cancer). Sunitinib blocks the tyrosine kinase activities of vascular endothelial growth factor receptor 2 (VEGFR2), is an oral small-molecule, multitargeted receptor tyrosine kinase (RTK) inhibitor.

Regorafenib is an orally bioavailable small molecule with potential anti-angiogenic and antineoplastic activities. There are even checkpoint inhibitors which are monoclonal antibodies but they have a different action, which is why they are classified as specific immunotherapies. The goal of which is to restore an immune response that allows the immune system to attack abnormal elements. The goal is to find a natural inhibitor molecule with no side effects. Our molecular docking results with ctDNA coincided with clinical results; the oxygenated compounds were the most dominant with a

Table 11. Pharmacokinetics and Medicinal Chemistry properties for best molecule.

| Molecule | Pharmacokinetics | | Medicinal Chemistry | |
|------------------------|------------------|-----------------------------|---|-------------------------|
| | GI absorption | Log K_p (skin permeation) | Lead likeness | Synthetic accessibility |
| Davanone L13 | High | -5.38 cm/s | No; 1 violation: MW < 250 | 3.96 |
| Davanol D1 L18 | High | -5.17 cm/s | No; 2 violations: MW < 250, XLOGP3 > 3.5 | 4.27 |
| 2-Hydroxy davanone L20 | High | -6.50 cm/s | Yes Pains; o alert Brenk:2 alerts: isolated_alkene. michael_acceptor_1 | 4.04 |
| Tetradecanoic acid L24 | High | -3.35 cm/s | No; 3 violations: MW < 250. Rotors > 7. XLOGP3 > 3.5 | 2.09 |
| Hexadecanoic acid L25 | High | -2.77 cm/s | No; 2 violations: Rotors > 7. XLOGP3 > 3.5 | 2.31 |

Table 12. Energy balance of complexes formed with ctDNA under other clinical and our results for essential oils of the *S. grandiflorus*.

| Drugs | ctDNA | Voie |
|-------------------------------|--------|--------------------------------------|
| Immunotherapy (Clinic) | | |
| Lung cancer | | |
| Afatinib | -8.630 | Injectable |
| Gefitinib | -8.365 | orally at a pre determine dose daily |
| Erlotinib | -7.481 | Injectable |
| Osimertinib | -7.811 | orally at a pre determine dose daily |
| Kidney cancer | | |
| Sunitinib | -8.019 | Oral |
| Liver cancer | | |
| Regorafenib | -8.578 | Oral |
| Our Results | | |
| Davanone Lig13 | -6.223 | Oral |
| Hexadecanoic acid Lig25 | -7.210 | Oral |

percentage 76.6–97.2%. Our ligand natural, hexadecanoic acid L25 (13.2%) better stabilized the system with its energy of -7.210 Kcal/mol, we compared with the components of Immunotherapy Clinic (see Table 12). The latter presents violations and toxicity, it cannot be presented as the best ligand, but davanone (Ligand13) with energy binding of -6.223 Kcal/mol could be excellent candidate as drugs because represents better the absorption 96.829 (see Table 8) among others ligands and the volume of distribution (VDss) suggest that the drug will be distributed in plasma. Moreover, the latter does not represent any violation (see Table 9) by contribution to the other ligands and again from the Table 10. Davanone was characterized by a high lipophilicity and high coefficient of skin permeability log K_p . Therefore, we propose ligand 13 as the best ligand which allows the inhibition of Vascular Endothelial Growth Factor receptors (VEGFR) and in the meantime, we suggest Ligand L13 davanone presented in essential oils of the root of the *S. grandiflorus* with its validated activity Score (-3.992, -6.529 and -4.778), respectively, for (VEGF, VEGFR-1 and VEGFR-2) as a new oral ligand despite obeying Lipinski's rule.

The present bioinformatic analysis MDs simulations were used to scrutinize novel oxygenated sesquiterpenes as inhibitor of Vascular Endothelial Growth Factor receptors (VEGFR). Preceding studies has indicated that davanone has been shown to have antitumor activity *in vitro*, many studies were focused on the inhibitory effect of the (*Eugenia jambolana*, *Musa paradisiaca* and *Coccinia indica*) extracts and date palm pollen (DPP), to key enzymes linked to cancer therapy, vascular endothelial growth factor receptors (Rasouli et al., 2018).

The IC_{50} value calculated for the hydro-alcoholic extract of (DDP) examined in the angiogenesis model was 260 μ g/mL. The results showed a decrease in the proliferation of endothelial cells. The results showed that the expression of the VEGF, MMP-2 and MMP-9 genes was significantly low. Harsha

Raj et al. (2017), we proved that the molecule from EA extracts of *E. Jambolana* and *M. Paradisiacal* can be developed into anticancer drugs. EA extracts of *E. Jambolana* and *M. paradisiacal* exhibited the highest cytotoxicity ($IC_{50} = 25$ and 60 L g/mL), inhibited cell proliferation (up to 81%). The same acid that we have validated (*N*-hexadecanoic acid) has proven to be of major interest in previous research. This molecule extract from the leaves of *Kigelia pinnata* showed significant cytotoxicity against human colorectal carcinoma cells (HCT-116) with an IC_{50} value of 0.8 μ g/mL (Ravi & Krishnan, 2016). The researches of Mansoor et al we have shown that always our best validated molecule (*N*-hexadecanoic acid) proved its anticancer effect (Saljooghianpour & Javaran, 2013). The study of Hosseinzadeh et al. (2019) we have exposed that sesquiterpenoids (hydroperoxide of davanone (1) and hydroxy davanone (2)) were identified as the active constituents responsible for the cytotoxic property of the petroleum ether extract of *A. aucheri*. This research has shown that 24h treatment with IC_{50} concentration of compound 2 increased caspase-3 activation in A2780 and MCF-7 cell lines and compounds 1 increased activity of caspase 3 in A2780 and SK-N-MC cell lines, between these compounds, compound 1 exhibited more potent activity against the MCF-7, SK-N-MC and A2780 cell lines with IC_{50} values of $8.45 \pm 0.81 \mu$ g/mL, $9.60 \pm 1.32 \mu$ g/mL and $10.9 \pm 2.03 \mu$ g/mL in A2780, MCF-7 and SK-N-MC cells, respectively. Compound 1 inhibited the growth of human cancer cells by induction of apoptosis (Hosseinzadeh et al., 2019). In our research the software package (MOE) does not detect any mark of the hydrophobic interactions between davanone and both the Vascular Endothelial Growth Factor receptors; what may be connected to the large size of this compound and the high number of torsion angles. The results were identified to have inhibitory activities against novel cDNA, VEGF and its receptors. Of these ligands, davanone (Ligand 13) has a stronger bond and high absorption in the intestines with good bio-availability. Therefore, the study carried out in this research reveals many secrets conveyed by the use of magic plants. Currently, herbal medicine offers solutions to heal with plants. It is a solution that is both alternative and complementary to the treatments of classical medicine, which are more and more popular and whose effectiveness is increasingly recognized.

4. Conclusion

In conclusion, the results showed that plant is a good source of davana derivatives and oxygenated components. The chemical composition of *S. grandiflorus* essential oil showed

a significant variability. Results showed the positive correlations between the amounts of components and altitude. The inhibition of Vascular Endothelial Growth Factor receptors was theoretically investigated by two methods of computational chemistry: molecular docking analyzes MD simulations, ADME properties and pharmacological knowledge. Our ligand and natural inhibitor davanone has an affinity to interact with cDNA, VEGF and its receptors. This model showed a significant decrease of energy (score) and there by an increase of the inhibition activity. However, the combination between docking simulation and MD simulations proved the stability of the complex formed by ligand L13. Although two compounds L13 and L25 have potent binding affinity with VEGF receptors in the docking simulation. Moreover, the ADMET properties and BOILED-Egg plot validated the compound 13 and 25 that passed the brain barrier and have high absorption in the intestines with good bioavailability. Davanone has the highest binding affinity among all the inhibitors, it is proposed as a natural orally active drug and reliable treatment during the first stage of cancerous cells.

Ethics approval and consent to participate

Not applicable.

Consent For publication

Not applicable.

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In silico design of enzyme α -amylase and α -glucosidase inhibitors using molecular docking, molecular dynamic, conceptual DFT investigation and pharmacophore modelling

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is characterized by elevated blood glucose levels and can lead to serious complications such as nephropathy, neuropathy, retinopathy and cardiovascular disease. The aim of this work is to identify and investigate the inhibition mechanism of natural flavonoids and phenolics acids against, the α -amylase (α A) and α -glucosidase (α G). Therefore, we used different approaches; such as conceptual DFT and pharmacophore mapping in addition to molecular mechanics, dynamics and docking simulations. Whereas, a close agreement was found out to decide that Linarin (Flavones) provides more optimized inhibition of α A and α G enzymes. Our results have shown that Linarin could be useful as preventative agent, and possibly therapeutic modality for the treatment of metabolic diseases.

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1. Introduction

The incidence of type 2 diabetes is increasing at an alarming rate (Fleury-Milfort, 2008). Recent evidence suggests that high postprandial plasma glucose concentration is associated with an increased risk of developing type 2 diabetes and metabolic syndrome. Therefore, control of postprandial blood glucose level has been suggested as an important and effective way to mitigate and prevent the illnesses of hyperglycemia and diabetes.

α -Amylase (α A) is a key enzyme in the digestive system and catalyzes the initial step in the hydrolysis of starch (Vadivelan et al., 2012). It has been proposed that inhibition of α A can effectively control the postprandial elevation of the blood glucose level (Yuan et al., 2014). α -Glucosidase (α G), which is located in the brush border of the enterocytes of the jejunum, is the most important enzyme in carbohydrates digestion (Proença et al., 2017). It catalyzes the hydrolysis of 1,4- α bonds of the unabsorbed oligo- and disaccharides, and converts them into monosaccharides, namely, glucose, which are absorbed in the upper jejunum, resulting in hyperglycemia. Inhibitors of α G can retard the decomposition and absorption of dietary carbohydrates by restricting the breakdown of linear or branched oligosaccharide units like α -limit dextrins, maltose and maltotriose to produce glucose, thereby preventing glucose absorption into blood stream and suppress the postprandial hyperglycemia

(PPHG) (Proença et al., 2017). Among the therapeutic approach for treating type 2 diabetes is to decrease the (PPHG) by slowdown the intestinal absorption of glucose through the inhibition of the enzymes, α A and α G (Ali Asgar, 2013). Polyphenols are the important inhibitors of intestinal enzymes which are able to suppress carbohydrate digestion, delay glucose uptake and consequently, reduce blood sugar levels. Although drugs such as acarbose, voglibose and miglitol inhibit α G and α A in practice, they produce undesired side effects such as bloating, abdominal discomfort, diarrhea and flatulence (Alqahtani et al., 2019). So, it is vital to search for other compounds with acceptable α A and α G inhibitory activity but without serious side effects. Flavonoids are promising modulators of this enzyme's activity (Alqahtani et al., 2019).

The study of natural products is one of the strategies for the discovery of new drugs that can be used in type 2 diabetes mellitus therapy. Many phenolic compounds such as gallic acid, tannic acid, morin, luteolin, kaempferol, myricetin, quercetin and apigenin have been shown to exhibit inhibitory effects against α A and α G (Abdelli et al., 2020; Meng et al., 2016).

The aim of this research was the bioinformatical study of α A and α G inhibition of PPs in all two main classes: by flavonoids and phenolics acids, compared to acarbose, voglibose and miglitol which are oral drugs inhibitors of those enzymes. The main interest was to develop new potential

inhibitors of the α A and α G interaction and finally discussed with the bioactivity scores, drug likeness, pharmacokinetics, molecular docking and molecular dynamic analysis of major components. The more we know about these interactions, the more we can do with that knowledge. Although many efforts have been made to produce the natural and reliable treatment during the first stage of type 2 diabetes mellitus (T2DM).

2. Material and methods

2.1. Selection of receptors and ligands

In this study, 29 compounds were mainly incorporated; the interaction of best flavonoids and phenolic acids obtained from the simulation of molecular docking with the lowest energy scores from compounds, as described in Table 1, were investigated. The structures of inhibitors (see Supplementary Table 1a) were downloaded from the PubChem server (<https://pubchem.ncbi.nlm.nih.gov>) (Wang et al., 2017). The PDB database (<https://www.rcsb.org/>) was used to obtain the complete structures of α A and α G enzymes 4GQR and 3L4Y, respectively (Figure 1). In general, the protein structure with a resolution between 1.5 and 2.5 Å have a good quality for further studies (Clément & Slenzka, 2006; Didierjean & Tête-Favier, 2016) whereas, the resolution value of α A and α G belongs to this interval.

2.2. Molecular docking

At first, we minimized the energy of the obtained 3D structures of the enzymes and the ligands and their geometries were conducted using Hamiltonian AM1 implanted in MOE software and then isolation of the active site of the targets was carried out. The most stable geometry of each ligand was minimized by the same method (AM1) and then, all simulations were run by using all explicit salvation models using TIP3P water.

In the end, positioning the ligands into the enzyme's active site was done using the docking module implanted in MOE software and, the binding energy (score) between ligands and targets was calculated using molecular mechanics (Molecular Operating Environment (MOE), 2013).

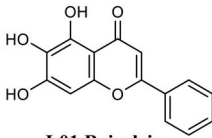
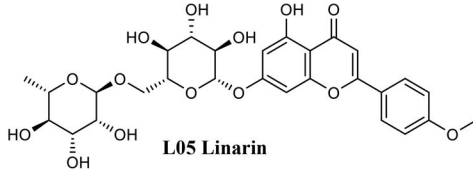
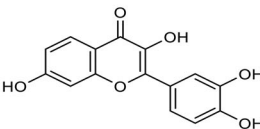
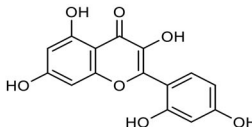
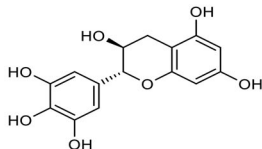
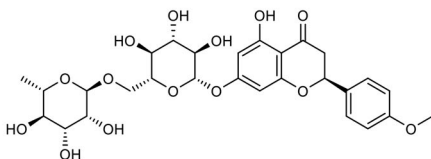
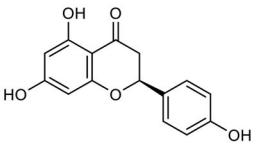
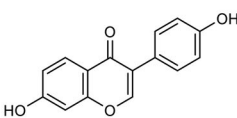
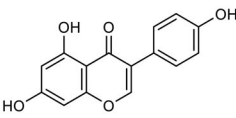
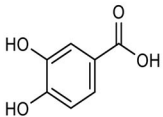
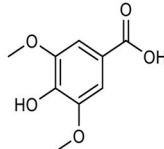
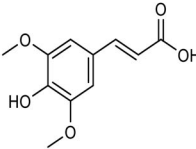
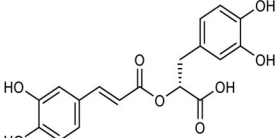
2.3. Molecular dynamics simulation, DFT calculations, ADMET, PASS cytochromes P450 and pharmacophore mapping

The best conformer of α A and α G enzyme with ligands was subjected to molecular dynamics simulations MD was performed for both the complex (4GQR–Ligand, 3L4Y–Ligand) using the MOE 2014.09 software (*Molecular Operating Environment (MOE)*, 2013). Molecular dynamics (MD) aims to numerically simulate condensed phases of a molecular system in order to understand, predict and calculate the properties of a studying system (Adcock & McCammon, 2006). The stability of the targets with the best selected natural product compound was determined using MD simulations.

