REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE

MINISTERE DE L'ENSEIGNEMENT SUPERIEURE ET DE LA RECHERCHE SCIENTIFIQUE

UNIVERSITE ABOU-BEKR BELKAID - TLEMCEN

MEMOIRE

Présenté à :

FACULTE DES SCIENCES – DEPARTEMENT DE CHIMIE

Pour l'obtention du diplôme de :

MASTER EN CHIMIE

Spécialité : Chimie Pharmaceutique

Par : Mr. **Nourene Irobey Itnene**

Sur le thème

Synthèse de bases de Mannich de N-(1-(4-méthoxyphényl) -3-oxo-3-phénylpropyl) acétamide et 3-(4-méthoxyphényl) -1- phényl-3-(pipéridin-1-yl) propan-1-one et l'étude de leurs toxicités, de leurs activités antimicrobiennes et antioxydantes.

Soutenu via Teams Le 21/04/2022 à Tlemcen devant le jury composé de :

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Année universitaire: 2020-2021

PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

UNIVERSITY ABOU BEKR BELKAID OF TLEMCEN

MASTER THESIS

Introduced to:

FACULTY OF SCIENCES - DEPARTMENT OF CHEMISTRY

As mean to obtain the degree of:

MASTER IN CHEMISTRY

Speciality: Pharmaceutical Chemistry

By: **Mr. Nourene Irobey Itnene**

On the Topic

Synthesis of Mannich bases of N-(1-(4-methoxyphenyl)-3-oxo-3-phenyl propyl) acetamide and 3-(4-methoxyphenyl)-1-phenyl-3-(piperidin-1-yl) propan-1-one and investigation of their toxicity, antimicrobial and antioxidant activities

Defended via Teams on 21/04/2022 in Tlemcen in front of the jury composed of:

Dr. NEGADI Latifa	Professor	University of Tlemcen	Chair
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Academic year: 2020-2021

Dedicacy

To my parents

To my brothers and sisters

To all who are dear to me

Acknowledgments

First of all, I would like to thank God, the almighty and merciful, who granted us the strength and patience to do this work.

This work was carried out at the Organic Chemistry of Natural Substances Laboratory, University of Ibadan, Nigeria, under the supervision of **Dr. Ganiyat K. Oloyede,** in the frame of the Intra-Africa Mobility Project. Hence, I would like to express all my gratitude to my co-supervisor **Dr. Ganiyat K. Oloyede** at the University of Ibadan for her follow-up supervision, advice, encouragement, support and availability during my stay in Nigeria.

I would like primarily to express my sincere thanks to my main supervisor, **Prof. Chewki Ziani-Cherif** at the University of Tlemcen for his excellent supervision throughout my research work and throughout the completion of this thesis. I am very grateful for his help, support, availability, encouragement, trust, understanding, and modesty.

My sincere thanks also go to the coordinator of the ACADEMY Intra-Africa project, **Prof. Latifa Negadi** for her unfailing support and help in making this work possible.

I would also like to warmly thank **Dr Keniche Assia** at the University of Tlemcen, for her advice, encouragement, and availability during my two years of master's training.

Separately, I would also like to thank the members of the jury, **Prof. Latifa Negadi**, **Dr. Benabdallah Med,** and **Prof. Rahmoun Nadjib**, who honored me by judging this research work.

I address my sincere thanks without limits to my parents, for their constant support, their encouragement, and especially their judicious advice, which contributed to the success of my university studies.

Finally, I would like to express my sincere thanks to all the professors, lecturers and all the people who, through their words, writings, advice and criticisms, have guided my reflections and have agreed to meet me and answer my questions during this research work.

Contents

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Abstract

Mannich bases of N-(1-(4-methoxyphenyl)-3-oxo-3-phenylpropyl) acetamide (A) and 3-(4-methoxyphenyl)-1-phenyl-3-(piperidin-1-yl) propan-1-one (B) were synthesized and their purities were confirmed by melting point and Thin Layer Chromatography. The compounds were characterized by NMR, IR and UV. Antimicrobial analyses were carried out using the Agar well diffusion method. The antioxidant activity was assessed using the 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) and hydrogen peroxide (H_2O_2) methods. Toxicity was carried out following the Brine Shrimp Toxicity assay. Results of toxicity analysis gave LC_{50} values of 119.91 mg/mL (A) and 178.14 mg/mL (B) which showed that both compounds were non-toxic compared to the standard (cyclophosphamide). Both compounds possessed significant antimicrobial activity against bacterial and fungal strains (*Escherichia coli, Staphylococcus aereus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonellae typhi, Candida albican, Rhizopus stolonifer, Aspergillus Niger,* and *Penicillium notatum*) as compared to the standards, gentamicin and thioconazole for bacteria and fungi respectively. *In vitro* antioxidant screening by DPPH free radical scavenging method and H_2O_2 scavenging effect showed that the compounds possessed notable antioxidant activity compared to the antioxidant standards (ascorbic acid (vitamin C), vitamin A and butylated hydroxyanisole). The results showed that these two Mannich bases are synthetic drug promoters and could be the basis for synthesis of other antimicrobial and antioxidant drugs.

Keywords: N-(1-(4-methoxyphenyl)-3-oxo-3-phenylpropyl) acetamide,3-(4-methoxyphenyl)-1-phenyl-3-(piperidin-1-yl) propan-1-one, solubility, antimicrobial, antioxidant, toxicity.

Résumé

Les bases de Mannich de N-(1-(4-methoxyphényl) -3-oxo-3-phenylpropyl) acétamide (A) et de 3-(4-methoxyphényl) -1-phényl-3-(pipéridin-1-yl) propan-1-one (B) ont été synthétisées et leur pureté a été confirmée par point de fusion et chromatographie sur couche mince. Les structures ont été caractérisées dans un premier temps par RMN, IR et UV. L'analyse antimicrobienne a été effectuée a été effectuée par méthode de diffusion en puits d'agar ; celle oxydante fut effectuée en utilisant les méthodes du radical 2,2-diphényl-1-picryl hydrazyl (DPPH) et du peroxyde d'hydrogène (H_2O_2). La toxicité a été évaluée par test de toxicité de la crevette de saumure. Les résultats de l'analyse de toxicité ont donné des valeurs de LC_{50} de 119,91 mg/mL (A) et 178,14 mg/mL (B) qui ont montré que les deux composés n'étaient pas toxiques par comparaison à la référence (cyclophosphamide). Les deux composés possèdent une activité antimicrobienne significative contre les souches bactériennes et fongiques (*Escherichia coli, Staphylococcus aereus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonellae typhi, Candida albican, Rhizopus stolonifer, Aspergillus Niger,* et *Penicillium notatum*) par rapport aux standards, gentamicine et thioconazole pour les bactéries et les champignons respectivement. Le criblage antioxydant in vitro par la méthode de piégeage des radicaux libres DPPH et l'effet de piégeage sur le peroxyde d'hydrogène (H_2O_2) ont montré que les composés possédaient une activité antioxydante notable par rapport aux normes antioxydantes (acide ascorbique (vitamine C), vitamine A et hydroxyanisole butylé). Les résultats ont montré que ces deux bases de Mannich sont des médicaments synthétiques promoteurs et pourraient constituer la base de synthèse d'autres médicaments antimicrobiens et antioxydants.

