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كلية علوم الطبيعة والحياة، وعلوم الأرض والكون
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Department of Biology

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Presented by

M^r BELLIFA Aymen

M^r CHIRANI Zakaria

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President	Mr Seghir Abdelfettah	Professor	University of Tlemcen
Supervisor	Mrs Baba Ahmed-Kazi Tani Zahira Zakia	Professor	University of Tlemcen
Examiner	Mr Aissaoui Mohamed	MCA	University of Tlemcen
Examiner	Ms Hassaine Lahfa Imane	MCB	University of Tlemcen

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Dedication



First and foremost, I am grateful to ALLAH for His guidance, health, strength, and blessings throughout this journey. Alhamdulillah for everything.

To my dear parents,

For your unconditional love, sacrifices, patience, and unwavering support throughout my life. Thank you for everything you have done to help me reach this achievement. May God protect you and grant you health, happiness, and a long life.

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Dedication



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Abstract

This study investigated the antibacterial activity of *Cinnamomum verum* bark essential oil, used alone and in combination with colistin, against clinical isolates and a reference strain of *Klebsiella pneumoniae*. The essential oil was extracted by hydrodistillation, with a mean recovery of 0.878%. The evaluation of its antibacterial activity by the agar disc diffusion method showed inhibition zones ranging from 13.33 to 17.00 mm. The determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) revealed bactericidal activity of the essential oil, with MIC/MBC values of 9.76 /19.53 µg/mL for the clinical isolates and 4.88/9.76 µg/mL for the reference strain. Colistin showed stronger antibacterial activity, with MIC values of 0.61 µg/mL for the clinical isolates and 0.076 µg/mL for the reference strain. However, combination therapy using *C. verum* essential oil and colistin showed no significant enhancement of antibacterial activity against the tested strains. Overall, *C. verum* essential oil demonstrated notable antibacterial activity against *K. pneumoniae*, although its activity was lower than that of colistin. The absence of a synergistic effect following combination highlights the need to investigate the activity of other essential oils and isolated bioactive constituents, such as cinnamaldehyde, and their potential effects against bacterial biofilms.

Keywords: *Cinnamomum verum*, Essential oil, *Klebsiella pneumoniae*, Colistin, Antibacterial activity, MIC, MBC.

الملخص

تُقيم هذه الدراسة النشاط المضاد للبكتيريا للزيت العطري المستخلص من قشور القرفة (*Cinnamomum verum*)، المستخدم بمفرده ومزجه مع الكوليستين، ضد عزلات سريرية وسلالة مرجعية لبكتيريا *Klebsiella pneumoniae*. استخلص الزيت العطري عن طريق التقطير المائي، بمتوسط مردود بلغ 0.878%. أظهر تقييم نشاطه المضاد للبكتيريا بواسطة طريقة الانتشار عبر الأقراص أقطار تثبيط تراوحت بين 13.33 و 17.00 مم. وكشف تحديد التركيز الأدنى المثبط (MIC) والتركيز الأدنى القاتل للبكتيريا (MBC) عن نشاط قاتل للبكتيريا للزيت العطري، بقيم MIC/MBC بلغت 19.53/9.76 µg/mL للعزلات السريرية و 9.76/4.88 µg/mL للسلالة المرجعية. أظهر الكوليستين نشاطاً مضاداً للبكتيريا أشد فاعلية، بقيم MIC بلغت 0.61 µg/mL للعزلات السريرية و 0.076 µg/mL للسلالة المرجعية. ومع ذلك، فإن العلاج المدمج باستخدام الزيت العطري لـ *C. verum* والكوليستين لم يظهر أي تحسن ملموس في النشاط المضاد للبكتيريا ضد السلالات المختبرة. وبشكل عام، أظهر الزيت العطري لـ *C. verum* نشاطاً ملحوظاً مضاداً للبكتيريا ضد *K. pneumoniae*، على الرغم من أن نشاطه كان أقل من نشاط الكوليستين. إن عدم وجود تأثير تآزري بعد الجمع يسلط الضوء على الحاجة إلى دراسة نشاط زيوت عطرية أخرى والمركبات الحيوية النشطة المنفصلة، مثل Cinnamaldéhyde، وآثارها المحتملة على البيوفيلم البكتيري.

الكلمات المفتاحية: *Cinnamomum verum*، الزيت العطري، *Klebsiella pneumoniae*، الكوليستين، النشاط المضاد للبكتيريا، MIC، MBC.

Résumé

Cette étude porte sur l'évaluation de l'activité antibactérienne de l'huile essentielle d'écorce de *Cinnamomum verum*, utilisée seule et en association avec la colistine, vis-à-vis d'isolats cliniques et d'une souche de référence de *Klebsiella pneumoniae*. L'huile essentielle a été extraite par hydrodistillation, avec un rendement moyen de 0,878 %. L'évaluation de son activité antibactérienne par la méthode de diffusion sur disque a montré des diamètres de zones d'inhibition compris entre 13,33 et 17,00 mm. La détermination de la concentration minimale inhibitrice (CMI) et de la concentration minimale bactéricide (CMB) a révélé une activité bactéricide de l'huile essentielle, avec des valeurs CMI/CMB de 9,76/19,53 µg/mL pour les isolats cliniques et de 4,88/9,76 µg/mL pour la souche de référence. La colistine a présenté une activité antibactérienne plus importante, avec des valeurs de CMI de 0,61 µg/mL pour les isolats cliniques et de 0,076 µg/mL pour la souche de référence. Cependant, l'association de l'huile essentielle de *C. verum* avec la colistine n'a montré aucune amélioration significative de l'activité antibactérienne vis-à-vis des souches testées. Globalement, l'huile essentielle de *C. verum* a présenté une activité antibactérienne notable contre *K. pneumoniae*, bien que son activité soit inférieure à celle de la colistine. L'absence d'un effet synergique après association souligne la nécessité d'étudier l'activité d'autres huiles essentielles ainsi que de constituants bioactifs isolés, tels que le cinnamaldéhyde, et leurs effets potentiels sur les biofilms bactériens.

Mots-clés : *Cinnamomum verum*, Huile essentielle, *Klebsiella pneumoniae*, Colistine, Activité antibactérienne, CMI, CMB.

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List of Abbreviations

K. pneumoniae : *Klebsiella pneumoniae*

MDR : multidrug-resistant

XDR : extensively drug-resistant

PDR : pandrug-resistant

CLSI : the Clinical and Laboratory Standards Institute

ATCC : American Type Culture Collection

C. verum : *Cinnamomum verum*

MBC : minimum bactericidal concentration

MIC : minimum inhibitory concentration

Part I : Literature
Review

Klebsiella pneumoniae is an opportunistic Gram-negative, non-motile, encapsulated, facultative anaerobic bacterium belonging to the family Enterobacteriaceae and is recognized as one of the most important pathogens responsible for healthcare-associated infections worldwide. Although it commonly colonizes the gastrointestinal tract and the nasopharynx of healthy individuals without causing disease, disruption of host defenses may allow this microorganism to invade sterile body sites and produce severe infections. Patients admitted to intensive care units, those undergoing invasive medical procedures, individuals receiving prolonged antibiotic therapy, and immunocompromised patients are particularly susceptible to *K. pneumoniae* infections (Martin & Bachman, 2018; Dong et al., 2022; Ullah et al., 2024).