The MD simulations were conducted by using Nose–Poincaré–Andersen (NPA) equations in 20 ns (Bond et al., 1999; Sturgeon & Laird, 2000). The Berendsen thermostat is an algorithm to rescale the velocities of particles in molecular dynamics simulations to control the simulation temperature (Berendsen et al., 1984). The coordinates were stored every 0.2 ps to get an accurate view of molecular movement. In all simulations the van der Waals cut-out distance was set to 8 Å. Energy minimization process was applied by using MMFF94x force field (Parikesit et al., 2015). The default protocols and steps of the MD were used to optimize the system's equilibrium for 100 ps and the production run in 600 ps. The MD simulations have been carried out by heating the complex system from 300 up to 330 K (Berendsen et al., 1984). During the production step, the root mean square deviation (RMSD) obtained was observed to determine the stability of the complex (Parikesit et al., 2015). Here, we have shown the detailed analysis of MD simulation results of best compound LinarinL5 with target α A and α G enzyme were shown in (Figures 6 and 7) because these compounds show better binding affinity for both receptors. To identify the potential of these compounds, molecular dynamics studies were performed for canonical NVT units using MOE. In the end and according to the molecular dynamics simulation analysis among these two compounds, the most active compounds were L5 and L14 in α A and α G enzymes. The molecular operating environment (MOE) software was used for our study because it has proven its performance in several recent studies; we can cite some example of work: Sadia Naz et al. (2021), Mourad Stitou et al. (2021), Daoud Ismail et al. (2018), Mehreen Ghufraan et al. and Mesli Fouzia and Ghalem (2017). Furthermore, the stable conformation obtained in the MD simulation between the best two ligands and α A and α G enzyme was performed by iMODS. iMODS is a fast and easy server for determining and measuring the protein flexibility (Awan et al., 2017; Lopéz-Blanco et al., 2011). It can be used to investigate the values of deformability, eigenvalues, variance, co-variance map and elastic network. After that, The *Gaussian 09* software (Frisch et al., 2009) is used for DFT calculation, Becke's three-parameter exchange potential and Lee–Yang–Parr correlation functional (B3LYP) theory with 6-311 G(d,p) basis set, were used (Lopéz-Blanco et al., 2014; Lopéz-Blanco et al., 2011). The DFT calculation was done for the two best ligand molecules. The result of DFT calculation is summarized in Table 10 and Figure 11.

The molecular structures of the best ligands were analyzed using SWISSADME server (<http://www.swissadme.ch/>). The results of ADME/T for best ligand molecules are summarized in Table 8. In the drug-likeness property test, Lipinski's rule of five or not, along with some other properties were predicted. The drug likeness properties of the selected compounds were scrutinized using SWISSADME server as well as the OSIRIS Property Explorer (Filimonov et al., 2014; Geronikaki et al., 1999). The results of drug likeness property analysis are depicted in Table 9. Lipinski's rule of five (Lipinski et al., 1997), Veber's rule (Veber et al., 2002), Egan's rule and Polar Surface area (TPSA), number of rotatable were calculated using SwissADME properties calculation online.

Table 1. Various drugs used in the *in silico* docking studies.

| I- Flavonoids | |
|---|---|
|  L01 Baicalein IC ₅₀ αG = 32 μM ⁶² IC ₅₀ αA = 446.4 ± 73.9 μM ⁶³ |  L05 Linarin |
| Flavones | |
|  L08 Fisetin IC ₅₀ αG = 08 μM ⁶⁴ IC ₅₀ αA = 19.6 ± 6.4 μM ⁶⁵ |  L10 Morin IC ₅₀ αG = 4.48 μM ⁶⁶ IC ₅₀ αA = 23 ± 1 % 200 μM ⁶⁷ |
|  L12 Gallocatechin IC ₅₀ αG = 119.34 μM ⁶⁸ IC ₅₀ αA = 328.06 μM ⁶⁸ | |
| Flavanones | |
|  L14 Didymin IC ₅₀ αG = 4.20 ± 0.6 μM ⁶⁹ IC ₅₀ αA = 31.62 ± 2.8 μM ⁶⁹ |  L15 Naringenin IC ₅₀ αG = 50 μM ⁶⁹ IC ₅₀ αA = >0.50 10 ³ μM ⁶⁹ |
| Isoflavones | |
|  L19 Daidzen IC ₅₀ αG = 14 μM ⁶⁹ IC ₅₀ αA = >0.50 10 ³ μM ⁶⁹ |  L20 Genistein IC ₅₀ αG = 7 μM ⁶⁹ IC ₅₀ αA = >0.50 10 ³ μM ⁶⁹ |
| II-Phenolic acids | |
| Hydroxy benzoic acids | |
|  L21 protocatechuic acid IC ₅₀ αG = 411.17 ± 26.6 μM ⁶⁴ IC ₅₀ αA = 11.54 ± 0.45 10 ³ μM ⁶⁴ |  L24 Syringic acid IC ₅₀ αG = 369.78 ± 25.73 μM ⁶⁴ IC ₅₀ αA = 44.81 ± 2.57 μM ⁶⁴ |
| Hydroxy cinnamic acids | |
|  L26 Sinapic acid IC ₅₀ αG = 6.1 ± 0.8 10 ³ μM ⁵⁸ IC ₅₀ αA = 12.3 ± 0.3 10 ³ μM ⁵⁸ |  L29 Rosmarinic acid IC ₅₀ αA = 1.4 10 ³ μM ⁵⁸ |

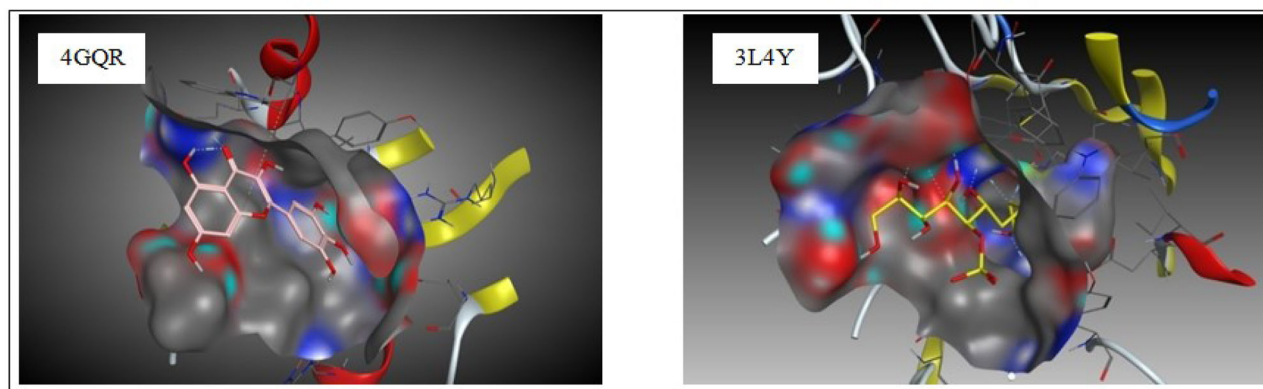


Figure 1. The active site of the isolated enzyme α -amylase and α -glucosidase.

Table 2. Some properties of best compounds for anti-diabetic drug.

| Ligand Compounds | Toxic | LogP | Energies (kcal/mol) | LogS | Hdon + Hacc | TPSA |
|-------------------------|-------|-------|---------------------|-------|---------------|--------|
| L01 Baicalein | No | 2.42 | 3.97320e + 001 | -3.46 | don:3; acc:4 | 86.99 |
| L05 Linarin | No | -0.95 | 1.65973e + 002 | -3.89 | don:7; acc:13 | 217.97 |
| L08 Fisetin | No | 2.31 | 6.34194e + 001 | -3.14 | don:4; acc:5 | 107.22 |
| L10 Morin | No | 2.01 | 5.06763e + 001 | -2.77 | don:5; acc:6 | 127.45 |
| L12 Gallocatechin | No | 1.35 | 5.57867e + 001 | -1.37 | don:6; acc:7 | 130.61 |
| L14 Didymin | No | -0.77 | 1.51010e + 002 | -2.88 | don:7; acc:14 | 214.06 |
| L15 Naringenin | No | 2.61 | 4.96034e + 001 | -2.45 | don:3; acc:5 | 86.99 |
| L19 Daidzein | No | 2.71 | 5.19326e + 001 | -3.54 | don:2; acc:3 | 66.76 |
| L20 Genistein | No | 2.42 | 4.48374e + 001 | -3.17 | don:3; acc:4 | 86.99 |
| L21 Protocatechuic acid | No | 0.80 | 1.16516e + 001 | -0.63 | don:3; acc:4 | 77.76 |
| L22 Syringic acid | No | 1.11 | 3.84830e + 001 | -1.09 | don:2; acc:5 | 75.99 |
| L26 Sinapic acid | No | 1.51 | 3.54787e + 001 | -1.60 | don:2; acc:5 | 75.99 |
| L29 Rosmarinic acid | No | 1.76 | 4.24150e + 001 | -2.65 | don:5; acc:7 | 144.52 |

The PASS approach has been previously reported in detail ((Filimonov et al., 2014), and there are also many publications where PASS predictions were approved by subsequent synthesis and biological testing (Filimonov et al., 2014; Singh et al., 2014). The PASS (Prediction of Activity Spectra for Substances) predictions of the two best selected ligands were conducted using PASS-Way2Drug server.

In the PASS prediction study, both possible biological activities of the selected ligands were predicted. Table 11 lists the results of the PASS prediction studies. The P450 sites of metabolism (SOM) of the three selected ligand molecules were determined by online tool, RS-WebPredictor 1.0 (Release Maestro, version 11.8, 2018). The LD50 values of the best three ligands were determined from admetSAR server. The server predicts the acute oral toxicity class of a compound and the LD50 value can be derived from the predicted category (Lee et al., 1988). Table 13 lists the results of P450 SOM study.

The pharmacophore mapping study of the three best ligands was carried out by online server PharmMapper (Parr & Yang, 1989). The pharmacophore mapping experiment was done for the two best ligand molecules among the 29 selected ligands use for the creation of new drugs (Figures and 14 and Table 13). The pharmacophore modelling of the two best ligands was performed using MOE software (*Molecular Operating Environment (MOE)*, 2013). However, the PASS prediction, P450 SOM prediction, pharmacophore mapping and modelling, solubility prediction and DFT calculations were carried out to determine and compare the biological activities of the two best ligands.

3. Results and discussion

The enzyme's active sites with co crystallization molecule are shown in Figure 1. The ligands' flavonoids and phenolic acids minimized toxicity and energy obtained by MOE software is shown in Table 2 and for the properties of the other ligands see Supplementary Table 2a.

As stated in Table 2, we find that molecules L05 and L14 have a low value of log P and log S compared to other molecules and also the results obtained show that these ligands (L05 and L14) have a low value of torsion angle relative to other compounds. As well, we note that the growth of the torsion angle depends on the binding number of the molecule.

3.1. Affinity of compounds with two targets

Results of docking calculations and bonds between atoms of best compounds and residues of the active site are given in Table 3.

3.2. Molecular docking analysis

3.2.1. Interaction with α A

We note that the result obtained (Tables 3 and 4), out of the best compounds studied, Linarin (Ligand 5) (Figure 2) was predicted to be the strongest (α A enzyme binder that forms a complex with the most stability with the lowest energy -8.386 kcal/mol) and that interacted with a four amino acids (HIS 299, ASP300, GLU233 and THR163) strong or low, and

Table 3. Results of bonds between atoms of best compounds and active site residues of two targets.

| Compounds | S-score (kcal/mol) | Bonds between atoms of compounds and residues of the active site | | | | | |
|-------------------------------------|--------------------|--|-------------------------|----------------------------|--------------------------|---------------|---------------------|
| | | Atom of compound | Involved receptor atoms | Involved receptor residues | Type of interaction bond | Distances (Å) | Energies (kcal/mol) |
| <i>α-Amylase (PDB ID: 4GQR)</i> | | | | | | | |
| L5 | -8.386 | O 10 | OD2 | ASP 300 | H-donor | 3.21 | -1.5 |
| | | O 44 | OG1 | THR 163 | H-donor | 2.86 | -0.9 |
| | | O 68 | OE2 | GLU 233 | H-donor | 3.18 | -2.2 |
| | | O 70 | OE2 | GLU 233 | H-donor | 2.84 | -2.1 |
| | | O 72 | NE2 | HIS 299 | H-acceptor | 3.00 | -1.8 |
| L14 | -8.007 | O 4 | OE2 | GLU 233 | H-donor | 2.97 | -2.6 |
| | | O 9 | OD1 | ASP 197 | H-donor | 3.16 | -2.4 |
| | | O 7 | NH2 | ARG 195 | H-acceptor | 3.35 | -1.0 |
| | | O 15 | NE2 | HIS 201 | H-acceptor | 2.75 | -3.7 |
| | | C 39 | NE2 | TRP 59 | H-pi | 3.89 | -1.3 |
| | | 6-ring | | GLN 63 | pi-H | 3.63 | -1.1 |
| L20 | -5.218 | - | - | - | - | - | - |
| <i>α-Glucosidase (PDB ID: 3L4Y)</i> | | | | | | | |
| L5 | -6.638 | O 68 | OD2 | ASP 327 | H-donor | 2.76 | -3.9 |
| | | O 70 | OD2 | ASP 327 | H-donor | 3.02 | -2.1 |
| | | O 68 | NE2 | HIS 600 | H-acceptor | 2.74 | -3.0 |
| | | O 74 | OD2 | ASP 443 | H-acceptor | 3.19 | -1.3 |
| L14 | -6.384 | O 13 | OD1 | ASP 327 | H-donor | 2.92 | -1.2 |
| | | O 15 | OD2 | ASP 327 | H-donor | 2.74 | -4.2 |
| | | C 26 | SD | MET 444 | H-donor | 4.18 | -0.7 |
| | | O 3 | OD2 | ASP 443 | H-acceptor | 3.16 | -1.4 |
| | | O 15 | NE2 | HIS 600 | H-acceptor | 2.69 | -3.0 |
| | | O 19 | NH2 | ARG 202 | H-acceptor | 2.88 | -4.4 |
| L20 | -5.619 | - | - | - | - | - | - |

the existence of two electric force (GLN63, ASP197), suggesting that Linarin can inhibit α A enzyme and interfere with HIS 299, ASP300, GLU233 and THR163. We note that the interactions between the residue of the active site of 4GQR and Linarin formed a stable complex with a strong interaction H-donor and H-acceptor.