Mots clés : N-(1-(4-méthoxyphényl)-3-oxo-3-phénylpropyl) acétamide, 3-(4-méthoxyphényl)-1-phényl-3-(pipéridin-1-yl)propan-1-one,solubilité,antimicrobien, antioxydant et la toxicité.

ملخص

قواعد Mannich لـ A) N- (1- (4-methoxyphenyl) -3-oxo-3-phenylpropyl) acetamide) و 3- (4-methoxyphenyl) -1-phenyl-3- (piperidin-1-yl) propan) تم تصنيع -1 - واحد (ب) وتأكد نقاوتهم بنقطة الانصهار وكروماتوغرافيا الطبقة الرقيقة. تميزت الهياكل لأول مرة بالرنين المغناطيسي النووي ، والأشعة تحت الحمراء والأشعة فوق البنفسجية. تم إجراء تحليل مضادات الميكروبات بطريقة الانتشار في آبار الآجار. تم إجراء الطريقة المؤكسدة باستخدام طريقة 2،2-ثنائي فينيل -1 بيكريل هيدروزيل (DPPH) وبيروكسيد الهيدروجين (H_2O_2). تم تقييم السمية عن طريق اختبار سمية الأرتيميا. أعطت نتائج تحليل السمية قيم التركيز المميت النصفي بلغت 119.91 مجم / مل (أ) و 178.14 مجم / مل (ب) مما أظهر أن المركبين ليسا سامين مقارنة بالمرجع (سيكلوفوسفاميد). يمتلك كلا المركبين نشاطًا هامًا مضادًا للميكروبات ضد السلالات البكتيرية والفطرية (Escherichia coli، Staphylococcus aereus، Bacillus subtilis، Pseudomonas aeruginosa، Klebsiella pneumoniae، Salmonellae typhi، Candida Albican، Rhizopus stolonifer، Penicillus niger، and notinamic stolonifer، Aspergillus niger ، للبكتيريا والفطريات على التوالي. أظهر فحص مضادات الأكسدة في المختبر بواسطة طريقة إزالة الجذور الحرة DPPH وتأثير الكسح على بيروكسيد الهيدروجين (H2O2) أن المركبات تمتلك نشاطًا مضادًا للأكسدة ملحوظًا مقارنة بمعايير مضادات الأكسدة (حمض الأسكوربيك (فيتامين ج) وفيتامين أ وبوتيلات هيدروكسي الأيزول). أظهرت النتائج أن قاعدتي Mannich هاتان هما محفزات عقاقير اصطناعية ويمكن أن تشكل الأساس لتخليق عقاقير أخرى مضادة للميكروبات ومضادات الأكسدة.

الكلمات المفتاحية: N- (1- (4-Methoxyphenyl) -3-oxo-3-phenylpropyl) acetamide، 3- (4-Methoxyphenyl) -1-phenyl-3- (piperidin-1-yl) propan-1-one، الذوبان ومضادات الميكروبات ومضادات الأكسدة والسمية

List of Abbreviations

Abs	Absorbance
BHA	Butylated hydroxyanisole
DPPh	2,2-diphenyl 1-picrylhydrazyl
DMSO	Dimethylsulfoxide
g	gramme
KBr	Potassium bromide
IR	Infrared
H_2O_2	Hydrogen peroxide
LC_{50}	Median Lethal Concentration
NaOH	Sodium hydroxide
NMR	Nuclear Magnetic Resonance
nm	nanometer
Mg	milligram
mL	milliliter
PBS	Phosphate-Buffered Saline
Rf	Frontal Ratio
TLC	Thin Layer Chromatography
UV-Visible	UltraViolet-Visible

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General Introduction

Organic synthesis, a branch of chemical synthesis has helped scientists to construct, extend structures, or add new components to a given molecule from smaller subunits thereby leading to new chemical substances [1]. Mannich reaction is a chemical reaction that consists of a condensation of ammonia, primary or secondary amine, with an aldehyde and a compound containing an active hydrogen atom. The Mannich reaction is a simple, fast, effective, and economical reaction leading to new chemical compounds called Mannich base. Mannich base synthesis undertaken at the Organic Synthesis Laboratory at the University of Ibadan, Nigeria is hereby presented.

Mannich bases represent extremely promising candidates for the development of drugs against many bacterial and parasitic infections which are a resurgent public health problem.

There are still so many deadly diseases and infections that continuously pose a threat to human existence, hence the need to study the Mannich base reaction as a mean to prevent recurrent diseases.

The aim

The aim of this research is to synthesize new Mannich bases with therapeutic activities and evaluate their toxicity, antimicrobial and antioxidant activities.

The Objectives

The objectives of this research work are:

- To synthesize new Mannich bases

- To determine the purity of the synthesized compounds by analytical procedures such as melting point determination and thin layer chromatography;

- Elucidate the structures of the synthesized compounds using spectroscopic methods such as Infrared, NMR, and UV-visible.

- To study the antimicrobial, antioxidant and toxicity activities of the synthesized compounds;

Chapter 1 : Literature Review

1.1 Introduction

The incidence of fungal and bacterial infections of all kinds has been steadily increasing over the last decades and has become one of the major causes of morbidity and mortality, especially in patients with weakened immune systems. The development of resistance to currently used antifungal and antibacterial drugs is also a major concern, and the discovery of new antibacterial and antifungal agents is becoming one of the highest priorities of the pharmaceutical industry [2].

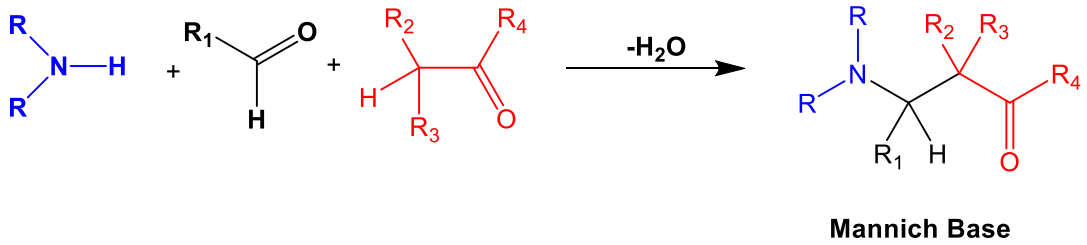
As a result, an impressive number of papers dealing with the antibacterial activity of Mannich bases have been published in the last decade. From a structural point of view, the Mannich bases reported in these studies are derived from almost all major types of substrates capable of undergoing aminomethylation. These Mannich bases have been evaluated against both Gram-positive and Gram-negative bacteria belonging to different families [3].

These facts explain the interest in developing new potent antibacterial drugs on the Mannich base to generate minimal bacterial resistance.

1.2 The Mannich reaction

1.2.1 Introduction

The Mannich reaction is a multi-component condensation between a non-enolizable aldehyde, a primary or secondary amine and an enolizable carbonyl compound, affording a β-amino-carbonyl compound also known as Mannich base. The iminium derivative of the aldehyde is the acceptor in the reaction. The reaction is named after the chemist Carl Mannich [4].