Clinically, *K. pneumoniae* is associated with a broad spectrum of infections, including hospital-acquired and ventilator-associated pneumonia, urinary tract infections, bloodstream infections, surgical site infections, liver abscesses, meningitis, and septicemia. These infections are frequently associated with prolonged hospitalization, increased healthcare costs, and high mortality rates, particularly when caused by multidrug-resistant (MDR) isolates. In recent years, the emergence of hypervirulent strains capable of causing invasive community-acquired infections has further increased the clinical importance of this pathogen (Navon-Venezia et al., 2017; Russo & Marr, 2019; Wyres et al., 2020;).

The pathogenic success of *K. pneumoniae* is closely related to the expression of several virulence determinants that promote bacterial colonization, persistence, and evasion of host immune responses. Among these, the polysaccharide capsule represents the major virulence factor by protecting bacterial cells against phagocytosis, complement-mediated killing, and antimicrobial peptides. Additional factors, including lipopolysaccharide, fimbrial adhesins, siderophore-mediated iron acquisition systems, and the hypermucoviscous phenotype (Figure1), further contribute to bacterial survival and dissemination within the host. Collectively, these virulence determinants considerably enhance the pathogenic potential of *K. pneumoniae* and facilitate the establishment of persistent infections (Clegg & Murphy, 2016; Paczosa & Meccas, 2016; Zhu et al., 2021).

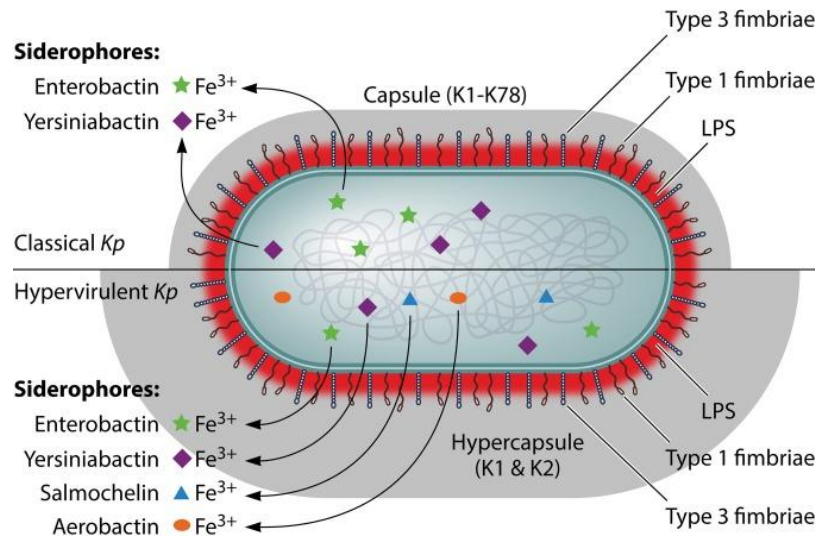


Figure 1: Major virulence factors of *K. pneumoniae* (Paczosa & Mecsas, 2016)

Among these virulence determinants, biofilm formation has attracted particular attention because of its major role in chronic and recurrent infections. Biofilms are highly organized microbial communities embedded within a self-produced extracellular polymeric matrix composed mainly of polysaccharides, proteins, lipids, and extracellular DNA. This three-dimensional structure firmly adheres to both living tissues and abiotic surfaces, including medical devices, while providing an efficient physical and biochemical barrier against host immune defenses and antimicrobial agents (Costerton et al., 1999; Donlan, 2002; Flemming & Wingender, 2010).

Biofilm development generally proceeds through four successive stages, including reversible attachment, irreversible adhesion accompanied by bacterial multiplication, maturation of the biofilm architecture, and finally dispersion, during which bacterial cells detach and colonize new surfaces (Figure 2). The capsule, lipopolysaccharide, and especially type 3 fimbriae play fundamental roles throughout these developmental stages. As a consequence, biofilm-producing *K. pneumoniae* isolates exhibit markedly increased tolerance to antimicrobial agents compared with planktonic bacteria, often requiring antibiotic concentrations several-fold higher to achieve bacterial eradication. This increased tolerance contributes significantly to therapeutic failure, recurrent infections, and the persistence of MDR strains in healthcare settings (Vuotto et al., 2014; Flemming et al., 2016; Guerra et al., 2022).

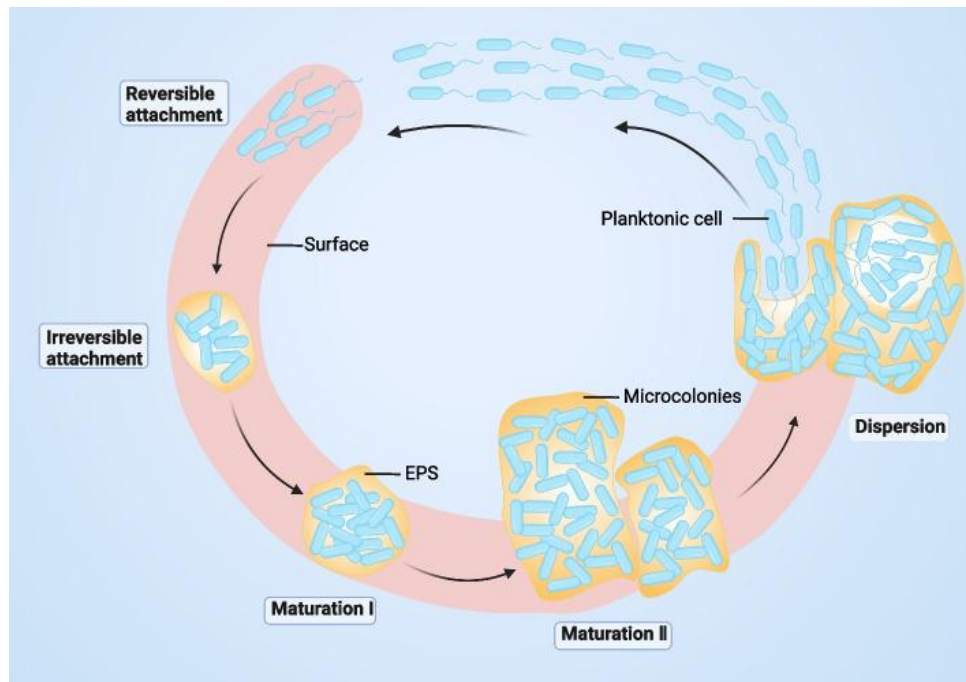


Figure 2: Stages of biofilm formation (Wang et al., 2023)

The remarkable pathogenicity of *K.pneumoniae* has been further aggravated by its extraordinary capacity to develop and disseminate antimicrobial resistance. Owing to its high genomic plasticity, this bacterium readily acquires resistance determinants through plasmids, transposons, integrons, and other mobile genetic elements, in addition to horizontal gene transfer. Consequently, *K. pneumoniae* has progressively evolved from a susceptible opportunistic pathogen into one of the leading causes of MDR healthcare-associated infections worldwide (Navon-Venezia et al., 2017; Partridge et al., 2018; Wyres & Holt, 2018).