The second best binder was Didymine (Ligand 14) with the energy of -8.007 kcal/mol. The results of second best compounds bonds is shown in Tables 3 and 4 that interacted with a six amino acids (GLU233, ASP197, ARG195, HIS201, TRP59 and GLN63) at a distance of (2.97, 3.16, 3.35, 2.75, 3.89 and 3.63 Å) strong or low. Knowing that the interactions between 2.5 and 3.1 Å are considered to be strong and those between 3.1 and 3.55 Å are assumed to be average. Interactions greater than 3.55 Å are weak (Imberty et al., 1991). This suggests that Didymine can inhibited α A enzyme and interfere with GLU233, ASP197, ARG195, HIS201, TRP59 and GLN63 (H-donor, H-acceptor and Pi-H) (see Figure 3).

3.2.2. Interaction with α G

We note that Linarin (Ligand 5) (Figure 4) was predicted to be the strongest α G binder that formed a complex with the most stability with the lowest energy -6.638 kcal/mol) that interacted with a four amino acids (ASP327, HIS600 and ASP443 at a distance of 2.76, 3.02, 2.74 and 3.19 Å) strong or low interaction respectively, with the existence of four electric force (ASP203, ASP542, ARG526 and TRP539), suggesting that Linarin can inhibited α G and interfere with ASP327, HIS 600 and ASP443. We note that the interactions between the residue of the active site of 3L4Y and the ligand formed a stable complex with a strong interaction (H-acceptor and H-donor).

The second best binder was Didymine (Ligand 14) with the energy of -6.384 kcal/mol, that interacted with six amino acids (ASP327, MET444, ASP443, HIS600 and ARG202) at a distance of 2.92, 2.74, 4.18, 3.16, 2.69 and 2.88 Å, strong or low interaction, with the existence of four electric force (ASP542, ASP203, ARG526 and TRP539). Results of energy balance of best complexes formed with two targets are given in Table 4. For the Energy of the other compound see Supplementary Table 4a (see Figure 5).

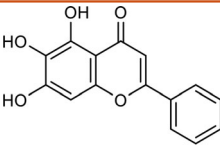
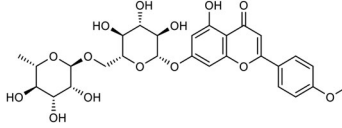
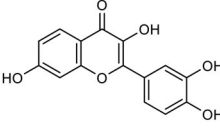
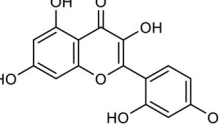
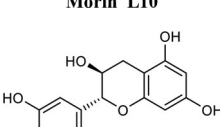
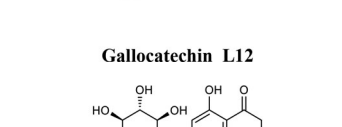
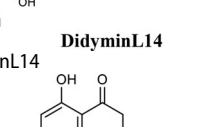
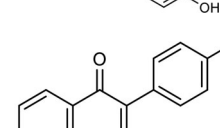
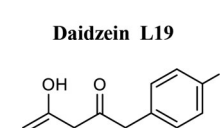
The molecules that had the lowest binding energy of docking score were considered the best molecule and inhibiting the target receptor as the lower binding energy corresponds to higher binding affinity (Simon et al., 2017). We compared all the values score complexes; we found that complex formed by (4QGR-Linarin, 3L4Y-Linarin) L5 has the lowest value energy and gives the best docking score compared to Didymine L14 inhibitor and Genistein L20. Therefore, we can consider that the two complexes (4QGR-Linarin, 3L4Y-Linarin) and (4QGR-Didymine, 3L4Y-Didymine) are stables with higher binding affinity. According to these docking results, we can classify Linarin L5 as the good inhibitor of the enzymes, α A and α G compared to the all ligands studied.

3.3. Molecular dynamics analysis

3.3.1. Thermodynamic properties

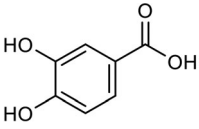
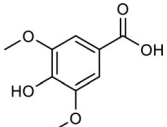
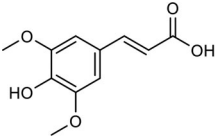
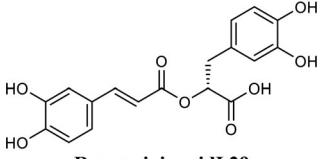
For the thermodynamic properties using the MD simulation approach, we have studied the evolution thermodynamic properties of ligand 5 and ligand 14 in NVT ensemble (see Table 5).

Table 4. Results of energy balance of best complexes formed with anti-diabetic drug molecules.

| Targets | Chemical Structure | Binding energy (kcal/mole) | Rmsd -refine | Energy-Conf | Energy-Place | Energy-Refine |
|--------------|--|--------------------------------|----------------|---------------------|----------------------|--------------------|
| 4QGR 3L4Y |  Baicalein L01 | -4.660 -4.750 | 1.118 0.674 | -31.547 30.279 | -67.792 -90.878 | -12.756 -14.474 |
| 4QGR 3L4Y |  Linarin L05 | -8.386 -6.638 | 1.663 3.572 | 217.4777 234.822 | -101.815 -68.026 | -35.474 -10.996 |
| 4QGR 3L4Y |  FisetinL08 | -4.917 -4.978 | 1.893 1.123 | 40.060 39.057 | -93.245 -80.057 | 12.622 -13.020 |
| 4QGR 3L4Y |  Morin L10 | -5.111 -4.688 | 5.117 2.055 | 31.272 35.000 | -102.046 -103.729 | -19.133 -7.355 |
| 4QGR 3L4Y |  Gallocatechin L12 | -4.817 -5.1024 | 2.595 2.099 | 17.979 20.011 | -88.556 -72.242 | -17.873 -9.245 |
| 4QGR 3L4Y |  DidymninL14 | -8.007 -6.384 | 1.496 3.318 | 221.3972 224.164 | -125.053 -85.915 | -38.649 -14.478 |
| 4QGR 3L4Y |  Daidzein L19 | -4.539 -4.676 | 3.293 1.463 | 9.716 9.5282 | -94.066 -92.089 | -15.304 -15.555 |
| 4QGR 3L4Y |  Genistein L20 | -4.657 -4.344 | 1.368 1.331 | 30.988 32.421093 | -59.764 -76.514 | 14.424 -8.092 |
| 4QGR 3L4Y |  Genistein L20 | -5.218 -5.619 | 6.093 1.928 | -4.921 -9.069 | -74.805 -77.703 | -16.506 -17.148 |

(continued)

Table 4. Continued.

| Targets | Chemical Structure | Binding energy (kcal/mole) | Rmsd -refine | Energy-Conf | Energy-Place | Energy-Refine |
|---|--|----------------------------|----------------|--------------------|--------------------|--------------------|
| 4GQR 3L4Y |  | -3.805 -3.882 | 2.280 1.482 | -71.64 -73.024 | -42.146 -42.197 | 5.342 -2.656 |
| Protocatechuic acid L21 | | | | | | |
| 4GQR 3L4Y |  | -4.360 -4.812 | 3.679 0.828 | -38.061 -51.307 | -55.482 -75.322 | -9.441 0.131 |
| Syringic acid L22 | | | | | | |
| 4GQR 3L4Y |  | -5.150 -4.822 | 0.851 1.466 | -56.536 -31.629 | -76.736 -56.733 | -7.176 -0.587 |
| Sinapic acid L26 | | | | | | |
| 4GQR 3L4Y |  | -6.089 -5.528 | 4.608 1.652 | -44.407 -38.807 | -76.613 -76.678 | -24.948 -14.188 |
| Rosmarinic acid L29 Rosmarinic acid L29 | | | | | | |

The enegie binding of the best ligands L5-L7 and L20 are mentioned in bold.

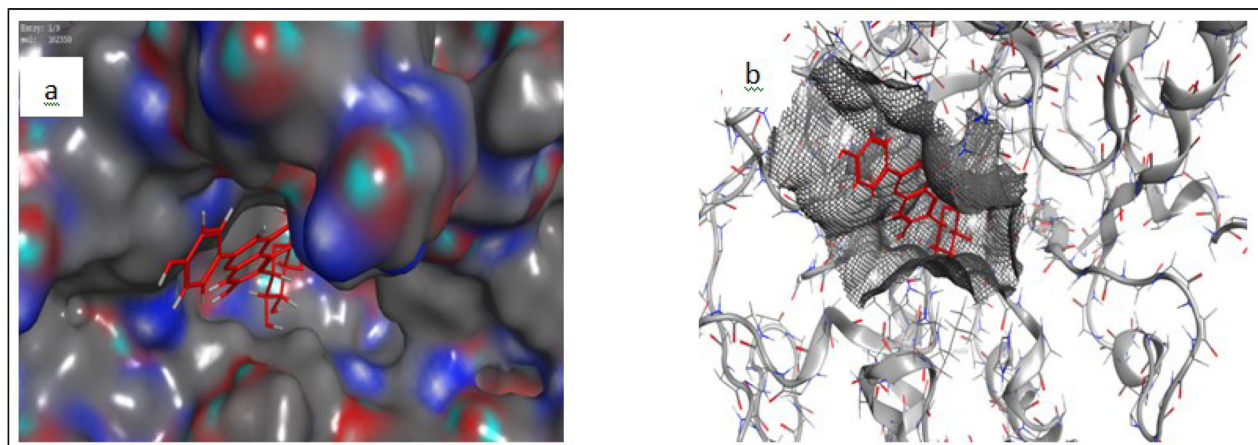


Figure 2. (a) The top scoring compound Linarin. (b) A novel inhibitor L-5 identified by molecular docking is shown in the active site.

In contrast to the complex formed by L14 their energies and enthalpy obtained were low. By against on pressure fluctuations were significant for the complex formed by L5 is of order 0.00251–0.0524 which explains the instability of the system by its strong therefore the movement rotational and vibration energy is important oscillation. In regard to variation in the average temperature of translation is fixed as at the outset in considering isochors–isotherms ensemble. In addition, the equations of motion were solved with a constant of integration step $\Delta t = 5E^{-15}$. Therefore, L5 was predicted to be the most interactive system. These results are in

total agreement with the docking prediction results (Tables 3 and 4) (see Figures 6 and 7).

3.3.2. Structural properties

For the structural dynamics properties using the MD simulation approach, we have studied the evolution structural dynamics of the best ligand molecule L5 (see Table 5) by IMODS.

The normal mode analysis (NMA) of the prepared α A-Linarin and α G-Linarin, respectively, complex was

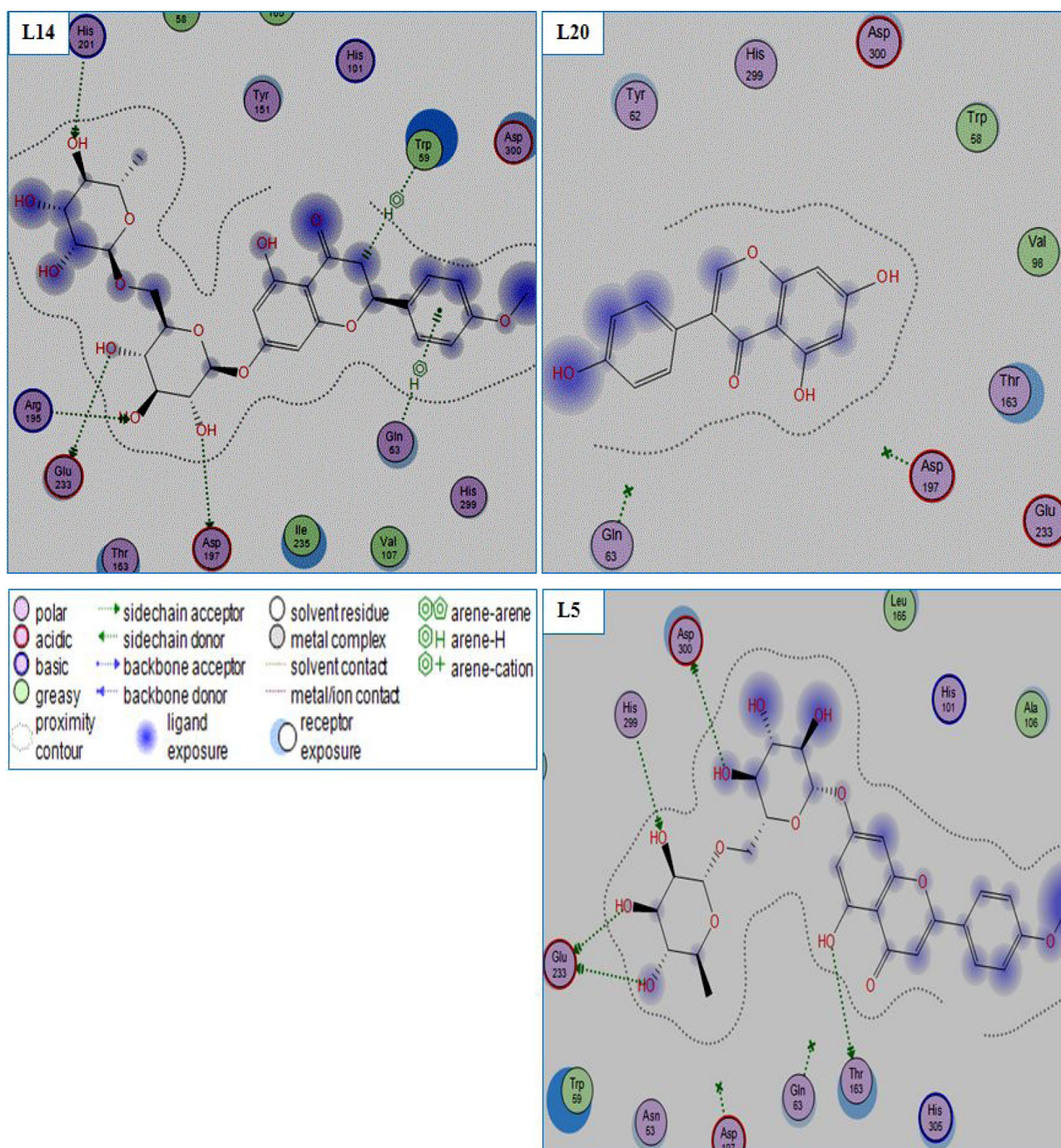


Figure 3. 2D representations of the best pose interactions between the ligands and their receptor. L5 interaction between Linarin and α A, L14. Interaction between Didymin and α A, L20. Interaction between Genistein and α A. The 2D representations of the best pose interactions between the ligands and their respective receptors were visualized using Molecular Operating Environment (MOE).

illustrated in Figures 8(a) and 9(a). From the molecular dynamics study of the prepared Linarin- α A and α G docked complex, it was clear that the prepared enzyme-ligand complex had quite high eigenvalue of $2.436689e-04$ and $1.646004e-04$, respectively, the eigenvalue is illustrated in Figures 8(b) and 9(b), respectively. However, the variance map showed high degree of cumulative variances than individual variances (Figures 8(c) and 9(c)). The co-variance and elastic network map also produced quite satisfactory results (Figures 8(d, e) and 9(d, e), respectively). The two selected ligand molecules can be used as potential agents to treat

(T2DM). Overall, in our study, Linarin emerged as the most potent anti- α A and α G agent. However, more *in vitro* and *in vivo* researches should be performed on the Linarin best ligands to finally confirm the findings of this study.