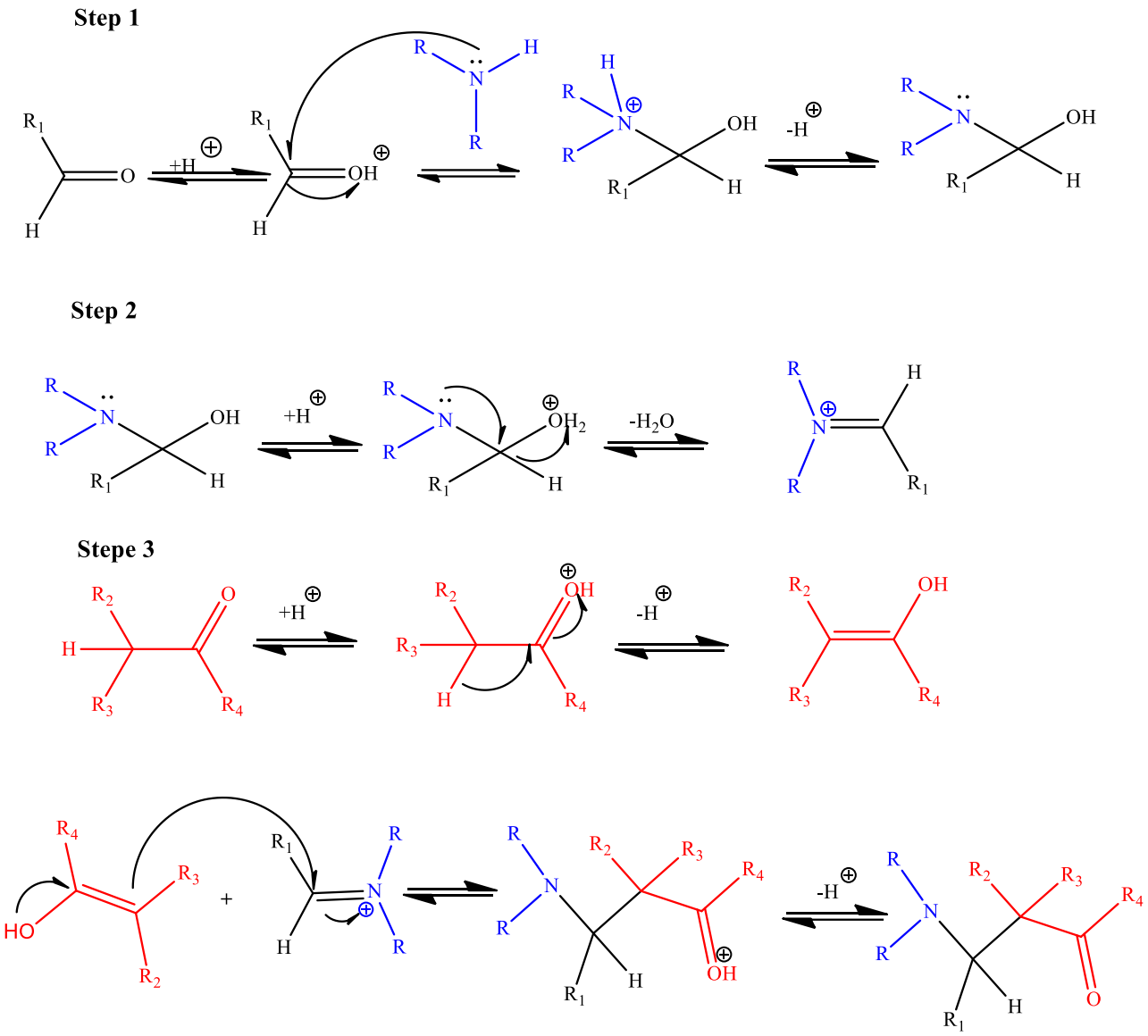
Scheme 1 : The example of the Mannich Reaction

The Mannich reaction is an example of nucleophilic addition of an amine to a carbonyl group followed by dehydration of the Schiff base. The Schiff base, or imine, is an electrophile that reacts in the second step of an electrophilic addition with an enolizable compound when submitted to an acidic medium.

In addition, the Mannich reaction is one of the most important and significant methodologies for the formation of carbon-carbon bonds in organic synthesis and a key step in the synthesis of many pharmaceutical and natural products. In this part, we try to follow and discover the synthesis of the Mannich base using the Mannich reaction.

1.2.2 Mechanism of the Mannich reaction

The original mechanism proposed for the Mannich reaction is detailed below:



Noteworthy, as shown below, variations of conditions for the Mannich reaction such as the nature of the catalyst have been documented recently, which may affect slightly the original mechanism.

1.2.3 Field of application of the Mannich reaction

The Mannich reaction is used in many areas of organic chemistry. Examples include [5]:

- Alkylamines;

- Peptides, nucleotides, antibiotics and alkaloids (*e.g.* tropinone);

- Agrochemicals, such as plant growth regulators;

- Polymers;

- Catalysts;

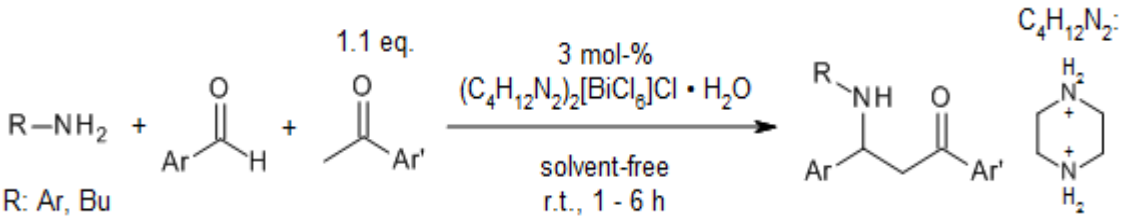
- Tissue cross-linking with formaldehyde;

- Pharmaceutical drugs (e.g. rolitracycline, the Mannich product of tetracycline and pyrrolidine), fluoxetine (antidepressant), tramadol and tolmetine (anti-inflammatory drug);

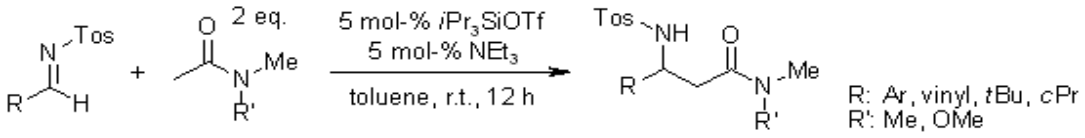
- Soap and detergents. These compounds are used in a variety of cleaning, automotive fuel processing applications.

1.2.4 Some examples of Mannich's reaction found in recent literature

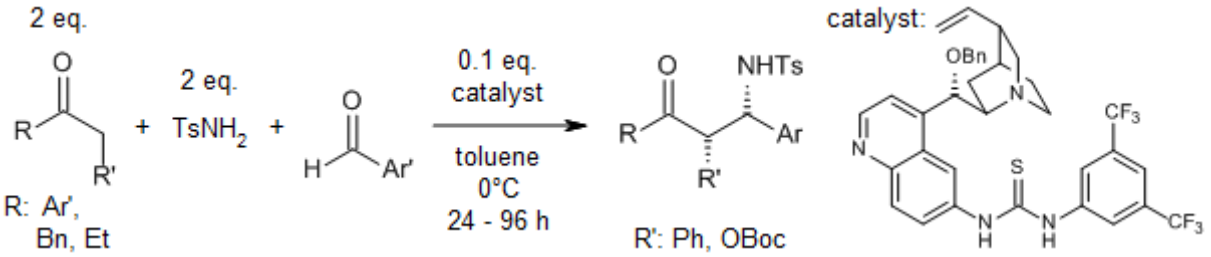
The following reactions represent examples of the Mannich reaction recently reported in the literature, the purpose being to emphasize the continuous importance of the reaction. With that kept in mind, yields will not be reported since they vary widely depending on the attached groups.