According to the international definitions proposed by Magiorakos et al. (2012), antimicrobial resistance in *K. pneumoniae* may be classified into MDR, extensively drug-resistant (XDR), and pandrug-resistant (PDR) phenotypes. MDR isolates exhibit resistance to at least one antimicrobial agent in three or more antibiotic classes, whereas XDR strains remain susceptible to only one or two antimicrobial categories. PDR isolates are resistant to virtually all currently available antimicrobial agents, leaving extremely limited therapeutic options and representing one of the greatest challenges in modern clinical practice.

Several complementary mechanisms contribute to antimicrobial resistance in *K. pneumoniae*. Among them, the production of extended-spectrum β -lactamases (ESBLs) and carbapenemases represents the most clinically significant mechanism. ESBL-producing isolates hydrolyze third-generation cephalosporins and monobactams, whereas carbapenem-resistant *K. pneumoniae* produces carbapenem-hydrolyzing enzymes, including KPC, NDM, OXA-48-like, IMP, and VIM, which inactivate carbapenems that were previously considered the antibiotics of choice for severe Gram-negative infections. In addition to enzymatic degradation, resistance is reinforced by reduced outer membrane permeability resulting from porin loss, overexpression of multidrug efflux pumps, modification of antibiotic targets, and the protective effect of biofilm formation. The coexistence of several of these mechanisms within the same isolate considerably compromises the efficacy of conventional antimicrobial therapy and contributes to therapeutic failure (Navon-Venezia et al., 2017; Wang et al., 2020; Li et al., 2023).

The rapid global dissemination of carbapenem-resistant *K. pneumoniae* has dramatically reduced the number of effective antimicrobial agents available for clinical use. Consequently, clinicians have been compelled to reconsider older antibiotics, particularly polymyxins, as one of the few remaining therapeutic alternatives against severe infections caused by MDR Gram-negative bacteria. Among these antibiotics, colistin has regained considerable clinical importance because of its potent bactericidal activity against carbapenem-resistant Enterobacterales, despite the important limitations associated with its clinical use (Falagas & Kasiakou, 2005; Li et al., 2006; Nordmann et al., 2011).

Colistin, also known as polymyxin E, is a cyclic cationic polypeptide antibiotic originally isolated from *Paenibacillus polymyxa subsp. colistinus*. Introduced into clinical practice during the late 1950s, its systemic use progressively declined following the development of safer antimicrobial agents because of its well-documented nephrotoxicity and neurotoxicity. However, the worldwide emergence of MDR Gram-negative bacteria and the scarcity of novel antibiotics have led to its reintroduction as a last-resort therapeutic option for the treatment of infections caused by carbapenem-resistant *K. pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Poirel et al., 2017; Baltogianni, et al., 2024).

Colistin exerts its antibacterial activity by interacting with the lipid A component of lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria. This interaction disrupts membrane integrity, increases membrane permeability, and ultimately causes leakage of intracellular components leading to rapid bacterial cell death (**Figure3**). Despite its potent bactericidal activity, the clinical use of colistin remains challenging because its effective therapeutic concentrations frequently approach toxic plasma levels. Furthermore, the emergence of plasmid-mediated *Mcr* genes and chromosomal mutations associated with colistin resistance has raised additional concerns regarding the long-term effectiveness of this antibiotic (**Sorlí et al., 2017; Ahmed et al., 2020;**).

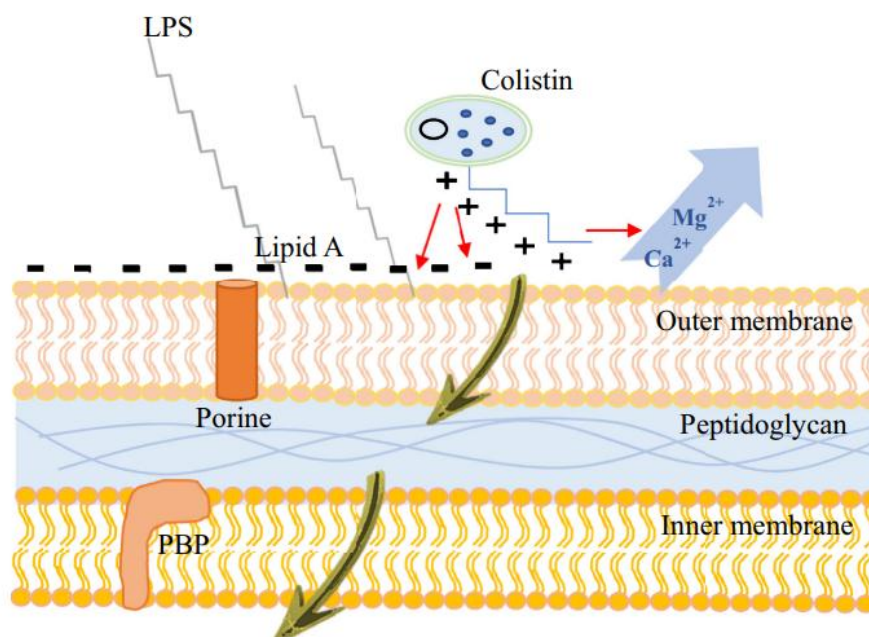


Figure 3: Mechanism of action of colistin against Gram-negative bacteria (**Silva et al., 2019**)

These limitations have encouraged the scientific community to investigate alternative therapeutic strategies capable of preserving the antibacterial efficacy of colistin while reducing its required therapeutic dose. Among the most promising approaches, combination therapy with natural products possessing intrinsic antimicrobial activity has attracted considerable attention, particularly the association of conventional antibiotics with plant-derived essential oils (**Wang et al., 2018; Topa et al., 2020; Jalil et al., 2023; Kong et al., 2023; Romo-Castillo et al., 2023**).

The continuous emergence of MDR bacteria, together with the limited availability of novel antimicrobial agents, has stimulated growing interest in medicinal plants as valuable sources of bioactive compounds with therapeutic potential. For centuries, medicinal plants have been widely used in traditional medicine for the treatment of infectious diseases, and numerous scientific investigations have confirmed that many plant-derived metabolites possess antibacterial, antiviral, antioxidant, and anti-inflammatory activities. Owing to their chemical diversity and multiple mechanisms of action, these natural products have become attractive candidates for the development of alternative or complementary antimicrobial therapies (**Silva & Fernandes Júnior, 2010; Anand et al., 2019; AlSheikh et al., 2020**).

Among plant-derived antimicrobial agents, essential oils have attracted particular attention because of their broad-spectrum biological activities. Essential oils are complex mixtures of volatile and hydrophobic secondary metabolites produced by aromatic plants and are generally obtained by steam distillation or hydrodistillation. Their chemical composition mainly includes terpenoids and phenylpropanoids, whose relative abundance depends on several factors such as plant species, geographical origin, climatic conditions, harvesting period, and extraction method. Unlike conventional antibiotics, which generally act on a single molecular target, essential oils contain numerous bioactive constituents capable of simultaneously affecting different bacterial structures and metabolic pathways, thereby reducing the probability of resistance development (**Bakkali et al., 2008; Burt, 2004; De Sousa et al., 2023; Nabi et al., 2025**).