3.4. Prediction of the relative global softness

The relative interaction scores (docking results) of Lref_i, Lig5, and Lig14 (see Tables 3 and 4) in the inhibition were

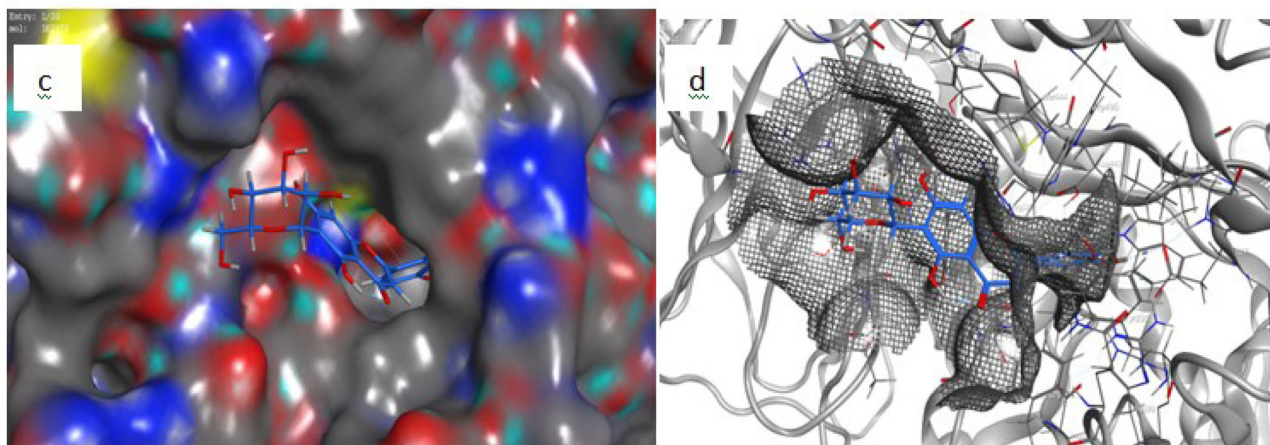


Figure 4. (c) The top scoring compound Linarin. (d) A novel inhibitor L-5 identified by molecular docking is shown in the active site.

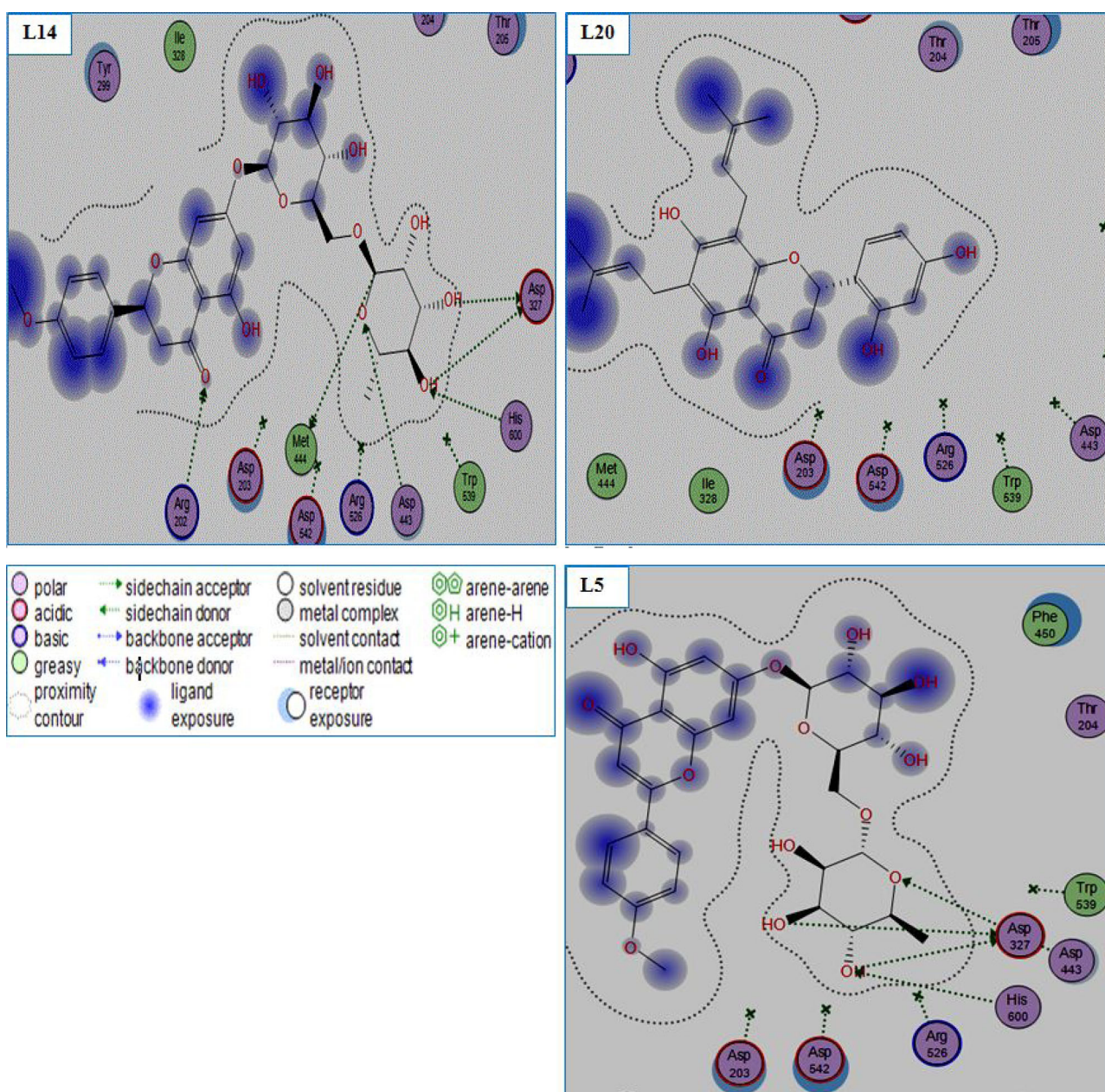


Figure 5. 2D representations of the best pose interactions between the ligands and their receptor. L5 interaction between Linarin and α G, L14. Interaction between Didymin and α G, L20. Interaction between Genistein and α G. The 2D representations of the best pose interactions between the ligands and their respective receptors were visualized using Molecular Operating Environment (MOE).

Table 5: Thermodynamic properties calculated in reels units.

| Stage | Method | H | U | EKT | P |
|--------|-----------------------|-------|----------|-----------|---------|
| SP_i | α -Amylase | | | | |
| | NVT Complex_5 | 9.634 | 8705.267 | 8544.281 | -62.247 |
| | NVT Complex_14 | 3.171 | 6167.721 | 8648.702 | 00.753 |
| | α -Glucosidase | | | | |
| | NVT Complex_5 | 7.524 | 7254.256 | 7542.0254 | -55.236 |
| | NVT Complex_14 | 6.235 | 5425.235 | 7452.256 | 2.125 |

Pressure $P = P^* \epsilon / \sigma^{-3}$, energy of configuration $U = U^* N \epsilon$, translation kinetic energy $EKT = EKT^* N \epsilon$ and enthalpy $H = H^* N \epsilon$.

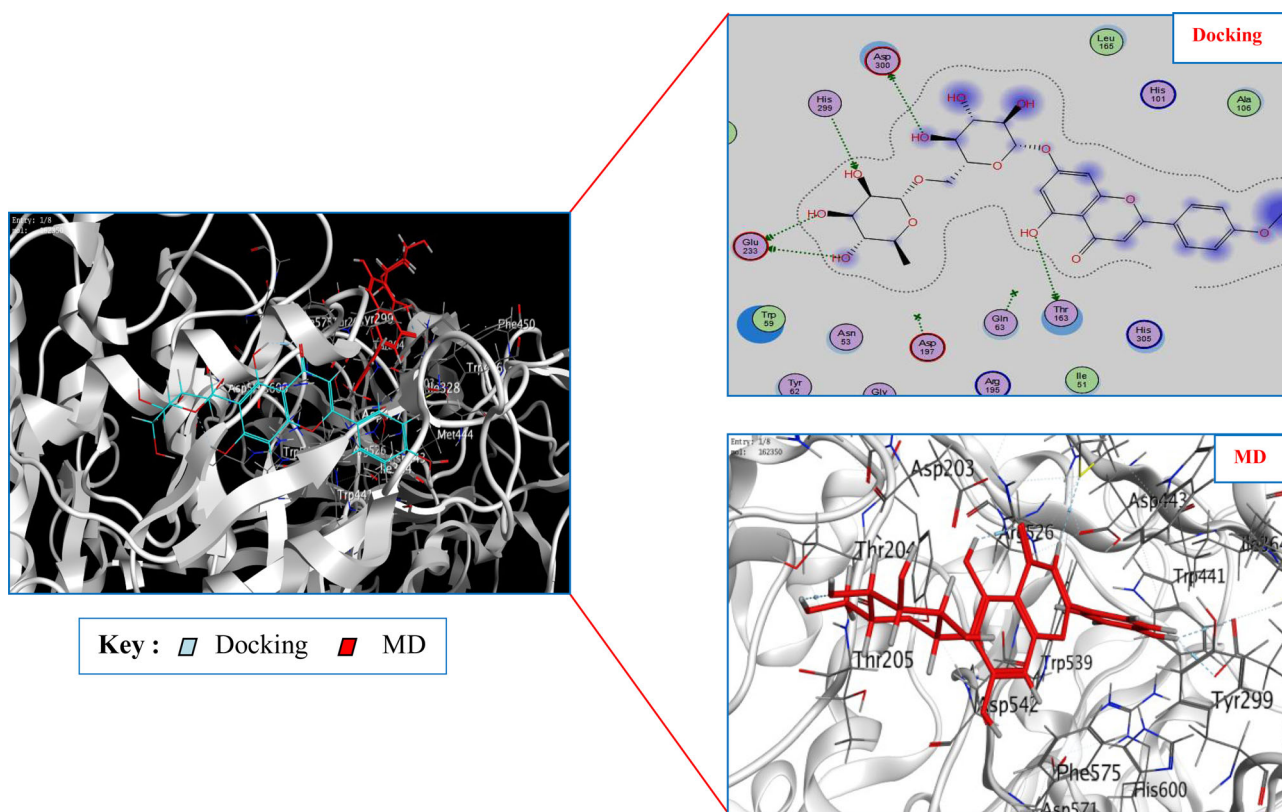


Figure 6. Compound 5 Linarin is docked without water well into the binding site of alpha-amylase and has the highest dock score; there is also a clear difference between the final ligand pose and the docking pose after a molecular dynamics (MD) simulation.

rationalized using the global softness S index. The numerical values of this quantity are given in Table 6.

Linarin (Flavones) (Ligand 5) was predicted to be characterized by a strong electrophilic power index (strong electron acceptor) and a high lipophilicity by providing Didymine (Ligand 14). The relative global reactivity of these systems was justified by means of electrophilic power index. The current study shows that the docking trends of the relative activities of these inhibitors agree with their predicted electrophilic power. These results are in total synchronicity with the docking prediction results (see Tables 3 and 5 and Figure 10).

3.5. In silico assessment of the ADME properties and drug-likeness

A computational study of the best compounds was performed for the assessment of ADME properties and is shown

in Table 7 and ADME analysis of other compounds is shown in Supplementary Table 7a.

The results showed that compound L5 (Figure 11) and compound L14 have low absorptions. We note that the molecular weight MW of L5 and L14 is in the range of 592.55–594.56 (>500), HBA in the range of 14–15 (>10) and HBD in the range of 7–8 (>5), these large molecules can hardly be administered orally. But, inhaled. An improvement in the lives of millions of diabetics has been proven by inhaling insulin, which is found in the lungs in the form of millions of small particles and can reach the blood stream (<https://www.doctissimo.fr/html/dossiers/diabete/articles/9043-diabete-insuline-spray-exubera.htm>). So, inhaling our best molecule linarin (flavones) (Ligand 5) can easily reach the blood stream and represents high affinity with αA and αG .