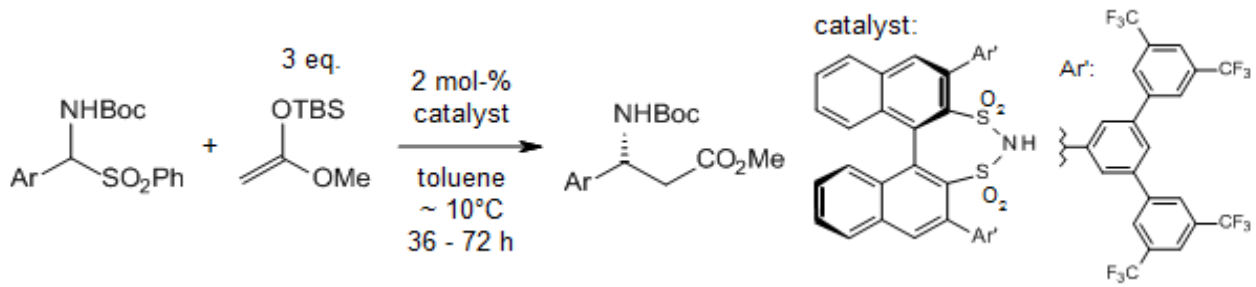
Scheme 2: An Efficent, Mild, Solvent-free, One-pot three-component Mannich reaction catalyzed by $(C_4H_{12}N_2)_2[BiCL_6]$ Cl.H2O [6].



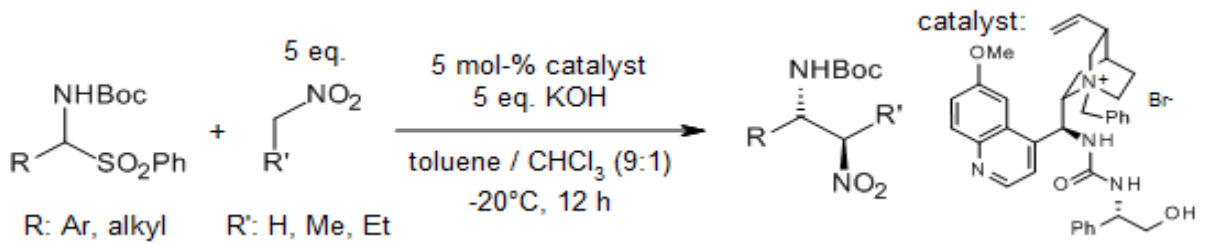
Scheme 3: Catalytic Silicon-Mediated Carbon-Carbon Bond-Forming Reaction of Unactivated Amides [7].



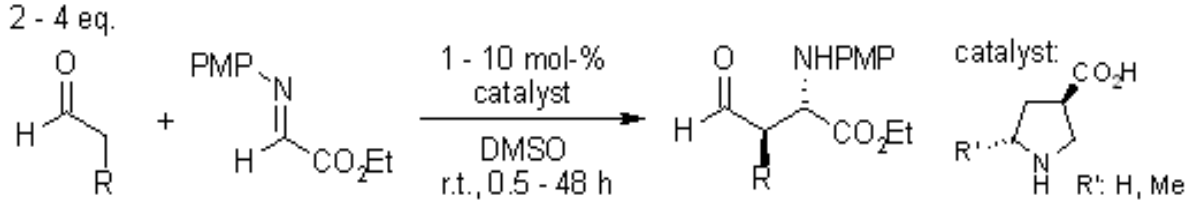
Scheme 4: Highly Enantioselective Three-Component Direct Mannich Reactions of Unfunctionalized Ketones Catalyzed by Bifunctional Organocatalysts [8].



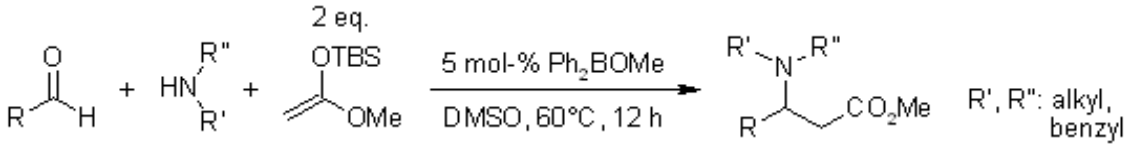
Scheme 5: Disulfonimide-Catalyzed Asymmetric Synthesis of β[3]-Amino Esters Directly from *N*-Boc-Amino Sulfones [9].



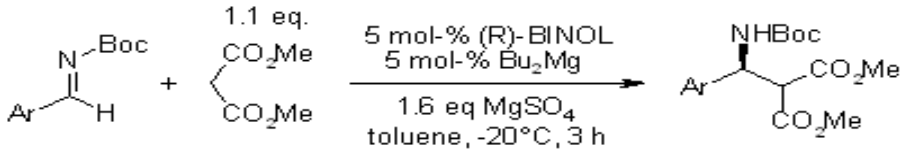
Scheme 6 : Asymmetric Phase-Transfer Catalysts Bearing Multiple Hydrogen-Bonding Donors: Highly Efficient Catalysts for Enantio- and Diastereoselective Nitro-Mannich Reaction of Amidosulfones [10]



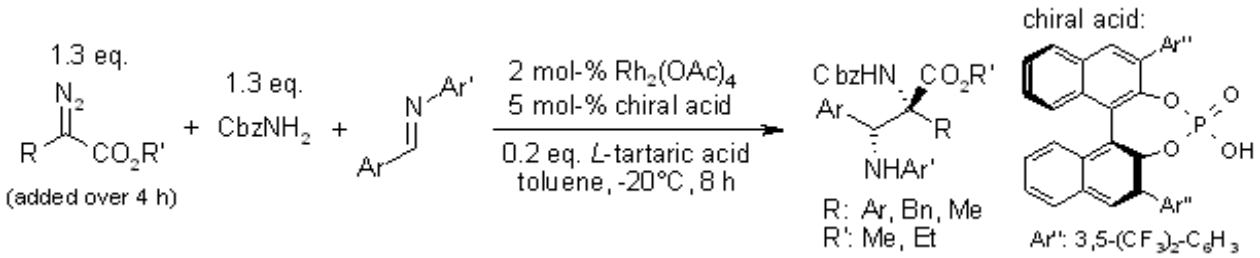
Scheme 5 : Catalysis of 3-Pyrrolidinecarboxylic Acid and Related Pyrrolidine Derivatives in Enantioselective *anti*-Mannich-Type Reactions: Importance of the 3-Acid Group on Pyrrolidine for Stereocontrol [11].



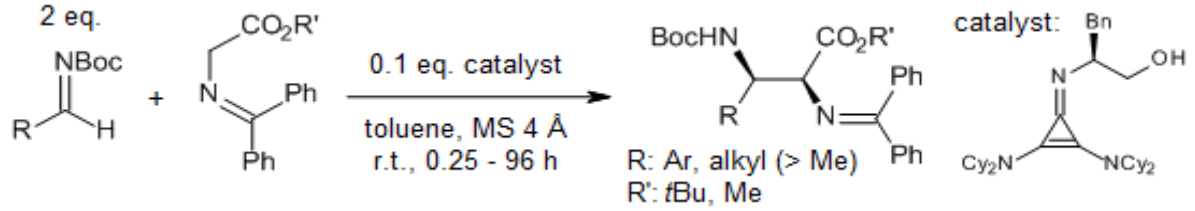
Scheme 6 : Diarylborinic Acid Derivatives as a Catalytic Iminium Ion Generator in the Mannich-Type Reaction Using Secondary Amines, Aldehydes, and Ketene Silyl Acetals [12].