The remarkable biological properties of *C. verum* are mainly attributed to the composition of its essential oil, which is particularly rich in trans-cinnamaldehyde, accompanied by other bioactive constituents including eugenol, linalool, β -caryophyllene, cinnamyl acetate, and several minor volatile compounds. Although trans-cinnamaldehyde represents the predominant constituent and is largely responsible for the characteristic aroma and antimicrobial activity of cinnamon, increasing evidence indicates that the biological activity of the essential oil results from the synergistic interaction of all its phytochemical constituents rather than from a single compound alone. Variations in chemical composition may nevertheless occur according to the geographical origin of the plant, environmental conditions, extraction procedure, and the plant organ used for essential oil production (**Rao & Gan, 2014; Kazemi & Mokhtariniya, 2016; Diniz do Nascimento et al., 2020; Usai & Di Sotto, 2023**).

Numerous studies have demonstrated that *Cinnamomum verum* essential oil exhibits significant antibacterial activity against both Gram-positive and Gram-negative bacteria, including clinically important MDR pathogens. Its antimicrobial effect is mainly related to the hydrophobic nature of its constituents, which facilitates their interaction with the bacterial cell membrane. Following membrane penetration, the essential oil alters membrane integrity and permeability, leading to leakage of intracellular constituents, disruption of ion homeostasis, inhibition of ATP synthesis, protein denaturation, and ultimately bacterial cell death. In addition, cinnamon essential oil has been shown to induce oxidative stress and interfere with quorum sensing systems involved in bacterial communication, virulence expression, and biofilm development (**Figure4**), thereby further reducing bacterial pathogenicity (Iseppi et al., 2024; Barbieri et al., 2026; El-Sakhawy et al., 2026).

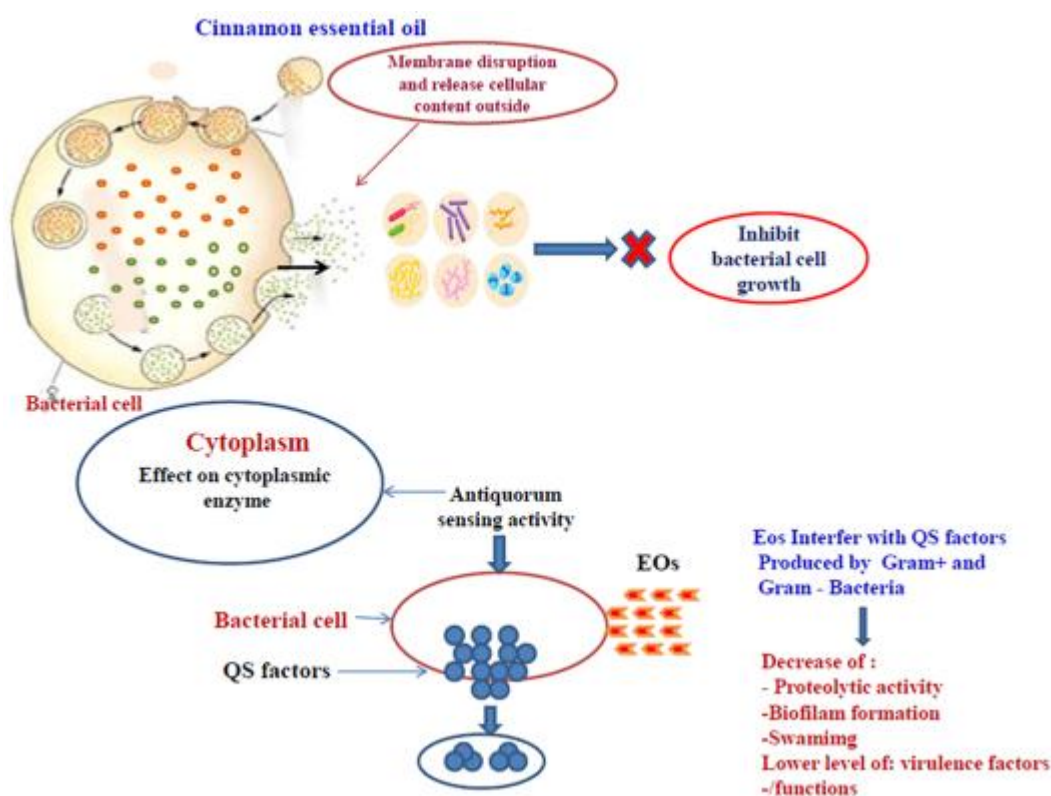


Figure 4: Antibacterial mechanisms of *Cinnamomum verum* essential oil (Singh et al., 2020)

Beyond its direct antibacterial activity, *C. verum* essential oil has demonstrated a remarkable capacity to inhibit biofilm formation. Experimental studies have shown that it interferes with the initial adhesion of bacterial cells, reduces the production of extracellular polymeric substances, disrupts biofilm maturation, and weakens established biofilms. These

properties are of particular clinical relevance in the management of *K.pneumoniae* infections, where biofilm formation plays a central role in bacterial persistence and antimicrobial tolerance. Consequently, the ability of cinnamon essential oil to impair biofilm development may contribute to improving bacterial susceptibility to conventional antimicrobial agents (**Adeosun et al., 2022; Didehdar et al., 2022; Mahrous et al., 2023**).

In this context, the search for alternative therapeutic strategies capable of enhancing antibacterial efficacy while reducing antibiotic toxicity has become a major area of research. Particular attention has been devoted to the investigation of medicinal plants and their essential oils because of their broad-spectrum antimicrobial properties and their potential to potentiate the activity of conventional antibiotics. The present study was therefore undertaken to evaluate the antibacterial activity of *Cinnamomum verum* essential oil, used alone or in combination with colistin, against clinical and reference strains of *Klebsiella pneumoniae*. More specifically, this work aimed to extract the essential oil of *C. verum*, evaluate its antibacterial activity, determine MIC and minimum bactericidal concentration (MBC) of both the essential oil and colistin, and investigate whether their combination could reduce the effective concentration of colistin required to inhibit bacterial growth.

***Part II : Materials
and Methods***

The experimental work was carried out at the Fin de Cycle Microbiology Laboratory (Genetics Pole), Department of Biology, Faculty of Natural and Life Sciences and Earth and Universe Sciences, Abou Bekr Belkaïd University – Tlemcen.

I. Materials

I.1 Plant Material

The study focused on a plant species widely used in human nutrition, cinnamon (*Cinnamomum verum*). The bark of this species, purchased from a local market in the wilaya of Tlemcen, served as the raw material for the extraction of the essential oil.



Figure 5: Bark sticks of cinnamon (*Cinnamomum verum*).

I.2 Biological Material

Antibacterial assays were performed using three strains belonging to the species *Klebsiella pneumoniae*. These included two clinical isolates, originating from a urinary tract sample and an oral cavity sample, respectively, as well as a reference strain (*Klebsiella pneumoniae* ATCC 700603).