So we can consider our natural Ligand L5 with its proven activity score -8.386 , -6.638 , respectively, for αA and αG , as a *new inhaled ligand* despite not obeying Lipinski's rule,

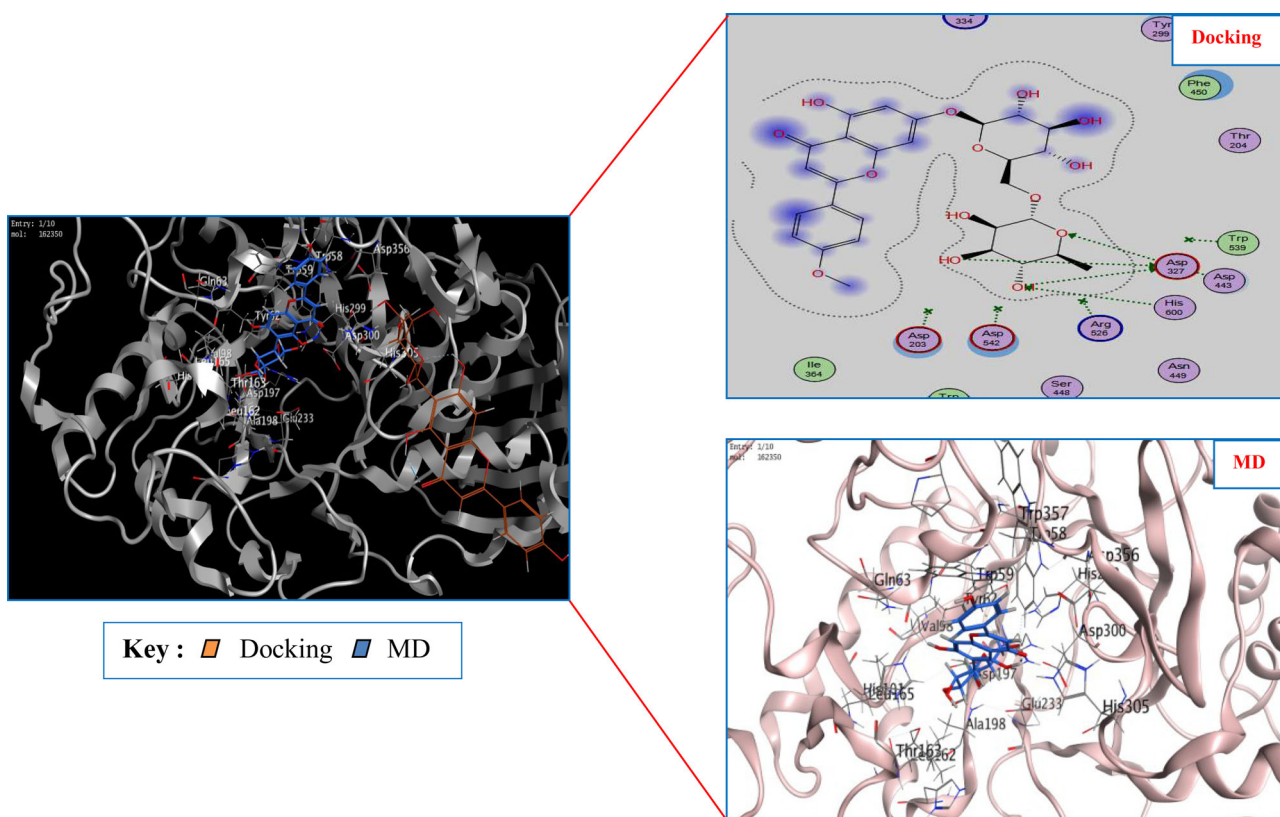


Figure 7. Compound 5 Linarin is docked without water well into the binding site of alpha glucosidase and has the highest dock score; there is also a clear difference between the final ligand pose and the docking pose after a molecular dynamics (MD) simulation.

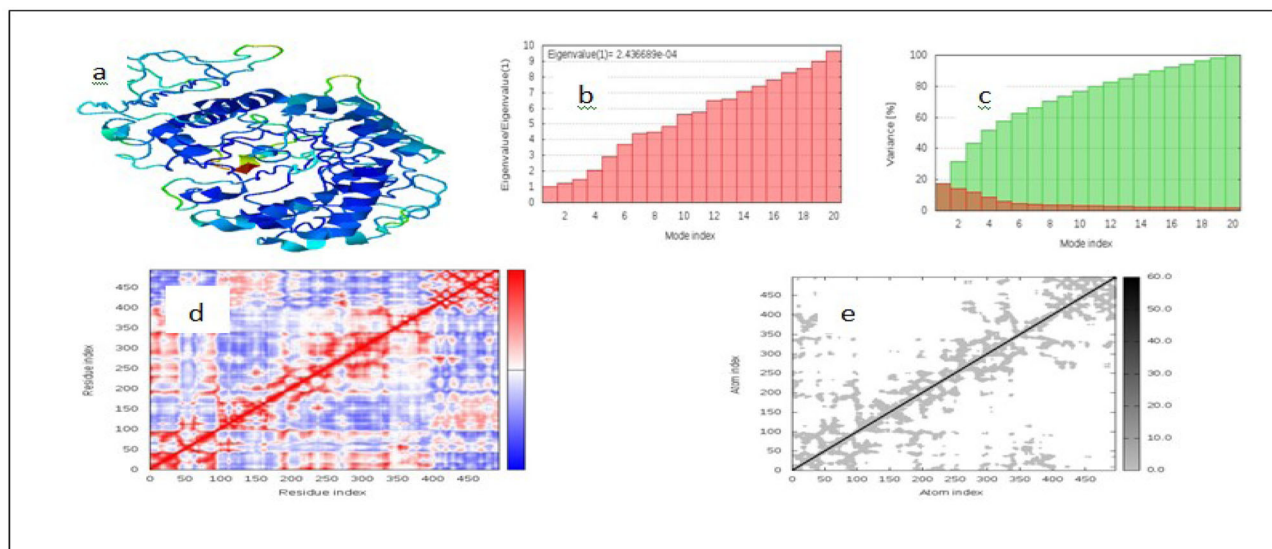


Figure 8. Results of molecular dynamics simulation of Linarin- α -amylase docked complex. (a) NMA mobility, (b) eigenvalue, (c) variance (red color indicates individual variances and green color indicates cumulative variances), (d) co-variance map (correlated (red), uncorrelated (white) or anti-correlated (blue) motions) and (e) elastic network (darker grey regions indicate more stiffer regions) of the complex.

because the Lipinski parameter is not a criterion for anchoring. 'Lipinski rule of 5' is an ADME filter for choosing the molecule for drug likeness. In addition, to study the binding interaction through docking, it is not necessary to see whether or not the molecule obeys the 'Lipinski rule of 5'. It was predicted that Linarin (flavones) presents better interaction of α A and α G enzymes and consequently can be the

best inhaled inhibitor candidate to be investigated *in vivo* and *in vitro*.

And in the meantime, we propose Ligand 20 present in (isoflavones) with its proven activity score -5.218 , -5.619 , respectively, for α A and α G enzymes as a *new oral ligand* despite obeying Lipinski's rule. It has a maximum of 3 H+ donors and 5 H+ acceptor atoms, as shown in Table 7. With respect to

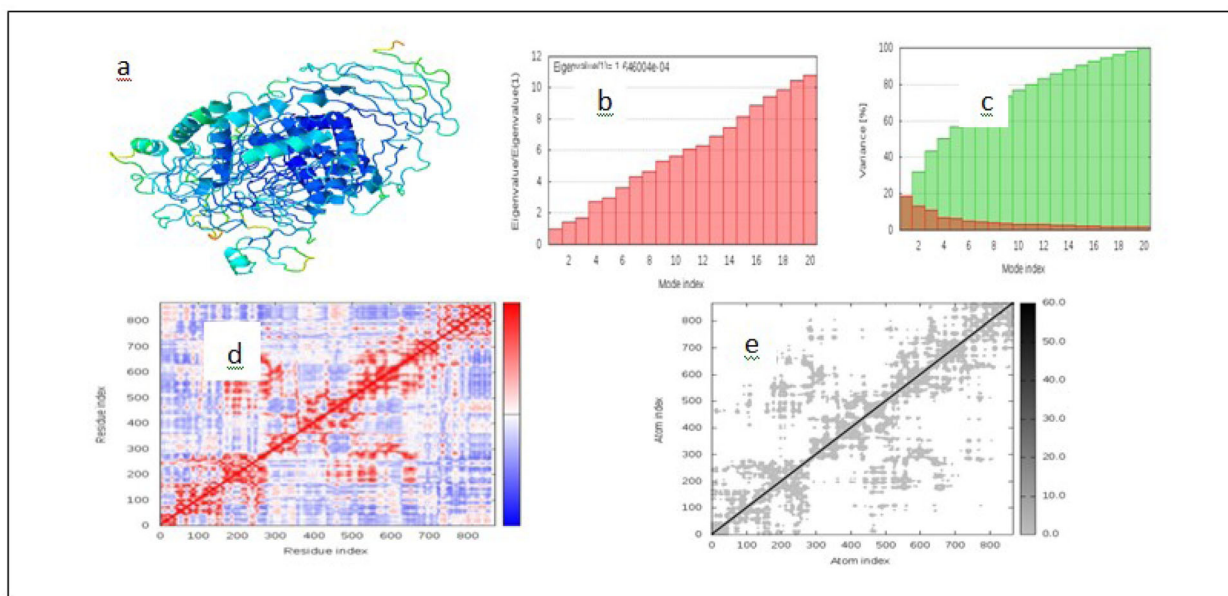


Figure 9. Results of molecular dynamics simulation of Linarin- α -glucosidase docked complex. (a) NMA mobility, (b) eigenvalue, (c) variance (red color indicates individual variances and green color indicates cumulative variances), (d) co-variance map (correlated (red), uncorrelated (white) or anti-correlated (blue) motions) and (e) elastic network (darker grey regions indicate more stiffer regions) of the complex.

Table 6. LUMO and HOMO energies, and global reactivity indices μ , η , ω for Ligref_i, Lig 5 and Lig14 at the B3LYP/6-311G(d,p).

| Compounds | HF | HOMO (au) | LUMO (au) | μ (au) | η (au) | S (au) | ω (au) |
|--------------------------------|-----------|---------------------|----------------------|------------|-------------|----------|---------------|
| Lref1 α -Amylase | -1172.716 | 0.0742 OA:83(a) | -0.309 OA:82(a) | 8.621 | 0.191 | -5.2113 | 193.674 |
| Lref2 α -Glucosidase | -1298.203 | 0.1196 OA:46(a) | -0.319 OA:45(a) | 1.044 | 0.219 | -4.555 | 2.486 |
| L5 | -2126.962 | 0.0674 OA:157(a) | -0.314 OA:156(a) | 1.600 | 0.191 | -5.231 | 6.697 |
| L14 | -2128.138 | 0.0904 OA:158(a) | -0.316 OA:157 (a) | 2.299 | 0.203 | -4.910 | 12.983 |

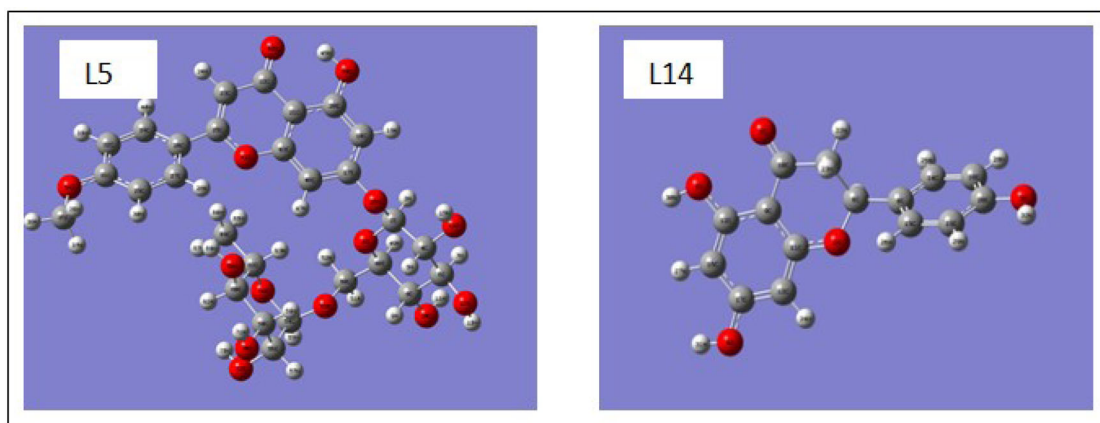


Figure 10. Structure of best ligands L5 and L14 (docking and MD results) at the B3LYP/6-311g(d,p).

pharmacokinetics, Ligand 20 present in isoflavones confirmed the safety of the compound for oral administration with good skin permeability, which allows applications in topical formulations. It was predicted that Genistein L20 present in (isoflavones) presents better interaction of α A and α G enzymes and consequently can be *the best oral inhibitor* candidate to be investigated *in vivo* and *in vitro*.

Regarding the absorption parameters, compounds L01, L04, L06, L20 and L19 present a promising oral availability, due to the optimal Caco-2 cell permeability and HIA (>0.9 and >90%, respectively, Table 8), and skin permeability (log $K_p < -2.5$, Table 8).

Minnow toxicity: < -0.3 ; high acute toxicity, VD_{55} : < -0.15 low, >0.45 high, BBB: >0.3 cross BBB, < -1 poorly

Table 7. Lipinski's rule of five for ADME analysis of best inhibitors.

| N° | Name | Lipinski's rule of five | | | | | Drug-likeness Lipinski's rule follows |
|----|---------------------|-----------------------------|---------------------------|-------------------------|----------------------------|--|---|
| | | Molecular weight (g/mol) | Lipophilicity (MLog P) | Hydrogen bond donors | Hydrogen bond acceptors | No. of rule violations | |
| | | Less than 500 Dalton | Less than 5 | Less than 5 | Less than 10 | Less than 2 violations | |
| 01 | Baicalein | 270.24 | 0.52 | 3 | 5 | 0 | Yes |
| 05 | Linarin | 592.55 | -2.76 | 7 | 14 | 3 violations: MW > 500, NorO > 10, NHorOH > 5 | No |
| 08 | Fisetin | 286.24 | -0.03 | 4 | 6 | 0 | Yes |
| 10 | Morin | 302.24 | -0.56 | 5 | 7 | 0 | Yes |
| 12 | Gallocatechin | 306.27 | -0.29 | 6 | 7 | 1 violation: NHorOH > 5 | Yes |
| 14 | Didymin | 594.56 | -2.57 | 7 | 14 | 3 violations: MW > 500, NorO > 10, NHorOH > 5 | No |
| 15 | Naringenin | 272.25 | 0.71 | 3 | 5 | 0 | Yes |
| 19 | Daidzein | 254.24 | 1.08 | 2 | 4 | 0 | Yes |
| 20 | Genistein | 270.24 | 0.52 | 3 | 5 | 0 | Yes |
| 21 | Protocatechuic acid | 154.12 | 0.40 | 3 | 4 | 0 | Yes |
| 22 | Syringic acid | 198.17 | 0.49 | 2 | 5 | 0 | Yes |
| 26 | Sinapic acid | 224.21 | 0.73 | 2 | 5 | 0 | Yes |
| 29 | Rosmarinic acid | 360.31 | 0.90 | 5 | 8 | 0 | Yes |

MW, molecular weight; MLogP, logarithm of partition coefficient of the compound between water and n-octanol, n-OHND, number of hydrogen bonds donors; n-ON acceptors, number of hydrogen bond acceptors; n-ROTB, number of rotatable bonds.

distributed to the BBB, CNS: > -2 penetrate CNS, < -3 unable to penetrate CNS, Low skin permeability: > -2.5 , Caco-2 permeability: > 0.9 , human intestinal absorption: > 90 .