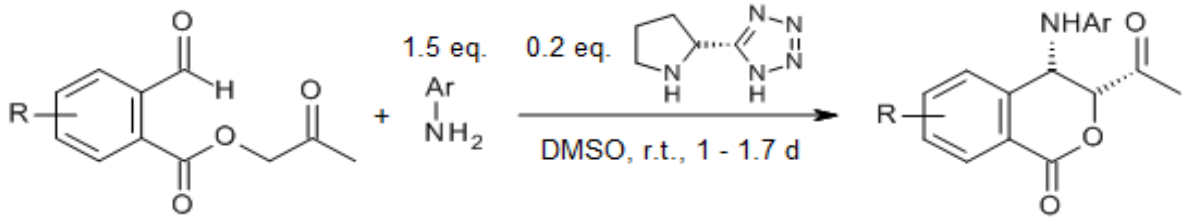
Scheme 7 : Magnesium(II)-Binaphtholate as a Practical Chiral Catalyst for the Enantioselective Direct Mannich-Type Reaction with Malonates [13].



Scheme 8 : Diastereoselectively Switchable Enantioselective Trapping of Carbamate Ammonium Ylides with Imines [14].



Scheme 9: Cyclopropenimine-Catalyzed Enantioselective Mannich Reaction of tert-Butyl Glycinates with N-Boc-Imines [15].



Scheme 10 : Asymmetric Organocatalytic synthesis of 4-Aminoisochromanones via a Direct One-pot Intramolecular Mannich Reaction [16].

Conclusion

In conclusion, this chapter has elucidated the Mannich reaction and its field of application with examples from recent literature in the pharmaceutical industry.

Therefore, in the next chapter, our work will consist in the synthesis of some Mannich bases and the evaluation of their biological activities.

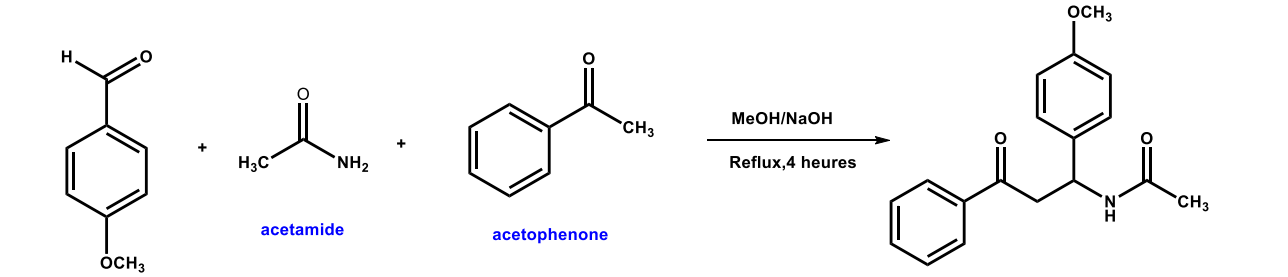
Chapter 2: Results and Discussion

Introduction

Considering our primary goal and objectives that were described above, we've decided to synthesize two Mannich bases, namely the N-(1-(4-methoxyphenyl)-3-oxo-3-phenylpropyl) acetamide and 3-(4-methoxyphenyl)-1-phenyl-3-(piperidin-1-yl) propan-1-one, and to evaluate their toxicity, antibacterial and antioxidant activities. The following represent the results obtained, which will be followed by a brief discussion.

1.1 Synthesis of the Mannich Bases

Following a modified protocol earlier reported by Oloyede et al [1], the synthesis of the N-(1-(4-methoxyphenyl)-3-oxo-3-phenylpropyl) acetamide (compound A) was achieved using anisaldehyde, acetamide and acetophenone, under basic conditions, as shown in the following scheme.

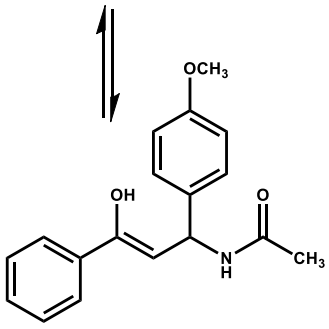


4-methoxybenzaldehyde

acetamide

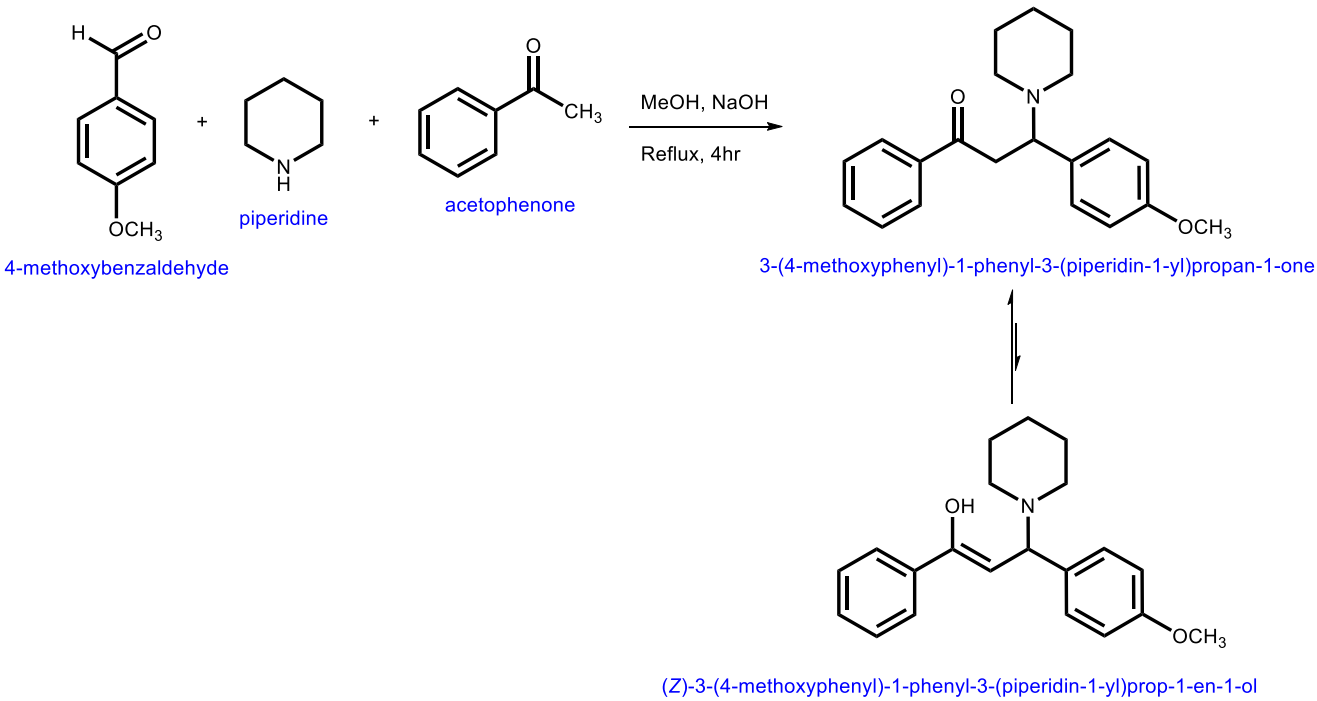
acetophenone

N-(1-(4-methoxyphenyl)-3-oxo-3-phenylpropyl)acetamide



N-(3-hydroxy-1-(4-methoxyphenyl)-3-phenylallyl)acetamide

Following the same protocol described above, we also proceeded to synthesize 3-(4-methoxyphenyl)-1-phenyl-3-(piperidin-1-yl) propan-1-one (compound B) using piperidine instead of acetamide, as shown below:

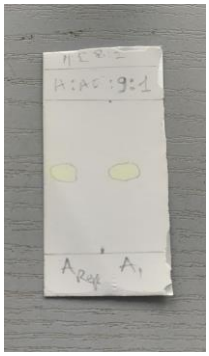


1.2 Some Characteristics of the synthesized compounds

	Compound A	**Compound B**
Appearance	lemon yellow solid	butter yellow solid
Yield (%)	69.10	74.39
Molecular Yield (g/moL)	297	323
Molecular Formula	$C_{18}H_{19}NO_3$	$C_{21}H_{25}NO_2$
Melting Point (°C)	288 to 289	296 to 297
Retention Factor Rf (*)	0.51	0.55

(*) **Eluent**: Hexane / ethyl acetate (9 /1)

Compound A= (1.9/3.7) Compound B= (2.9/5.2)



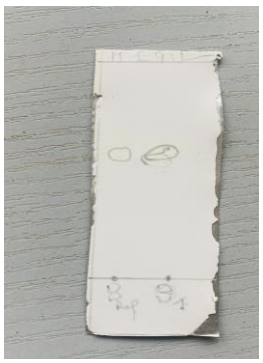


Figure 1: Chromatographic plates showing TLC of compounds A and B.

1.3. Determination of the solubility with different solvents

Table 1: Solubility properties of compounds A and B

Solvent	Compound A	Compound B
Water	Insoluble	Insoluble
Methanol	soluble	Soluble
Ethanol	soluble	Soluble
Hexane	Insoluble	Insoluble
Dichloromethane	soluble	Soluble
Acetone	Insoluble	Insoluble
Ethyl acetate	Sparingly soluble	Sparingly soluble
Dimethylsulfoxide	soluble	Soluble

1.4. Results of the spectroscopic analyses

1.4.1. Result of the Infrared of compounds A and B

The characteristic bands of the functions were assigned to the frequency absorption as follows:

Table 2 :Infrared result of compounds A and B

Compound A (cm^{-1})	Compound B (cm^{-1})	Binding	Intensity
3428.42	3435.98	O-H	broad
3312.7	-	N-H	medium
1600,1577.55 and 1512.42	1600.76, 1577.30 and 1512.62	C=C aromatic	strong
1213.61	1213.90	C-N	strong
1018.75	1018.70	C-O	srong
1658	-	C=O	strong
1658	1658.68	C=C	strong

- Absorption frequencies obtained using KBR Disc

Table 3 : UV/visible absorption data of compounds A and B

Wavelength of compound A (nm)	Absorbance	Wavelength of compound B (nm)	Absorbance
708.00	0.038	805.00	0.090
423.00	0.114	762.00	0.092
344.00	0.089	621.00	0.108
219.00	0.003	564.00	0.116
		421.00	0.107
		344.00	0.088

-Wavelength (nm) and absorbance frequencies

1.5. Antimicrobial activity

Table 4 : Zones of inhibition of compounds A and B

Conc. mg/ml	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. typhi*	*Kleb*	*C. albicans*	*A. niger*	*P. notatum.*	*R. stulonite*	Compound
100	29±1.4	27±1.4	29±1.4	28±2.8	28±2.8	29±1.4	19±1.4	20±0.0	19±1.4	19±1.4	
50	26±0.0	24±2.8	26±2.8	26±2.8	24±3.5	27±1.4	17±1.4	18±0.0	17±1.4	17±1.4	
25	22±0.0	21±2.1	23±2.8	23±2.8	21±4.2	23±1.4	16±0.0	15±1.4	15±1.4	15±1.4	**A**
12.5	18±0.00	17±1.4	20±2.8	20±2.1	18±5.6	20±2.1	14±0.0	14±0.0	12±0.00	13±1.4	
6.25	14±0.7	14±0.7	16±2.8	16±2.8	15±4.2	16±2.1	10±0.0	10±0.0	10±0.0	11±1.4	
100	25±1.4	25±1.4	23±1.4	23±1.4	23±1.4	25±1.4	17±1.4	18±0.0	14±0.0	14±0.0	
50	22±2.5	21±1.4	20±2.1	19±1.4	20±2.1	21±1.4	15±1.4	16±0.0	12±0.0	12±0.0	
25	20±2.1	18±0.0	17±1.4	17±1.4	17±1.4	18±0.0	13±1.4	14±0.0	10±0.0	10±0.0	**B**
12.5	16±2.8	14±0.0	14±0.0	15±1.4	14±0.0	14±0.0	11±1.4	12±0.0	-	-	
6.25	12±2.8	10±0.0	11±1.4	11±1.4	10±0.0	10±0.0	10±0.0	10±0.0	-	-	
-Ve	-	-	-	-	-	-	-	-	-	-	
+Ve	38	38	40	40	38	40	28	28	26	28	Controls

Note: *S. aureus* = *Staphylococcus aureus* (gram positive bacteria), *E. coli* = *Escherichia coli* (gram negative bacteria), *B. subtilis* = *Bacillus subtilis* (gram positive bacteria), *P. aeruginosa* = *Pseudomonas aeruginosa* (gram negative bacteria), *S. typhi* = *Salmonellae typhi* (gram negative bacteria), *Klebsiellae pneumonae* = *Kleb* (gram negative bacteria), *C. albicans* = *Candida albicans* (fungi), *A. niger* = *Aspergillus niger* (fungi), *P. notatum.* = *P. notatum* (fungi), *R. studonite*=*Rhizopus studonite* (fungi).
-Ve=Negative control = Methanol
+Ve=Positive control = Gentamicin 10 µg/mL (bacteria), Thioconazole 70% (fungi)
The result represents mean ± standard deviation of duplicate analysis.

The values in the table are expressed in millimeters.

Table 5 :Minimum Inhibitory Concentration results of compounds A and B

Conc mg/ml	*S. aureus*	*B. subtilis*	*E.coli*	*P. aeruginosa*	*S. typhi*	*Kleb*	*C. albicans*	*A. niger*	*P. notatum.*	*R. stulonite*	Compound
50	-	-	-	-	-	-	-	-	-	-	
25	-	-	-	-	-	-	-	-	-	-	
12.5	-	-	-	-	-	-	-	-	+	+	**A**
6.25	-	-	-	-	±	+	+	+	+	+	
50	-	-	-	-	-	-	-	-	-	-	
25	-	-	-	-	-	-	-	+	+	+	**B**
12.5	-	-	-	-	+	+	+	+	+	+	
6.25	+	+	+	+	+	+	+	+	+	+	

- =Inhibition occurred, no growth of the organism;

+ =Growth of the organism has occurred, i.e. no inhibition;

± = Inhibition is not significant.