II. Methods

II.1 Essential Oil Extraction

The extraction of essential oil was conducted at the Natural Products Laboratory (LAPRONA) of Abou Bekr Belkaid University of Tlemcen. The extraction was performed using classical hydrodistillation of dried ground *cinnamon* bark, as shown in **(Figure 6)**. Three independent extractions were conducted using 420 g, 305 g, and 400 g of plant material. Each sample was mixed with 2 liters of water and subjected to hydrodistillation for 3 hours. To prevent any deterioration caused by air or light, the essential oils were stored in glass tubes at a temperature of 4 ± 1 °C and protected from light



Figure 6: Hydrodistillation apparatus for the extraction of *Cinnamomum verum* essential oil

II.2 Evaluation of Antibacterial Activity

The antibacterial activity of *Cinnamomum verum* essential oil, colistin, and their combination was evaluated against three *K. pneumoniae* strains, including two clinical isolates (urinary and oral cavity isolates) and one reference strain. Antibacterial activity was assessed using the agar disc diffusion method and the broth microdilution method for the determination of MIC, following the recommendations of the Clinical and Laboratory Standards Institute (CLSI).

II.2.1 Agar Disc Diffusion Method

A fresh bacterial culture (18–24 h) was suspended in sterile physiological saline (0.9% NaCl). The bacterial suspension was adjusted to an absorbance ranging from 0.08 to 0.10 at 625 nm, corresponding to approximately 10^8 CFU/mL. Sterile paper discs (6 mm in diameter) were impregnated separately with *Cinnamomum verum* essential oil, colistin solution, and a combination of *Cinnamomum verum* essential oil and colistin at a final concentration of **512 µg/disc**. The impregnated discs were then placed onto Mueller–Hinton agar plates previously inoculated with the standardized bacterial suspension. All assays were performed in triplicate. Following incubation at 37°C for 24 h, the diameters of the inhibition zones were measured in millimeters to evaluate the antibacterial activity according to **the NCCLS (2006)**.

II.2.2 Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentrations (MIC) of *Cinnamomum verum* essential oil and colistin were determined using the broth microdilution method in sterile 96-well microplates according to the Clinical and Laboratory Standards Institute (**CLSI, 2009**).

A fresh bacterial culture (18–24 h) was used to prepare a bacterial suspension adjusted to approximately 1×10^6 CFU/mL in Mueller–Hinton broth. Due to its hydrophobic nature, *Cinnamomum verum* essential oil was dissolved in DMSO and Tween 80, and serial two-fold dilutions ranging from 10 mg/mL to 0.61 µg/mL were prepared. Colistin was dissolved in sterile distilled water, and serial two-fold dilutions ranging from **1250 µg/mL** to **0.076 µg/mL** were prepared. Subsequently, 100 µL of each dilution was dispensed into the appropriate wells of a sterile 96-well microplate containing 100 µL of the standardized bacterial suspension. Wells containing only Mueller–Hinton broth served as the negative control, whereas wells containing the bacterial suspension without the tested agents served as the positive control.

The microplates were incubated at 37°C for 24 h. The MIC was defined as the lowest concentration of the tested agent that completely inhibited visible bacterial growth.

Following MIC determination, the minimum bactericidal concentration (MBC) was evaluated according to standard guidelines (**CLSI, 1999**). Briefly, a 5µL aliquot was collected

from each well showing no visible bacterial growth (corresponding to the MIC and higher concentrations) and subcultured by spread plating onto the surface of sterile Mueller–Hinton agar (MHA) plates. The inoculated plates were then incubated at 37 °C for 24 h.

II.2.3 Checkerboard Microdilution Assay

The evaluation of the antimicrobial effect of the combination of *Cinnamomum verum* essential oil and colistin against planktonic cells of *K. pneumoniae* strains (urinary isolates, oral cavity isolates, and the reference strain ATCC 700603) was carried out using the checkerboard microdilution method (**Moody, 1991**).

The antimicrobial combination (*C. verum* / colistin) was used at concentrations ranging from 1/8×MIC, 1/4×MIC, 1/2×MIC, MIC, 2×MIC and 4×MIC of each agent.

***Part III : Results
and Discussion***

I. Essential oil extraction

I.1 Organoleptic Characteristics

Hydrodistillation of *Cinnamomum verum* bark yielded an essential oil with specific organoleptic characteristics, as shown in the following **Table1**.

Table 1: Organoleptic characteristics of *Cinnamomum verum* essential oil

Characteristics	Smell	Color	Aspect
<i>Cinnamomum verum</i>	Strongly aromatic	Pale yellow	Oily liquid



Figure 7: Extracted essential oil of *Cinnamomum verum*

I.2 Essential oil yield of *Cinnamomum verum*

Hydrodistillation of *Cinnamomum verum* bark yielded an essential oil with a mean yield of 0.878%. The extraction yield was calculated according to the following formula (**Kasim et al., 2014**):

$$\text{Yield (\%)} = (\text{mass of essential oil} / \text{mass of plant material}) \times 100$$

Table 2: Yield of *Cinnamomum verum* essential oil

Extraction	Mass (g)	Yield (%)	Mean yield (%)
First extraction	420	0.835	0.878
Second extraction	305	0.952	
Third extraction	400	0.848	

The mean essential oil yield obtained from *Cinnamomum verum* bark in the present study was 0.878%, based on three independent extractions. This value is lower than that reported by **Abbas & Maouche. (2019)** for *Cinnamomum zeylanicum* essential oil 1.67% obtained by hydrodistillation. Since *Cinnamomum zeylanicum* is considered a synonym of *Cinnamomum verum* **Singh et al. (2020)**, this comparison remains relevant.

The yield obtained in this study was also lower than that reported by **Beniaich et al. (2023)**, who recorded a yield of $2.11 \pm 0.15\%$ for *Cinnamomum verum* essential oil extracted by hydrodistillation from plant material collected in Morocco. In addition, **Malsawmtluangi et al. (2016)** reported a higher bark oil yield 2.13% for *Cinnamomum verum* from Mizoram, North-East India, using hydrodistillation with a Clevenger-type apparatus.

Furthermore, **Li et al. (2013)** reported a yield of 1.14% for *Cinnamomum verum* bark collected in China. However, their protocol differed from ours in several aspects, including the use of finely powdered bark, a 1hour soaking step, prolonged hydrodistillation (5 h), and an additional recovery step of volatile compounds from the aqueous phase using dichloromethane. These methodological differences may have contributed to the higher yield observed in their study.

In contrast, the yield obtained in the present study was higher than that reported by **Saeedi et al. (2022)**, who recorded a yield of 0.68% for *Cinnamomum verum* bark essential oil obtained by hydrodistillation using a Clevenger-type apparatus. This indicates that our result falls within the range of values already described in the literature for the same species (**Figure 8**).