The overall lecture of Table 12 highlights that compounds L20 oral could be an excellent candidate as drugs or, however, lead to further studies and manipulations. All compounds are not substrates of the renal organic cation transporter 2 (OCT2). Compound L16 did not pass the AMES test, whereas all others do not present any particular toxicity problems. The volume of distribution (VDss) and fraction unbound are two of the most important pharmacokinetic drug parameters. Values of the VDss > 0.45 indicate that the drug will be distributed in tissue whereas values < -0.15 indicate that the drug will be distributed in plasma. So, VDss describes the extent of drug distribution, and the fraction unbound describes the portion of free drug in plasma that may extravasate. Compounds 04, 05, 06, 10, 14 and 16 are entirely unable to penetrate the central nervous system (CNS). The absorption and distribution parameters, respectively, have been graphically represented by the extended and renewed version of the Edan-Egg model named Brain OrIntestinalEstimatedD (BOILED) permeation predictive model (BOILED-Egg).

The absorption and distribution parameters, respectively, have been graphically represented by the extended and renewed version of the Edan-Egg model named Brain Or Intestinal Estimate D (BOILED) permeation predictive model (BOILED-Egg) is shown in Figure 12.

The ADMET properties and BOILED-Egg (Figure 12) plot validate compounds 05 and 14 to be **unable** to pass the brain barrier and have **low** absorption in the intestines with **less** bioavailability. All ligand discussed here in satisfy the Lipinski's rule, except for 05, 14 and which significantly violates three parameters (MW > 500 , number of hydrogen bond donors > 5 and number of hydrogen bond acceptors > 10);

furthermore, these latter ligands also violate the BOILED-Egg method.

Compound 5 has the highest binding affinity among all the inhibitors, it is not proposed as an orally active drug due to violation of the Lipinski's rule.

Compound 14, the second best ligand, is not proposed as an orally active drug due to violation of the Lipinski's rule. The graph showed that ligands 19 are absorbed by the brain. The ligands L1, L8, L10, L12, L15, L20, L21, L22, L26 showed gastrointestinal absorption within acceptable limits, except for ligands L5 and L14 (TPSA: 217.97, 214.06 Å²), respectively (see Table 2) (TPSA > 131.6). Analysis of the interactions with the protein-ligand interaction profiler between tree enzyme (α A and α G enzymes) and ligand 5 revealed the best binding affinity.

By analyzing the drug's score (S-value), ligand 5 showed the lowest S-value (-8.386, -6.638) respectively for (α -A and α -G enzymes), resulting as the best ligand among our selected ligands to inhibit the activity of enzymes α A and α G. However, it violates three parameters (MW > 500 D, H-bond donors > 5 and H-bond acceptors > 10) of the Lipinski's rule, as well as Egan's parameters (1 violation: TPSA > 131.6). Although ligand 5 is proposed to be a potential therapeutic inhibitor of α A and α G enzymes, it may fail as an orally active drug because it deviates from the Lipinski's rule and from the Egan's rule, these large molecules can hardly be administered orally. But, *inhaled*. Indeed, further analysis indicates that drugs well beyond the limits of the rule of five, including hydrophilic macromolecules, can be administered by inhalation (Attique et al., 2019). Lipinski explicitly warned in his paper that the Rule-of-5 does not apply to natural products (<https://www.sciencedirect.com/topics/nursing-and-health-professions/lipinskis-rule-of-five>). In recent years, however, a number of macromolecular drugs that flagrantly violate the Rule of Five have been efficiently propagated, *via*

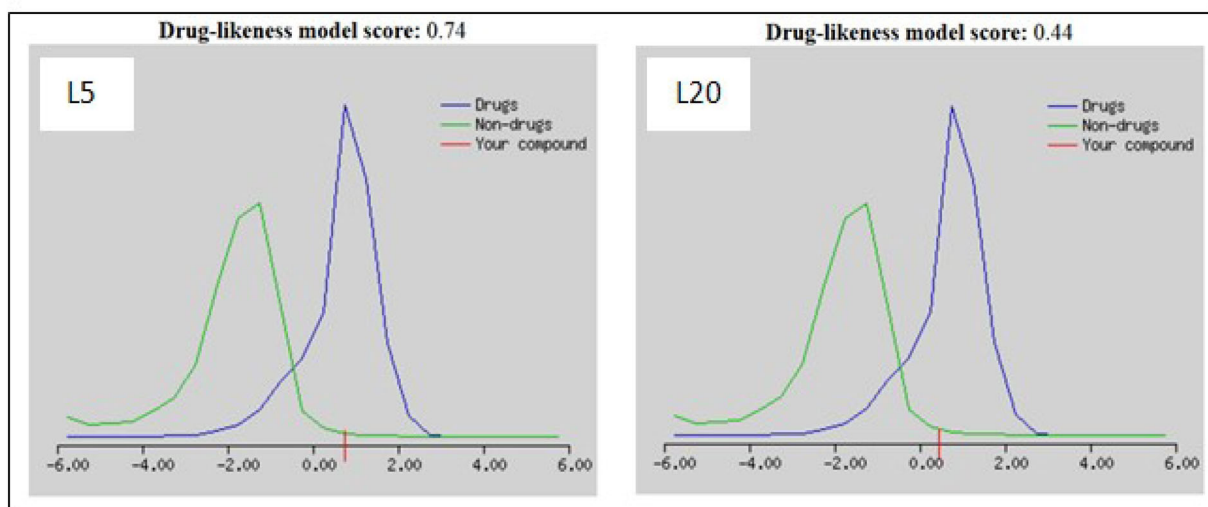


Figure 11. Drug-likeness model score: 0.74 for L5 and 0.44 for L20, drug likeness prediction.

the lung, such as insulin, due to the high permeability of alveolar epithelium (Petersson et al., 1988).

In this study, ligand 20 was predicted as the best targets inhibitor (with maximum binding affinity for two targets after L5) to be used as a potentially therapeutic *orally* active drug.

In addition to the Lipinski rule of five, other four drug-likeness rules named Ghose, Egan, Veber and Muegge, have been contemporarily satisfied by compound L20 with the exception of molecule L05 and L14. The results of drug likeness property analysis are summarized in Table 9.

Instead, the stringent lead-like criteria of Teague have been passed by compounds L20. Since lead-likeness tests are intended to provide leads with high affinity in high-throughput screens that allow for the discovery and exploitation of additional interactions in the lead-optimization phase, molecule L20 is excellent candidate to be investigated based on scaffold hopping approach. Finally, the outcome of the pan assay interference structures (PAINS) model, conceived to exclude small molecules that are likely to show false positives in biological assays, post no alert for the compound L20, concerning the presence of a molecule moiety.

On the other hand the two rules of Ghose et al. (1999), Egan et al. (2000), Muegge et al. (2001) are verified only for Genistein drug.

Two molecule is predicted not absorbed and not BBB permeant because outside of the range of the plot: L05 with a TPSA of 217.97 Å² and L14 with TPSA of 214.06 Å² (1 violation: TPSA > 131.6) (see Figure 12 and Table 2).

3.6. Pharmacokinetics and medicinal chemistry properties

The results of Medicinal Chemistry and Pharmacokinetics revealed that all compounds have high GI absorptions. We notice that there was a complement between our results for assessment of ADME properties (Table 9) and the predicted

results in medicinal chemistry and pharmacokinetics (Table 10).

The results of Medicinal Chemistry and Pharmacokinetics showed that compound L5 and compound L14 have low GI absorptions. We notice that there is a correlation between our results found by B3LYP/6-311G(d, p) (Table 11) and the predicted results in medicinal chemistry and pharmacokinetics (Table 10).

Didymin (Flavanones) (Ligand 14) is predicted to be characterized by a high lipophilicity and by high coefficient of skin permeability $\log K_p$ by providing Linarin from the Flavones (Ligand 5). We can conclude that the more negative the $\log K_p$ (with K_p in cm/s), the less the molecule is permeable to the skin (Kumbasar et al., 2015) which explains the reliability of our results. We quote, our previous research which confirms the stability of complexes and their affinities by MOE software (Fouzia & Salim, 2019; Mesli et al., 2019). So, Ligand L5 represents high affinity with αA and αG enzymes. Synthetic accessibility (SA) is a major factor to take into account in this selection process an acceptable value between 6.27 and 6.43 for ligands L14 and L5, respectively, these are more promising molecules which can be synthesized or subjected to bioassays or other experiments. According to its pharmacokinetic properties (Figure 13), L20 present in Isoflavones showed a high level of gastrointestinal absorption which contributes to *good oral* bioavailability.

The pink area represents the optimal range for each properties (lipophilicity: XLOGP3 between -0.7 and $+5.0$, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å², solubility: $\log S$ not higher than 6, saturation: fraction of carbons in the sp^3 hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds).

L5 was found less suitable to be proposed as oral drug because they violated at least two criteria, polarity and size in case of Linarin (L5) (MW >500 g/mol, polarity: TPSA >130 Å²).

The compound Genistein showed violation of only one criterion, that is, insaturation (L20), saturation: fraction of carbons

Table 8. Pharmacokinetic and toxicity evaluated parameters of best compounds.

| | Ligands | L5 | L14 | L4 | L06 | L20 | L12 | L8 | L2 | L22 | L26 | L1 | L10 | L15 | L16 | L19 |
|--------------|--------------------------------|--------|-----------|------------|------------|------------|--------|--------|------------|------------|--------|------------|------------|------------|------------|-----------|
| Absorption | Caco-2 permeability | 0.357 | -0.186 | 1.395 | 1.419 | 1.014 | -0.375 | 0.465 | 0.86 | 0.425 | 0.236 | 1.067 | 1.002 | 0.907 | 1.007 | 0.856 |
| | Human intestinal absorption | 36.679 | 26.62 | 98.50 1 | 98.48 8 | 90.85 1 | 54.128 | 85.622 | 84.39 2 | 82.41 2 | 96.401 | 91.23 3 | 70.93 2 | 89.34 5 | 75.36 4 | 93.04 |
| | Skin permeability | -2.735 | -2.735 | -2.738 | -2.74 | -2.837 | -2.735 | -2.736 | -2.739 | -2.735 | -2.625 | -2.785 | -2.735 | -3.009 | -2.746 | -2.764 |
| Distribution | VDss (human) | 0.919 | 0.124 | 0.443 | 0.318 | 0.483 | 1.301 | 0.123 | 0.103 | -1.18 | -0.851 | 0.174 | 0.269 | 0.278 | 0.075 | 0.206 |
| | Fraction unbound (human) | 0.115 | 0.322 | 0.099 | 0.111 | 0.153 | 0.274 | 0.049 | 0.051 | 0.609 | 0.42 | 0.065 | 0.126 | 0.227 | 0.072 | 0.12 |
| | BBB permeability | -1.636 | -1.634 | -1.18 | -0.97 | 0.979 | -1.377 | -1.171 | -1.151 | 0.067 | -0.19 | 0.967 | -1.483 | 0.917 | -1.06 | -0.13 |
| | CNS permeability | -4.674 | -4.826 | -3.313 | -3.075 | -2.156 | -3.507 | -2.367 | -2.465 | -2.963 | -2.715 | -2.31 | 3.403 | 2.221 | -3.219 | -1.94 |
| Excretion | Total clearance | 0.103 | 0.529 | 0.898 | 0.88 | 0.248 | 0.328 | 0.523 | 0.662 | 0.663 | 0.756 | 0.344 | 0.672 | 0.124 | 0.107 | 0.187 |
| | Renal OCT2 substrate | No | No | No | No | No | No | No | No | No | No | No | No | No | No | NO |
| Toxicity | AMES toxicity | No | No | No | No | No | No | No | No | No | No | No | No | No | Yes | No |
| | Oral rat acute toxicity (LD50) | 2.521 | 2.755 | 2.468 | 2.256 | 1.909 | 2.492 | 2.26 | 2.178 | 2.013 | 2.47 | 2.279 | 2.24 | 2.132 | 1.866 | 1.78 |
| | Minnowtoxicity | 4.316 | 5.47 1 | 0.09 1 | 0.06 8 | 0.93 | 4.235 | 1.045 | 0.97 | 2.55 9 | 2.321 | 1.47 8 | 2.18 5 | 0.64 | 1.36 7 | 1.03 2 |

All results have been obtained from the pkCSM web server.
Green = good, yellow = tolerable, red = bad.

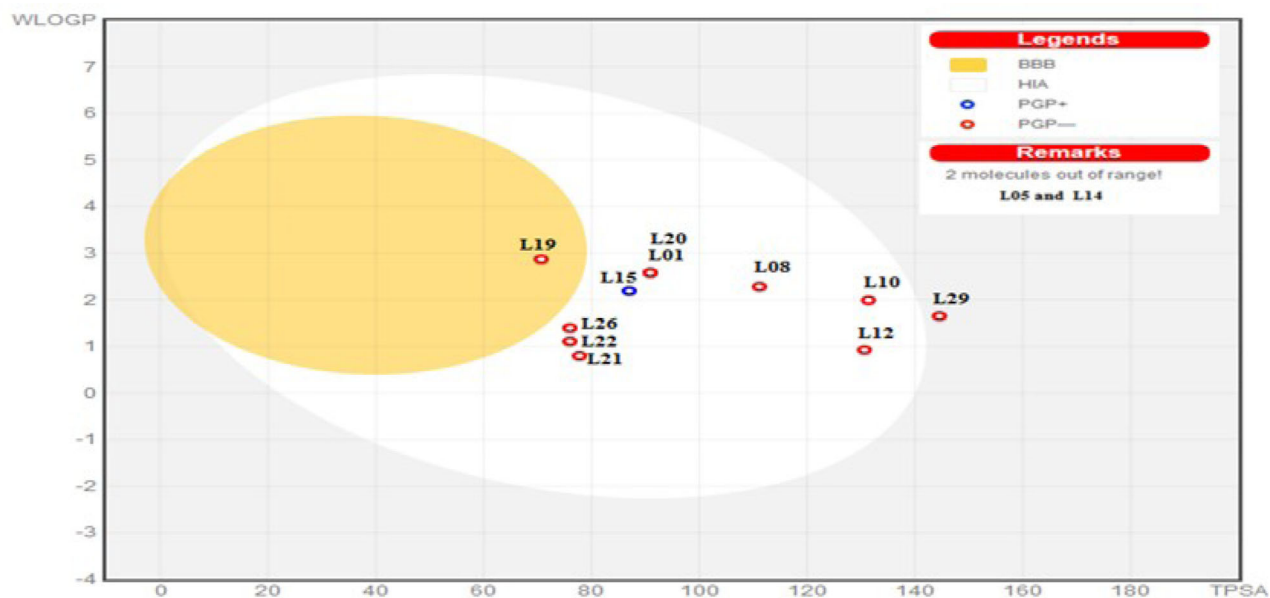


Figure 12. BOILED-Egg plot. Points located in the BOILED-Egg's yolk (yellow) represent the molecules predicted to passively permeate through the blood-brain barrier (BBB), whereas the ones in the egg white are relative to the molecules predicted to be passively absorbed by the gastrointestinal tract; the blue dots indicate the molecules for which it was expected to be effluated from the central nervous system (CNS) by the P-glycoprotein, whereas the red ones point-out to the molecules predicted not to be effluated from the CNS by the P-glycoprotein.

in the sp^3 hybridization less than 0.25, as observed in oral bio-availability radar (Figure 13), and its skin permeation coefficient (-6.05 cm/s) was also noticeably more than two top scored compounds L5 and L14. The order of skin permeation ability ($\log K_p$ value) of the ligands was as follows: L20

(-6.05 cm/s) > L5 (-9.56 cm/s) > L14 (-10.40 cm/s), and a small $\log K_p$ value represents less cutaneous permeability.