1.6. Antioxidant activity of the synthesized compounds

Table 6 reports the percentages of DPPH free radical inhibition. It measures the capacity of an antioxidant by hydrogen transfer.

Table 6 : Result of the free radical scavenging activity of compounds A and B on the DPPH (Absorbance at 517 nm)

Concentration (mg/mL)	Compound A (%)	Compound B (%)	Vitamin A (%)	BHA (%)
1	39.20±0.000	25.20±0.002	89.12±0.005	92.09±0.001
0.5	34.92±0.000	33.60±0.001	96.54±0.001	91.76±0.001
0.25	38.05±0.001	34.10±0.001	95.55±0.000	45.14±0.008

From the values obtained in table 6, the DPPH method indicated that the antioxidant activity of compounds A and B compared to the standards show low antioxidant activity.

Table 7 : Result of the free radical scavenging activity of compounds A and B on hydrogen peroxide (Absorbance at 285 nm)

Concentration (mg/ml)	Compound A (%)	Compound B (%)	Vitamin C (%)	Vitamin A (%)	BHA (%)
1	88.99±0.000	88.88±0.000	89.09±0.001	86.41±0.000	77.78±0.000
0.5	88.88±0.000	88.99±0.000	88.88±0.001	78.91±0.000	59.36±0.000
0.25	88.68±0.001	87.65±0.006	86.73±0.000	24.07±0.000	36.93±0.000

Absorbance control = 0.972

The values obtained in table 7 represent the percentages of hydrogen peroxide inhibition. In this method, both compounds A and B showed promising antioxidant activity given their hydrogen peroxide scavenging effects compared to the standards.

1.7. Toxicity of compounds A and B

Toxicity was evaluated using the brine shrimp lethality test**.** Shrimp eggs (*Artemia salina*) were used for this study, while Cyclophosphamide was used as control.

Table 8: Result of toxicity

Concentration (mg/mL)	Compound A (%)	Compound B (%)	Cyclophosphamide (%)
1000	100	76.67	80.00
500	83.33	86.67	75.00
250	63.33	50.00	70.00
125	50.00	43.33	60.00
62.5	36.67	26.67	40.00
LC_{50}	119.91	178.14	85.20

The values obtained in this table represent the percentage lethality of the harvested nauplii (shrimp). According to the values, both compounds A and B are non-toxic compared to the standard (cyclophosphamide) as well as their median lethal concentration.

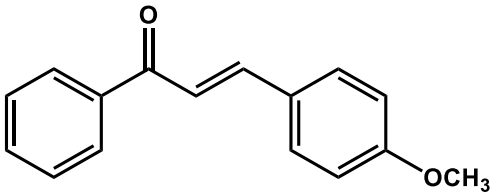
Discussion:

Mannich bases, N-(1-(4-methoxyphenyl)-3-oxo-3-phenylpropyl) acetamide (A) and 3-(4-methoxyphenyl)-1-phenyl-3-(piperidin-1-yl) propan-1-one (B) were synthesized by the Mannich reaction in this work. The Mannich reaction involves a enolizable compound and a primary or secondary amine (acetamide and piperidine) to initiate the reaction. Purity was determined by thin-layer chromatography and melting point. Single spots were obtained from the chromatographic plate of each of the two compounds in TLC analysis. Sharp melting points of compounds A and B which ranges between 288 to 289°C and 296 to 297°C respectively proved that the two compounds obtained are pure. The structures of the Mannich bases were confirmed originally only by Infrared (IR) and UV/visible spectroscopic analyses of functional groups and energy transitions. Those analyses were performed at the University of Ibadan, Nigeria. The Infrared absorption bands confirmed the presence of all the predicted functional groups. The broad bands at 3428.42 cm^{-1} to 3435.98 cm^{-1} for compound A and compound B respectively strongly suggested that these compounds undergone tautomerization and were therefore in the enolic form.

The bands obtained from the UV/visible absorption spectra of the two compounds A (421, 564, 621 and 762 nm) and B (344 nm) revealed that the compounds were strongly conjugated, aromatic with the absorption wavelength at 219 and 344 nm for compound A and 344 nm for compound B. The n-π^* transition is due to the presence of unbound electrons, such as those of the lone pair on oxygen and nitrogen which are present in both synthesized compounds. The π-π^* transition is due to the presence of double bonds associated with the presence of unsaturated groups or atoms with unshared electron pairs. (See pages 17-19).

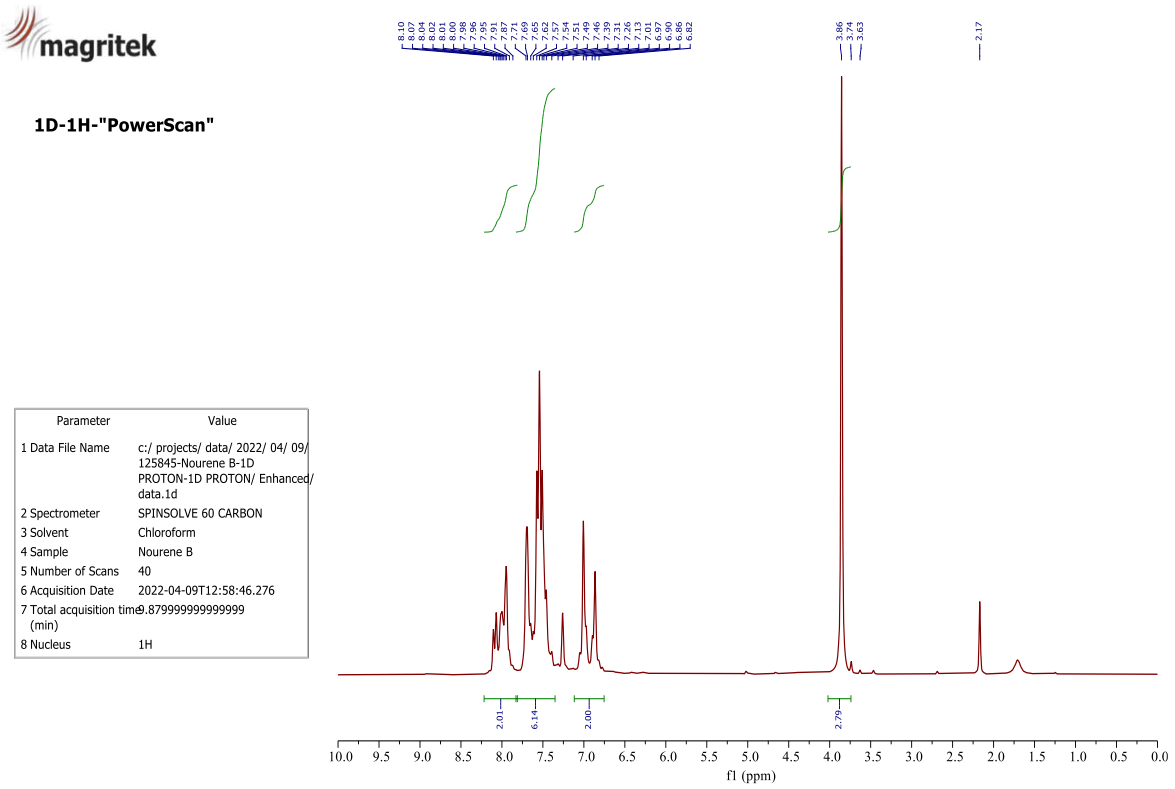
Despite all the assumed results, we proceeded with NMR confirmation once I returned to the University of Tlemcen.