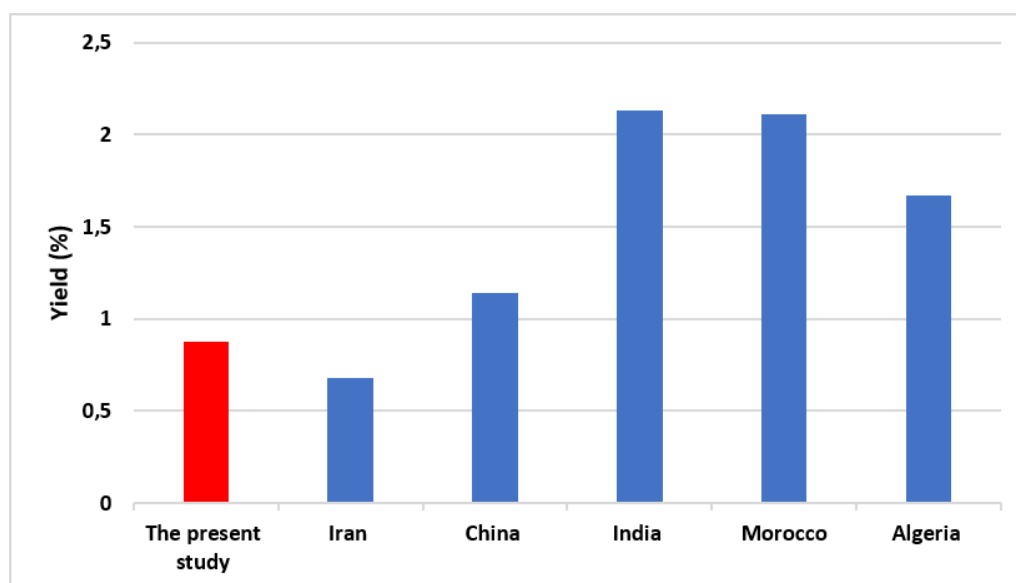


Figure 8: Variation in the essential oil yield of *C. verum* in different countries

The variability observed between our results and those reported in previous studies can be attributed to several factors. Indeed, both the yield and chemical composition of essential oils are influenced by multiple parameters, including plant species, geographical origin, harvesting conditions, harvest period, drying process, agronomic practices, and extraction techniques (Svoboda & Hampson, 1999).

II. Evaluation of Antibacterial Activity

II.1 Determination of Inhibition Zone Diameters

The antibacterial activity of the essential oil, colistin, and their combination against the three *K. pneumoniae* strains was evaluated using the disc diffusion method (**Figure 9**). The inhibition zone diameters are presented in **Table 3**.

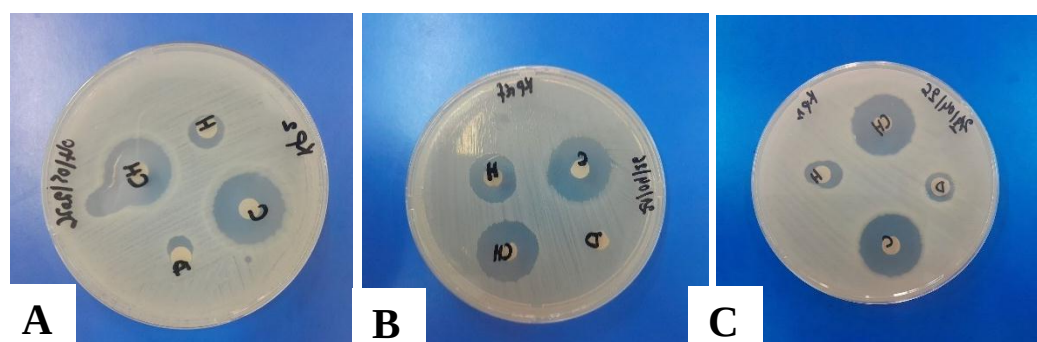


Figure 9 : Antibacterial activity of *Cinnamomum verum* essential oil (H), colistin (C), and their combination (CH) determined by agar disc diffusion against *K. pneumoniae* strains: **(A)** *K. pneumoniae* (oral cavity isolate, Kp2), **(B)** *K. pneumoniae* (urinary isolate, Kp1), and **(C)** *K. pneumoniae* ATCC 700603 (reference strain, Kp ref). (Where H: Essential oil, C: Colistin, CH: Essential oil + Colistin, D: DMSO control)

Table 3: Inhibition zone diameters (mm) of *Cinnamomum verum* essential oil, colistin, and their combination against *K. pneumoniae* strains.

Zone diameter (ID, mm)	<i>K. pneumoniae</i> (urinary)	<i>K. pneumoniae</i> (oral cavity)	<i>K. pneumoniae</i> ATCC 700603
Essential oil	13.33 ± 0.58	14.00 ± 1.00	17.0 ± 1.00
Colistin	21.00 ± 0.00	20.66 ± 1.52	19.67 ± 0.58
Essential oil + colistin	21.33 ± 0.58	19.66 ± 0.58	20.67 ± 0.58

The results showed that all three treatments exhibited antibacterial activity against the tested *K. pneumoniae* strains. For the urinary isolate, *C. verum* essential oil produced an inhibition zone of 13.33 ± 0.58 mm, whereas colistin exhibited greater antibacterial activity with an inhibition zone of 21.00 ± 0.00 mm. The combination of the essential oil and colistin resulted in a slightly larger inhibition zone (21.33 ± 0.58 mm) than colistin alone.

For the oral cavity isolate, the essential oil produced an inhibition zone of 14.00 ± 1.00 mm, while colistin exhibited an inhibition zone of 20.66 ± 1.52 mm. The combination of the essential oil and colistin produced an inhibition zone of 19.66 ± 0.58 mm, which was slightly lower than that obtained with colistin alone.

For the ATCC 700603 reference strain, the essential oil produced a larger inhibition zone (17.00 ± 1.00 mm) than those observed for the clinical isolates, indicating a higher susceptibility of the reference strain. Colistin exhibited an inhibition zone of 19.67 ± 0.58 mm, whereas the combination produced an inhibition zone of 20.67 ± 0.58 mm.

Overall, colistin exhibited the strongest antibacterial activity against all tested *K. pneumoniae* strains, whereas *C. verum* essential oil showed moderate antibacterial activity. The reference strain was the most susceptible to the essential oil, while the two clinical isolates exhibited comparable susceptibility profiles. Furthermore, combining the essential oil with colistin did not markedly improve the antibacterial activity compared with colistin alone, as only a slight increase in the inhibition zone was observed for the urinary isolate and the ATCC 700603 reference strain, whereas a slight decrease was recorded for the oral cavity isolate.

According to the qualitative classification of bacterial sensitivity proposed by **Ponce et al. (2003)**, inhibition zone diameters are classified as **not sensitive (<8 mm)**, **sensitive (9–14 mm)**, **very sensitive (15–19 mm)**, and **extremely sensitive (>20 mm)**. Based on this classification, *C. verum* essential oil showed **a sensitive (+)** antibacterial effect against both clinical isolates (13.33 and 14.00 mm), whereas the ATCC 700603 reference strain was classified as **very sensitive (++)** with an inhibition zone of 17.00 mm.

These findings are consistent with several previous studies reporting the antibacterial activity of *C. verum* essential oil against *K. pneumoniae* (**Zenati et al., 2014; Elgammal et al., 2020**).

II.2 Determination of Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs)

To confirm the antibacterial activity observed in the agar disc diffusion assay, MIC and minimum bactericidal concentration (MBC) of *Cinnamomum verum* essential oil and colistin were determined against the three *K. pneumoniae* strains. The results are presented in **(Table 4)**.

Table 4: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Cinnamomum verum* essential oil and colistin against three *K. pneumoniae* strains (in µg/mL).