Ligand 5 has a maximum of 7H+ donors and 14H+ acceptor atoms, as shown in Table 7. According to its pharmacokinetic properties, ligand 5 showed a low level of

Table 9. Drug-likeness, lead-likeness and PAINS parameters of best compounds.

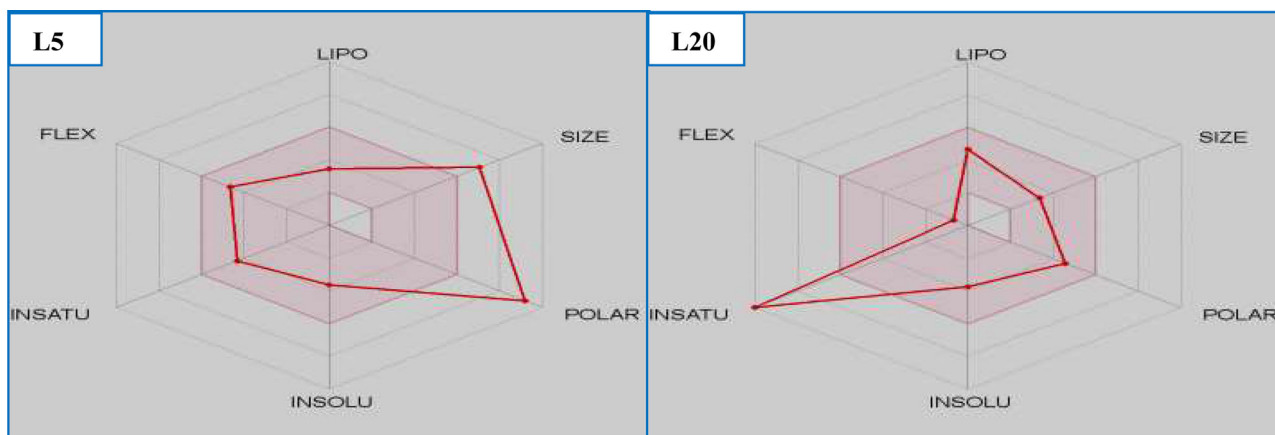
| Ligands | L05 | L14 | L04 | L06 | L20 | L12 | L08 | L02 | L22 | L26 | L01 | L10 | L19 | L15 | L9 |
|--------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| Drug-likeness | | | | | | | | | | | | | | | |
| Lipinski violations | 3 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ghose violations | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Veber violations | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Egan violations | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Muegge violations | 3 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lead-likeness violations | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| PAINS alerts | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |

Table 10. Pharmacokinetics and medicinal chemistry properties for tree top-scoring lead compounds.

| Molecules | Pharmacokinetics | | Medicinal chemistry | |
|----------------|------------------|------------------------------------|---|-------------------------|
| | GI absorption | Log K_p (skin permeation) (cm/s) | Leadlikeness | Synthetic accessibility |
| L5: Linarin | Low | -9.56 | No; 1 violation: MW > 350 | 6.43 |
| L14: Didymin | Low | -10.40 | No; 1 violation: MW > 350 | 6.27 |
| L20: Genistein | High | -6.05 | Yes; 0 violation: MW ≤ 350 XLOGP3 ≤ 3.5 Num. rotatable bonds ≤ 7 | 2.87 |

Table 11. The PASS prediction results showing the biological activities of the best two molecules.

| Sl. no. | Biological activities | Linarin L5 | | Genistein L20 | |
|---------|----------------------------|------------|-------|---------------|-------|
| | | Pa | Pi | Pa | Pi |
| 01 | Vasoprotector | 0.987 | 0.000 | 0.822 | 0.004 |
| 02 | CYP1A1 inhibitor | 0.970 | 0.001 | 0.893 | 0.004 |
| 03 | Free radical scavenger | 0.976 | 0.001 | 0.458 | 0.013 |
| 04 | Membrane integrity agonist | 0.970 | 0.002 | 0.913 | 0.008 |
| 05 | Anticarcinogenic | 0.954 | 0.001 | 0.715 | 0.008 |
| 06 | Chemopreventive | 0.952 | 0.002 | 0.648 | 0.004 |
| 07 | Hepatoprotectant | 0.932 | 0.002 | 0.680 | 0.008 |
| 08 | TP53 expression enhancer | 0.856 | 0.007 | 0.824 | 0.009 |
| 09 | Antioxidant | 0.815 | 0.003 | 0.765 | 0.004 |
| 10 | Antiviral | 0.746 | 0.004 | 0.430 | 0.024 |

**Figure 13.** Oral bioavailability radars of ligands L5, and L20. Colored zone is the suitable physicochemical space for oral bioavailability.

gastrointestinal absorption which contributes to *bad oral* bioavailability. But, *inhaled*.

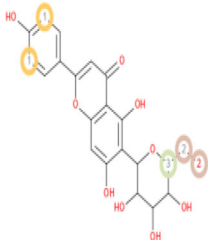
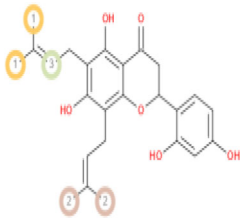
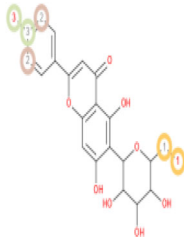
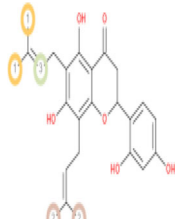
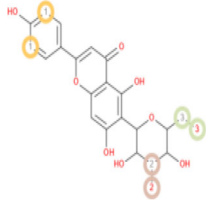
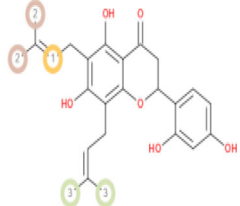
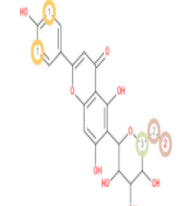
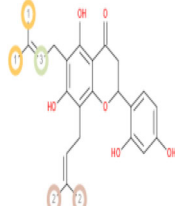
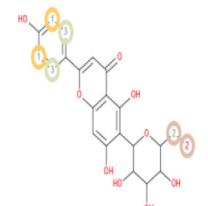
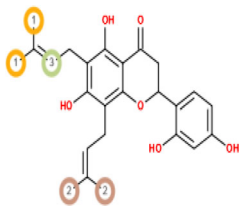
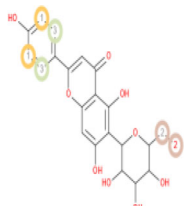
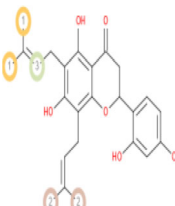
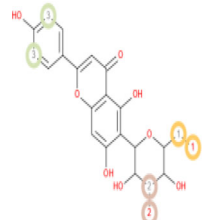
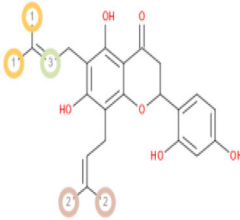
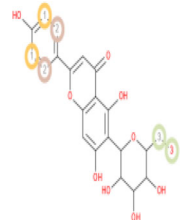
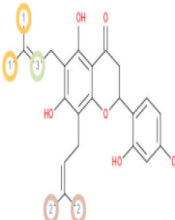
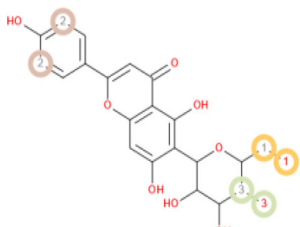
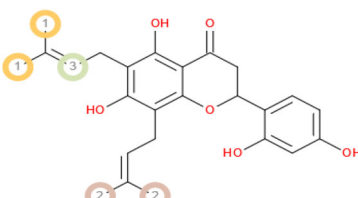
Ligand 5 according to pharmacokinetic parameters evaluated *in silico* showed no inhibition of cytochrome P450 isomers 1A2.

Ligand 20 has a maximum of 3H+ donors and 5H+ acceptor atoms, as shown in Table 7. According to its pharmacokinetic properties, ligand 20 showed a high level of

gastrointestinal absorption which contributes to *good oral* bioavailability.

Ligand 20 can inhibit CYP1A2, inhibition of these isoenzymes is certainly one major cause of pharmacokinetics-related drug-drug interactions leading to toxic or other unwanted adverse effects due to the lower clearance and accumulation of the drug or its metabolites. However, it should be noted that inhibition of CYP1A2 activity *in vitro*

Table 12. Results of the P450 sites of metabolism prediction study of the two best ligand molecules.

| Names of P450 isoenzymes | Linarin L5 | Genistein L20 | Names of P450 isoenzymes | Linarin L5 | Genistein L20 |
|--------------------------|---|--|--------------------------|--|--|
| 1A2 |  |  | 2C9 |  |  |
| 2A |  |  | 2C19 |  |  |
| 2B6 |  |  | 2D6 |  |  |
| 2C8 |  |  | 2E1 |  |  |
| 3A4 |  |  | | | |

does not necessarily imply drug interaction *in vivo*. Further studies will be needed to determine if this L20 can influence the CYP enzyme *in vivo*.

3.7. PASS and P450 site of metabolism (SOM) prediction

The PASS prediction study for best ligands was conducted to predict 10 intended biological activities. To carry out the PASS prediction experiment, $P_a > 0.7$ was kept, since this threshold give highly reliable prediction (Release, 2018). The PASS prediction results of all the best selected ligands are listed in Table 11. However, at $P_a > 0.7$, the LD50 and toxicity class was predicted using ProTox-II server (Pearson, 1986).

When analyzed by the admetSAR server, both Linarin and Genistein were predicted to have the acute oral toxicity class of 5, for this reason, they had the same predicted LD50 value (greater than 2000 mg/kg but less than 5000 mg/kg) (Becke, 1988). Prediction tests were also carried out for 10 biological activities, the results of which are in Table 11. We observe that all the compounds show some biological activities among the 10 tested.

3.8. Pharmacophore mapping

The possible sites of metabolism by CYPs 1A2, 2A6, 2B6, 2C19, 2C8, 2C9, 2D6, 2E1 and 3A4 of two best ligands

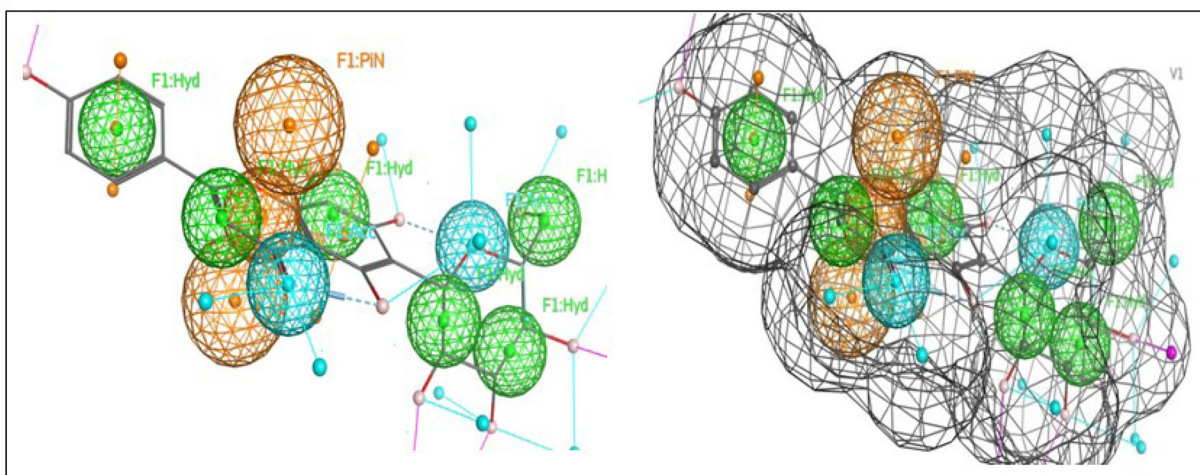


Figure 14. Pharmacophore mapping of Linarin L5. Here, cyan color – hydrogen bond acceptor, orange – aromatic, green color – hydrophobic.

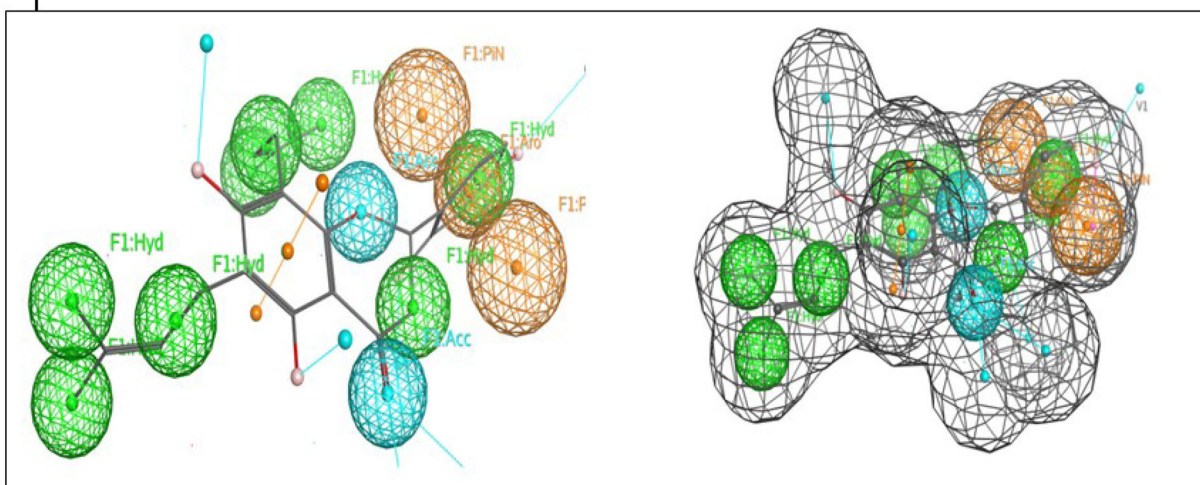


Figure 15. Pharmacophore mapping of Genistein L20. Here, cyan color – hydrogen bond acceptor, orange – aromatic, green color – hydrophobic.