Essential oil or antibiotic	Antibacterial parameters	<i>K. pneumoniae</i> (urinary)	<i>K. pneumoniae</i> (oral cavity)	<i>K. pneumoniae</i> ATCC 700603
<i>C. verum</i>	MIC	9.76	9.76	4.88
	MBC	19.53	19.53	9.76
	Effect	Bactericidal	Bactericidal	Bactericidal
Colistin	MIC	0.61	0.61	0.076
	MBC	1.22	1.22	0.15
	Effect	Bactericidal	Bactericidal	Bactericidal

Regarding *Cinnamomum verum* essential oil, the urinary and oral cavity isolates exhibited identical MIC and MBC values of **9.76 µg/mL** and **19.53 µg/mL**, respectively. In contrast, the reference strain showed lower MIC and MBC values (**4.88 µg/mL** and **9.76 µg/mL**, respectively), indicating greater susceptibility than the clinical isolates. Concerning colistin, both clinical isolates exhibited identical **MIC (0.61 µg/mL)** and **MBC (1.22 µg/mL)** values, whereas the reference strain showed lower **MIC (0.076 µg/mL)** and **MBC (0.15 µg/mL)** values. Overall, colistin demonstrated markedly lower MIC and MBC values than *C. verum* essential oil against all tested strains, confirming its stronger antibacterial activity. Furthermore, the MBC/MIC ratio was calculated to evaluate the nature of the antibacterial activity (**Table4**), where a ratio of ≤ 4 indicates a bactericidal effect, $4 < \text{MBC/MIC} \leq 16$ reflects a bacteriostatic effect, and a ratio > 16 indicates tolerance (**Fauchère & Avril, 2002**). In the present study, all calculated MBC/MIC ratios for both *C. verum* essential oil and colistin were equal to or lower than 2, confirming that both agents exert a true bactericidal effect against all tested *K. pneumoniae* strains.

The antibacterial activity observed for *C. verum* essential oil is in agreement with several previous studies reporting its efficacy against *K. pneumoniae*. **Wijesinghe et al. (2021)** demonstrated that *C. verum* leaf essential oil exhibited significant antibacterial and antibiofilm activity against *K. pneumoniae*, producing low MIC values and causing cell wall damage and cellular shrinkage. Similarly, **Vasconcelos et al. (2020)** reported that *Cinnamomum cassia* essential oil and its major constituent, cinnamaldehyde, inhibited carbapenemase-producing *K.*

pneumoniae by disrupting membrane integrity and inducing leakage of intracellular contents. More recently, **Selma et al. (2024)** also reported a pronounced bactericidal activity of *C. zeylanicum* essential oil against colistin-resistant Gram-negative clinical isolates, *including K. pneumoniae*. These findings support the antibacterial potential of cinnamon essential oil and are in agreement with the results obtained in the present study.

The high antibacterial activity of colistin observed in the present study aligns with previous literature. According to the CLSI guidelines ($\text{MIC} \leq 2 \mu\text{g/mL}$), all tested isolates were classified as susceptible to colistin. Similar high susceptibility rates have been widely documented in several studies on *K. pneumoniae* (**Simner et al., 2018; Atmani et al., 2023; Kazaz et al., 2025**), confirming that colistin remains one of the most effective therapeutic options against susceptible *K. pneumoniae* strains.

The lower susceptibility observed in the clinical isolates compared with the ATCC 700603 reference strain may be related to adaptive resistance mechanisms acquired in the hospital environment, such as outer membrane modifications or increased efflux pump activity (**Llobet et al., 2011; Masi & Pagès, 2013**).

II.3 Evaluation of the Combined Effect of Cinnamomum verum Essential Oil and Colistin

The minimum inhibitory concentrations (MICs) and the overall interactive effect of *C. verum* essential oil and colistin against the tested *K.pneumoniae* strains are summarized in **(Table 5)**.

Table 5: Minimum inhibitory concentration (MIC) of *Cinnamomum verum* essential oil and colistin used alone and in combination against *Klebsiella pneumoniae* isolates.

Bacterial Strains	<i>C.verum</i> (Essential oil)	Colistin	Combination (EO + Colistin)	Observation / Effect
	MIC (µg/mL)	MIC (µg/mL)	MIC (µg/ml)	
<i>K. pneumoniae</i> (urinary)	9.76	0.61	9.76 + 0.61	No obvious effect
<i>K. pneumoniae</i> (oral cavity)	9.76	0.61	19.53 + 1.22	No obvious effect
<i>K. pneumoniae</i> ATCC 700603	4.88	0.076	9.76 + 0.15	No obvious effect

The combination of *Cinnamomum verum* essential oil and colistin against planktonic *K. pneumoniae* did not enhance the antibacterial activity of either antimicrobial agent. For the urinary isolate, the MIC values obtained in combination remained identical to those of each agent used alone (9.76 + 0.61 µg/mL). In contrast, the oral cavity isolate and the ATCC 700603 reference strain required two-fold higher MIC values in combination (19.53 + 1.22 µg/mL and 9.76 + 0.15 µg/mL, respectively). These findings indicate that the association did not improve the antibacterial efficacy of the tested agents and, in some strains, even required higher inhibitory concentrations than those observed for the individual treatments.

Interestingly, these findings differ from those reported for purified phytochemicals. Previous studies have demonstrated that cinnamaldehyde and eugenol were able to potentiate the antibacterial activity of colistin against resistant Gram-negative bacteria (Wang et al., 2018; Topa et al., 2020; Kong et al., 2023).

The discrepancy observed in the present study may be explained by the fact that the whole essential oil is a complex mixture of numerous volatile constituents. Although trans-cinnamaldehyde is the major bioactive compound, interactions among the different minor constituents may influence its overall antibacterial activity and its interaction with colistin. Furthermore, variations in the phytochemical composition of the essential oil according to plant origin, extraction method, and environmental conditions may also contribute to these differences.

Part IV: Conclusion

The increasing prevalence of MDR *K. pneumoniae* and the emergence of resistance to last-resort antibiotics such as colistin represent a major public health concern. Moreover, the nephrotoxicity associated with colistin highlights the need to explore alternative therapeutic strategies capable of enhancing antibacterial efficacy while potentially reducing the required antibiotic dosage.

Therefore, the present study was undertaken with the following objectives:

- To extract the essential oil from *Cinnamomum verum*.
- To evaluate the in vitro antibacterial activity of *Cinnamomum verum* essential oil against *Klebsiella pneumoniae*.
- To determine the MIC and minimum bactericidal concentration (MBC) of *Cinnamomum verum* essential oil and colistin.
- To evaluate the antibacterial activity of the combination of *Cinnamomum verum* essential oil and colistin against *K. pneumoniae*.

The extraction of *Cinnamomum verum* essential oil yielded 0.878%, confirming the successful recovery of the volatile fraction. The essential oil exhibited noticeable antibacterial activity against all tested *K.pneumoniae* strains. The reference strain was more susceptible than the clinical isolates, whereas colistin demonstrated superior antibacterial activity against all tested strains. The MIC and MBC results confirmed the bactericidal activity of both the essential oil and colistin. Furthermore, the combination of *Cinnamomum verum* essential oil and colistin did not produce a marked enhancement of antibacterial activity under the experimental conditions of the present study. Although purified phytochemicals such as cinnamaldehyde and eugenol have been reported to potentiate the activity of colistin, this effect was not observed with the whole essential oil, suggesting that interactions among its different constituents may influence its antibacterial performance.

Overall, the findings of the present study demonstrate that *Cinnamomum verum* essential oil possesses promising antibacterial activity against *K. pneumoniae* and could be considered a potential natural source of antibacterial compounds. Nevertheless, no beneficial interaction with colistin was observed under the experimental conditions employed.

To further extend this work, it would be valuable to:

- Evaluate the antibiofilm activity of *C. verum* essential oil and its major phytochemical constituents, alone and in combination with colistin, against biofilm-forming *K. pneumoniae*
- Evaluate the antibacterial activity of *C. verum* essential oil against a larger collection of clinical *K. pneumoniae* isolates.
- Investigate purified bioactive constituents, particularly cinnamaldehyde and eugenol, in combination with colistin to determine their potential synergistic effects.
- Evaluate the efficacy and safety of these natural compounds through in vivo studies before considering their therapeutic application.
- Characterize the chemical composition of the essential oil in order to identify the compounds responsible for its antibacterial activity

Part V :

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Appendix

Annex01:

According to (Subasinghe et al., 2016), the botanical classification of *cinnamon verum* is as follows:

- **Kingdom:** Plantae
- **Subkingdom:** Viridiplantae
- **Infrakingdom:** Streptophyta
- **Superdivision :** Embryophyta
- **Division :** Tracheophyta
- **Subdivision :** Spermatophytina
- **Class:** Magnoliopsida
- **Superorder:** Magnolianaes
- **Order:** Laurales
- **Family:** Lauraceae
- **Genus :** *Cinnamomum Schaeff.*
- **Species :** *Cinnamomum verum*

Abstract

This study investigated the antibacterial activity of *Cinnamomum verum* bark essential oil, used alone and in combination with colistin, against clinical isolates and a reference strain of *Klebsiella pneumoniae*. The essential oil was extracted by hydrodistillation, with a mean recovery of 0.878%. The evaluation of its antibacterial activity by the agar disc diffusion method showed inhibition zones ranging from 13.33 to 17.00 mm. The determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) revealed bactericidal activity of the essential oil, with MIC/MBC values of 9.76 /19.53 µg/mL for the clinical isolates and 4.88/9.76 µg/mL for the reference strain. Colistin showed stronger antibacterial activity, with MIC values of 0.61 µg/mL for the clinical isolates and 0.076 µg/mL for the reference strain. However, combination therapy using *C. verum* essential oil and colistin showed no significant enhancement of antibacterial activity against the tested strains. Overall, *C. verum* essential oil demonstrated notable antibacterial activity against *K. pneumoniae*, although its activity was lower than that of colistin. The absence of a synergistic effect following combination highlights the need to investigate the activity of other essential oils and isolated bioactive constituents, such as cinnamaldehyde, and their potential effects against bacterial biofilms.

Keywords: *Cinnamomum verum*, Essential oil, *Klebsiella pneumoniae*, Colistin, Antibacterial activity, MIC, MBC.

المخلص

تُقيم هذه الدراسة النشاط المضاد للبكتيريا للزيت العطري المستخلص من قشور القرفة (*Cinnamomum verum*)، المستخدَم بمفرده ومزجه مع الكوليستين، ضد عزلات سريرية وسلالة مرجعية لبكتيريا *Klebsiella pneumoniae*. تم استخلاص الزيت العطري عن طريق التقطير المائي، بمتوسط مردود بلغ 0.878%. أظهر تقييم نشاطه المضاد للبكتيريا بواسطة طريقة الانتشار عبر الأقراص أقطار تثبيط تراوحت بين 13.33 و17.00 مم. وكشف تحديد التركيز الأدنى المثبط (MIC) والتركيز الأدنى القاتل للبكتيريا (MBC) عن نشاط قاتل للبكتيريا للزيت العطري، بقيم MIC/MBC بلغت 9.76/19.53 µg/mL للعزلات السريرية و4.88/9.76 µg/mL للسلالة المرجعية. أظهر الكوليستين نشاطاً مضاداً للبكتيريا أشد فاعلية، بقيم MIC بلغت 0.61 µg/mL للعزلات السريرية و0.076 µg/mL للسلالة المرجعية. ومع ذلك، فإن العلاج المدمج باستخدام الزيت العطري لـ *C. verum* والكوليستين لم يظهر أي تحسن ملموس في النشاط المضاد للبكتيريا ضد السلالات المختبرة. وبشكل عام، أظهر الزيت العطري لـ *C. verum* نشاطاً ملحوظاً مضاداً للبكتيريا ضد *K. pneumoniae*، على الرغم من أن نشاطه كان أقل من نشاط الكوليستين. إن عدم وجود تأثير تآزري بعد الجمع يسلط الضوء على الحاجة إلى دراسة نشاط زيوت عطرية أخرى والمركبات الحيوية النشطة المنفصلة، مثل Cinnamaldéhyde، وآثارها المحتملة على البيوفيلم البكتيري.

الكلمات المفتاحية: *Cinnamomum verum*، الزيت العطري، *Klebsiella pneumoniae*، الكوليستين، النشاط المضاد للبكتيريا، MIC، MBC.

Résumé

Cette étude porte sur l'évaluation de l'activité antibactérienne de l'huile essentielle d'écorce de *Cinnamomum verum*, utilisée seule et en association avec la colistine, vis-à-vis d'isolats cliniques et d'une souche de référence de *Klebsiella pneumoniae*. L'huile essentielle a été extraite par hydrodistillation, avec un rendement moyen de 0,878 %. L'évaluation de son activité antibactérienne par la méthode de diffusion sur disque a montré des diamètres de zones d'inhibition compris entre 13,33 et 17,00 mm. La détermination de la concentration minimale inhibitrice (CMI) et de la concentration minimale bactéricide (CMB) a révélé une activité bactéricide de l'huile essentielle, avec des valeurs CMI/CMB de 9,76/19,53 µg/mL pour les isolats cliniques et de 4,88/9,76 µg/mL pour la souche de référence. La colistine a présenté une activité antibactérienne plus importante, avec des valeurs de CMI de 0,61 µg/mL pour les isolats cliniques et de 0,076 µg/mL pour la souche de référence. Cependant, l'association de l'huile essentielle de *C. verum* avec la colistine n'a montré aucune amélioration significative de l'activité antibactérienne vis-à-vis des souches testées. Globalement, l'huile essentielle de *C. verum* a présenté une activité antibactérienne notable contre *K. pneumoniae*, bien que son activité soit inférieure à celle de la colistine. L'absence d'un effet synergique après association souligne la nécessité d'étudier l'activité d'autres huiles essentielles ainsi que de constituants bioactifs isolés, tels que le cinnamaldéhyde, et leurs effets potentiels sur les biofilms bactériens.

Mots-clés : *Cinnamomum verum*, Huile essentielle, *Klebsiella pneumoniae*, Colistine, Activité antibactérienne, CMI, CMB.