Linarin L5 and Genistein L20 are summarized in Table 12. The possible sites of a chemical compound, where the metabolism by the isoforms of CYP450 enzymes may be taken place, are illustrated by circles on the chemical structure of the molecule (Zaretski et al., 2013). So the Linarin L5 and Genistein L20 can be metabolized by these enzymes.

The P450 SOM predictions showed that Linarin L5 had six sites of metabolism (SOMs) for the CYP450 2B6, CYP450 2C8, CYP450 2C9, CYP450 2D6, CYP450 2E1, CYP450 3A4 and five sites for CYP450 1A2, CYP450 2C19.

The P450 SOMs predictions showed that Genistein L20 had five sites of metabolism (SOMs) for the CYP450 1A2, 2A6, 2B6, 2C19, 2C8, 2C9, 2D6, 2E1 and 3A4.

The pharmacophore mapping is conveyed for the Linarin L5 and Genistein L20 best *inhaled* ligand and *oral*, respectively, of the flavonoides showed for Linarin 2 hydrogen acceptor bonds, six hydrophobic groups and three aromatic rings and for Genistein 2 hydrogen acceptor bonds, eight hydrophobic groups and three aromatic rings. It also generated a good number of good contacts with the pharmacophore of αA and αG Figures 14 and 15. The pharmacophore of Linarin generates a

hypothesis which can be applied successfully in biological screening for further experiments (Dixon et al., 2006).

Validation of our results, for flavonoids and phenolics acids different Immunotherapy (Clinic) is mentioned in Table 13.

Our molecular docking results with αA and αG coincide with clinical results; the flavonoids compounds were the most dominant.

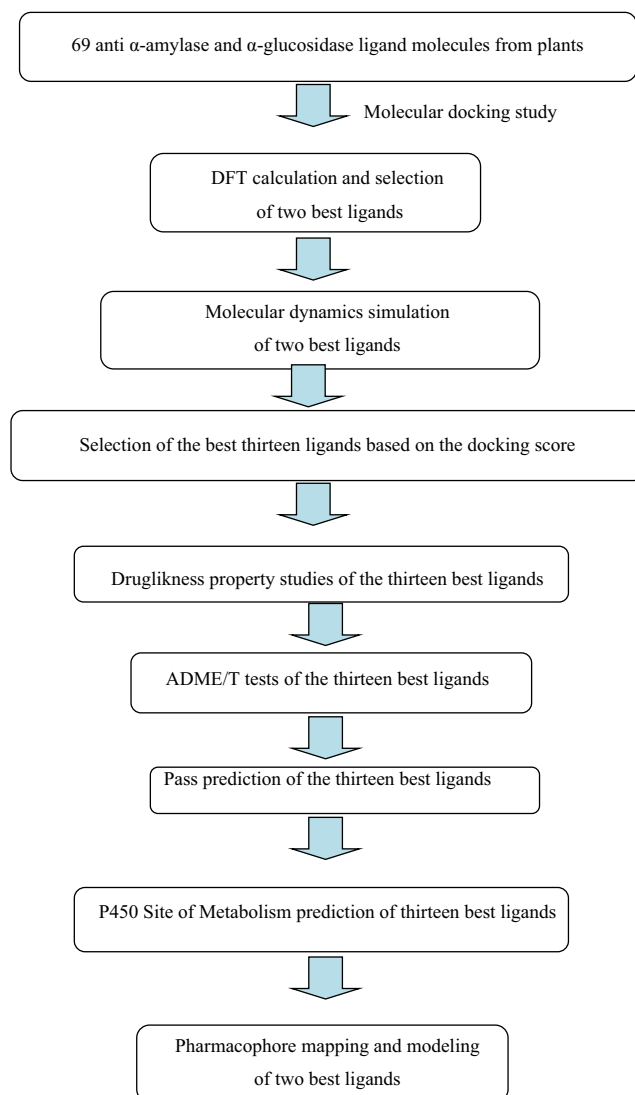
These three molecules (Acarbose, Miglitol and Voglibose) are pseudo-carbohydrates that competitively inhibit enzymes, αG and αA .

Our ligands natural Linarin L5 better stabilizes the system with its energy of αA and αG $-8.3867 \text{ kcal mol}^{-1}$ and -6.638 , respectively, we compare with the components of Clinical Drugs (see Table 13).

Linarin (Ligand5) could be excellent candidate as drugs because represents better energy (see Table 8) for all ligand molecules and the volume of distribution (VDs) suggest that the drug will be distributed in tissue. And again from the Table 5, Linarin L5 was characterized by a high lipophilicity and high coefficient of skin permeability $\log K_p$.

Table 13. Energy balance of complexes formed with α -amylase and α -glucosidase under other clinical and our results.

| Drugs | PDB ID: 4GQ and 3L4Y | Administration | Activity |
|-----------------------|--|----------------|---|
| <i>Clinical drugs</i> | | | |
| Acarbose | α -Amylase α -glucosidase -7.03730154 -5.61809444 | Oral | Inhibitor of <i>alpha-glucosidase</i> and pancreatic <i>alpha-amylase</i> with antihyperglycemic activity |
| Miglitol | - -3.71521044 | Oral | Inhibitor of <i>alpha-glucosidase</i> with antihyperglycemic activity |
| Voglibose | - -3.52416968 | Oral | Inhibitor of <i>alpha-glucosidase</i> with antihyperglycemic activity |
| <i>Our results</i> | | | |
| Linarin Lig5 | α -Amylase α -glucosidase -8.38637447 -6.63843393 | Inhaled | Inhibition with <i>alpha-glucosidase</i> and pancreatic <i>alpha-amylase</i> |
| Genistein Lig20 | -5.21851683 -5.61986256 | Oral | Inhibition with <i>alpha-glucosidase</i> and pancreatic <i>alpha-amylase</i> |

**Figure 16.** The flowchart of the work-plan of the experiment.

Therefore, we propose ligand 5 as the best ligand which allows the inhibition of α A and α G. And in the meantime, we suggest ligand L5 Linarin present in (flavonoid) with its validated activity score (-8.386, -6.638) respectively for α A and α G as a new *inhaled ligand*.

At the same time, we suggest ligand L20 Genistein present in Isoflavones as an oral inhibitor despite obeying Lipinski's rule without side effects. The latter has the same activity score -5.61986256 as Acarbose with α G. The present

bioinformatic analysis molecular dynamics simulations used to scrutinize novel (flavonoids). Linarin inhibitor of enzyme (α A and α G). Preceding studies has indicated that Linarin has been shown to have antidiabetic *activity*.

In vitro, many studies were focused on the inhibitory effect of the phenolic substances in two black legumes of different genera and the Mesocarp Tissue Extracts of Sugar Date Palm, on key enzymes linked to diabetic therapy, α A and α G.

The researches of Tan et al. (2017) proved that Myricetin exhibited the most potent α G inhibitory activity with IC50 (0.87 mg/mL = 2.73 mM), and had the highest inhibition for α A activity with IC50 values (1.19 mM = 0.38 mg/mL) lower than the commercial α A inhibitor (3.23 mg/mL).

The study of Das et al. (2017) showed the high inhibitory activities with IC50 values of (IC50 = 1.42 μ M for α A and IC50 = 1.8 μ M for α G), (IC50 = 0.23 μ M for α A and IC50 = 4.95 μ M for α G), (IC50 = 0.52 μ M, and 4-hydroxycinnamic amylase and IC50 = 2.58 μ M for α G) and IC50 = 4.91 μ M for α -acid (IC50 = 0.23 μ M) inhibited only α G for caffeic acid, 3,4-dihydroxy benzoic acid, Ferulic acid, 4-hydroxycinnamic and quinic acid, respectively (see Figure 16).

Dabur et al. (2018) reported that 5,6,7-trihydroxyflavone (baicalein), isolated from *Scutellaria baicalensis* acts as inhibitor of rat intestinal α G (IC50 = 32 μ M).

Jeong et al. (2012) reported that γ -aminobutyric acid and ferulic acid, isolated from *Triticum aestivum* L. Sprouts as inhibitor activities with IC50 values of 5.4 ± 0.2 and 9.5 ± 0.1 mM, respectively, against α A, and 1.4 ± 0.4 and 4.9 ± 0.3 mM against α G.

In our research the software package (MOE) does not detect any mark of the hydrophobic interactions between Linarin and both the α A and α G; what may be connected to the large size of this compound and the high number of torsion angles.

The results are identified to have inhibitory activities against novel α A and α G. Of these ligands, Linarin (ligand 5) has a stronger bond and high absorption in the intestines with good bioavailability. Therefore, the study carried out in this research reveals many secrets conveyed by the use of magic plants. Currently, herbal medicine offers solutions to heal with plants. It is a solution that is both alternative and complementary to the treatments of classical medicine, which are more and more popular and whose effectiveness is increasingly recognized.

4. Conclusion

In this investigation, the inhibition of α A and α G was theoretically examined by molecular docking analyses MD simulations, *in silico* assessment of the ADME and DFT calculation. Our calculations showed that the natural inhibitor Linarin of (flavones) provide more optimized inhibition of α -A and α -G for type-2 diabetic treatment. These interactions between α A and α G and those inhibitors are undergoing different interactions between (H-donor, H-acceptor and Pi-H) of natural ones. This model shows a considerable decrease of the complex energy and there by an increase of the inhibition activity. However, the docking simulation results are optimized under dynamic conditions by MD simulations to prove the stability of the interaction between both proteins and each ligand. Although compounds L5 and L14 have binding affinity with α -A and α -G in the docking simulation, the ligand-protein interactions mentioned in docking simulation are almost stable in dynamic conditions. Linarin inhibitor-L5 has a stronger bond and high affinity with α -A and α -G. We can

conclude that Linarin may be considered as effective α A and α G antidiabetic drugs.

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Disclosure statement

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ملخص

تتميز الجزائر بتنوع بيولوجي نباتي مهم يشكل مصدرا للمواد الطبيعية، لذلك للتعزير والحفاظ على هذه الثروة فوائد اقتصادية كبيرة لتنمية اقتصاد البلاد. وبالتالي، أولى هذا البحث اهتمامًا خاصًا بنبتتين من جنس *Carlina* و *Inula* لعائلة الأستراتييات (*Asteraceae*) ، وبشكل أكثر دقة توصيفًا كيميائيًا للمركبات المتطايرة باستخدام التقنيات التحليلية ودراسة الخصائص البيولوجية. كشف تحليل الزيت العطري عن غالبية مركب أسيتيلين في زيت جذور *Carlina hispanica* ومركبات أكسيجينية بالنسبة للزيت الأساسي من الأجزاء العلوية لـ *Inula montana*. زيادة عن ذلك أظهرت الدراسة البيولوجية أن أكسيد الكارلين والزيت الأساسي من *C. hispanica* ، يمتازان بخصائص مضادة للالتهابات واعدة وأخرى مضادة للأكسدة، كما أظهرت أن الجمع بين أكسيد الكارلين مع المضادات الإصطناعية قد أظهر تأثيرًا تآزرًا. مثلما أظهر الزيت الأساسي لـ *I. montana* تأثيرًا كمضاد للأكسدة. من ناحية أخرى، نتائج النشاط المضاد للميكروبات مثيرة للاهتمام. تم تطوير العمل المبدئي أيضًا على تثبيط مستقبلات إنزيم ديسموتاز الفائق عبر طريقتين بالكيمياء الحاسوبية مما أظهر أن كلا المركبين E-E acétate de farnésyle و E-acétate de nérolidol هما أفضل مثبطات ويمكن اقتراحهما كأدوية جديدة ذات خصائص مضادة للأكسدة.

الكلمات المفتاحية: الأستراتييات، الزيوت الأساسية، تحليل كيميائي، نشاط بيولوجي، النمذجة الجزيئية.

Résumé :

L'Algérie regorge d'une importante biodiversité floristique qui constitue une source potentielle de substances naturelles. De ce fait, la préservation et la valorisation de cette richesse inestimable peut avoir des retombés économiques considérables pour le développement de notre pays. En conséquence, ce travail de thèse a porté une attention particulière sur deux plantes du genre *Carlina* et *Inula* de la famille des *Asteraceae*, et plus précisément une caractérisation chimique de leurs fractions volatiles à l'aide des techniques d'analyses modernes et complémentaires (CPG, CPG-SM, RMN), et une étude des propriétés biologiques. L'analyse de l'huile essentielle des racines de *Carlina hispanica* a révélé la prédominance d'un composé acétylénique, et des sesquiterpènes oxygénés pour l'huile essentielle des parties aériennes de *Inula montana*. Cependant le volet biologique a montré que l'oxyde de carline ainsi que l'huile essentielle de *C. hispanica* sont dotées d'activité anti-inflammatoire et antioxydante prometteuse. Cependant, la combinaison de l'oxyde de carline avec les références a montré un effet synergique. L'huile essentielle de *Inula montana* a donné aussi un effet antioxydant intéressant. Les résultats de l'activité antimicrobienne ont été intéressants. Un travail initiatique sur l'inhibition des récepteurs de l'enzyme la superoxyde dismutase par les composés de l'huile essentielle de *Inula montana* via deux méthodes de chimie computationnelle a été élaboré et a montré que les deux composés E-E acétate de farnésyle et le E- acétate de nérolidol sont des meilleurs inhibiteurs et peuvent être proposés comme de nouveaux médicaments a potentiel antioxydant.

Mots clés : Asteraceae, huile essentielle, caractérisation chimique, activité biologique, modélisation moléculaire.

Abstract :

Algeria abounds in an important floristic biodiversity which constitutes a potential source of natural substances. Therefore, the preservation and development of this invaluable wealth can have considerable economic benefits for the development of the country. Therefore, this research focused on two plants of the genus *Carlina* and *Inula* of the *Asteraceae* family, and more precisely a chemical characterization of their volatile fractions using different analytical techniques (GC, GC-MS, NMR), and a study of biological properties. The analysis of the essential oil of the roots of *Carlina hispanica* revealed the predominance of an acetylenic compound, and on the other hand the dominant occurrence of oxygenated sesquiterpenes for the essential oil of the aerial parts of *Inula montana*. However, the biological aspects showed that carlina oxide as well as the essential oil of *C. hispanica* are endowed with promising anti-inflammatory and antioxidant activity, and the combination of carlina oxide with the references showed a synergistic effect. The essential oil of *Inula montana* also showed an interesting antioxidant effect. Furthermore, the results of the antimicrobial activity were moderate. An initial work on the inhibition of the receptors of the enzyme superoxide dismutase by the compounds of the essential oil of *Inula montana* via two methods of computational chemistry was elaborated and revealed that both compounds E-E farnesyl acetate and E- nerolidol acetate are better inhibitors and can be proposed as new drugs with antioxidant potential.

Keywords : Asteraceae, essential oil, chemical characterization, biological activity, molecular modeling.