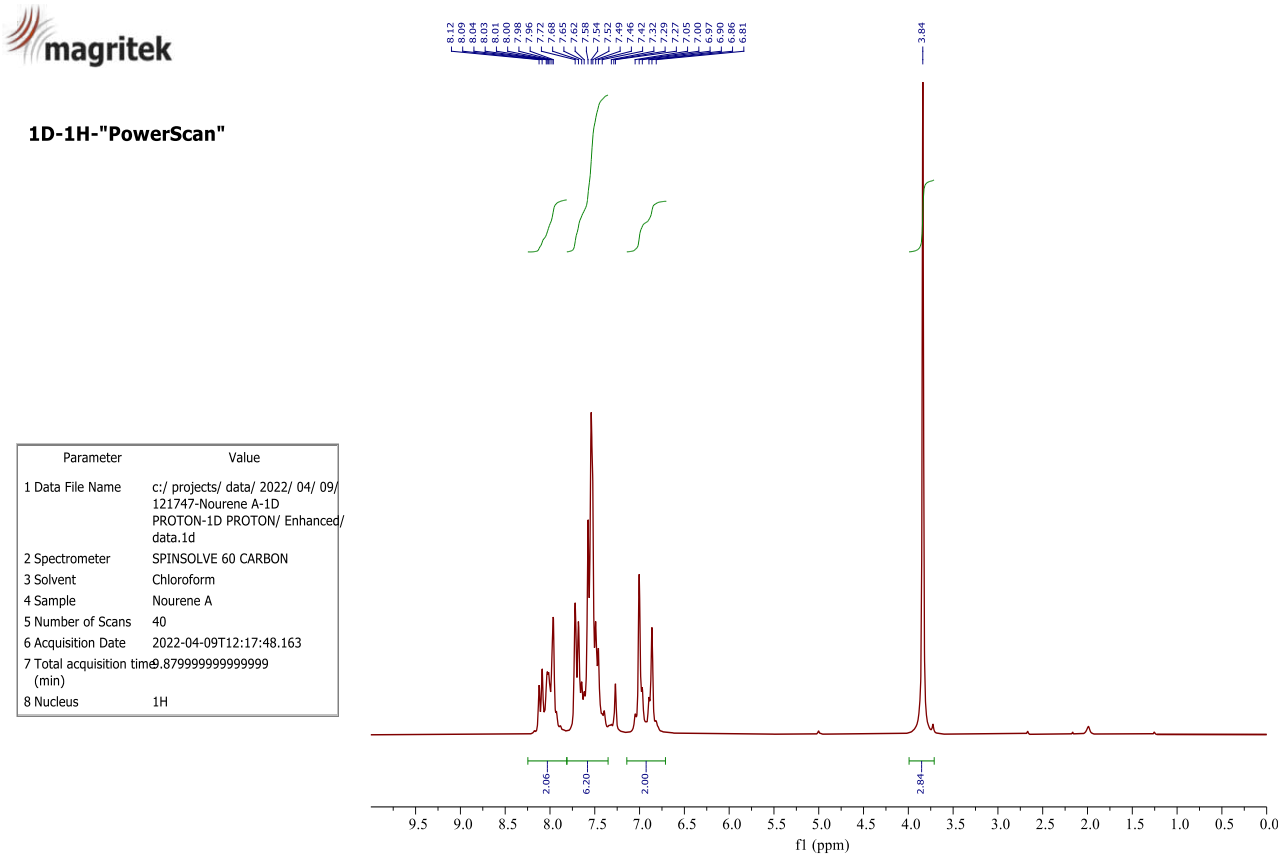
Unfortunately, NMR proved without any doubt that compounds A and B were but a single compound, namely the (E)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one.



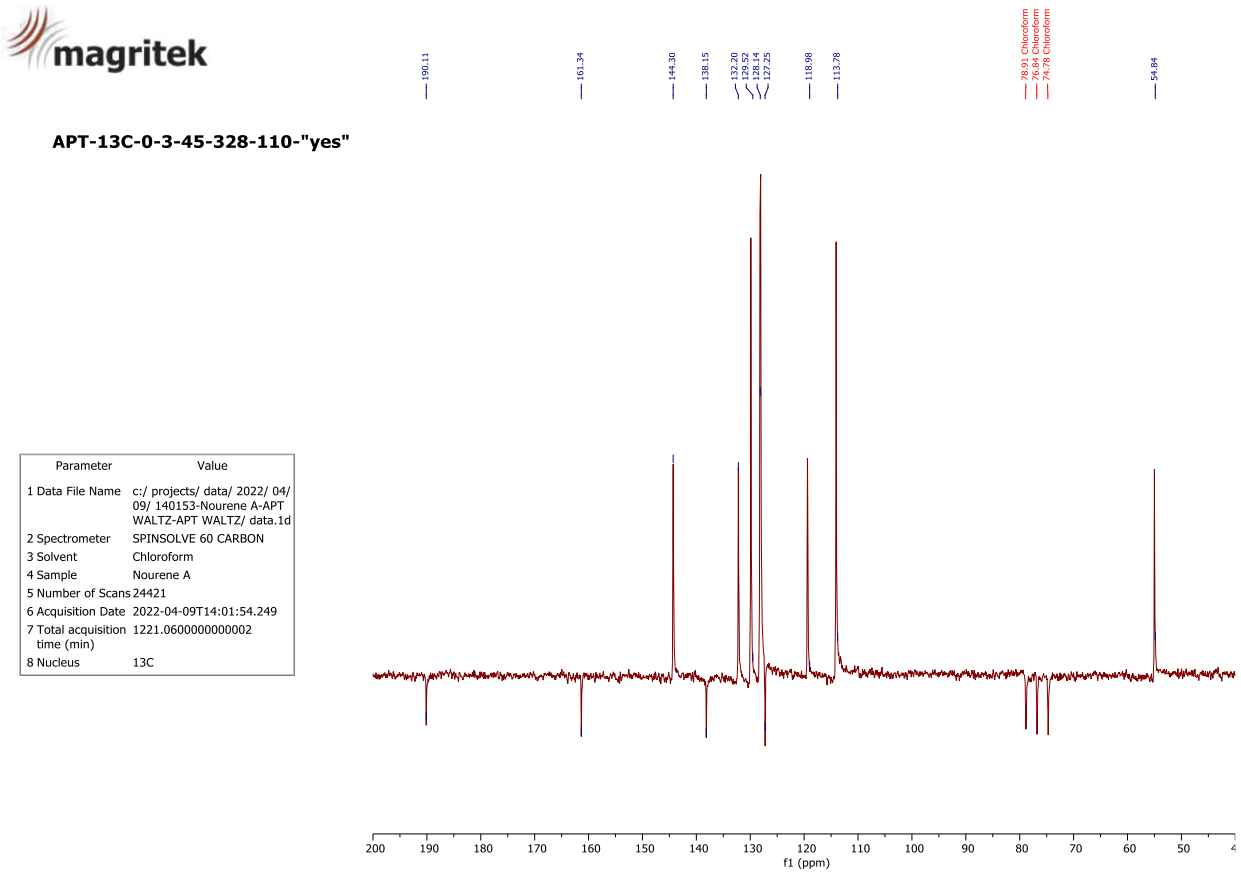
The confirmation was performed on the basis of the following arguments:

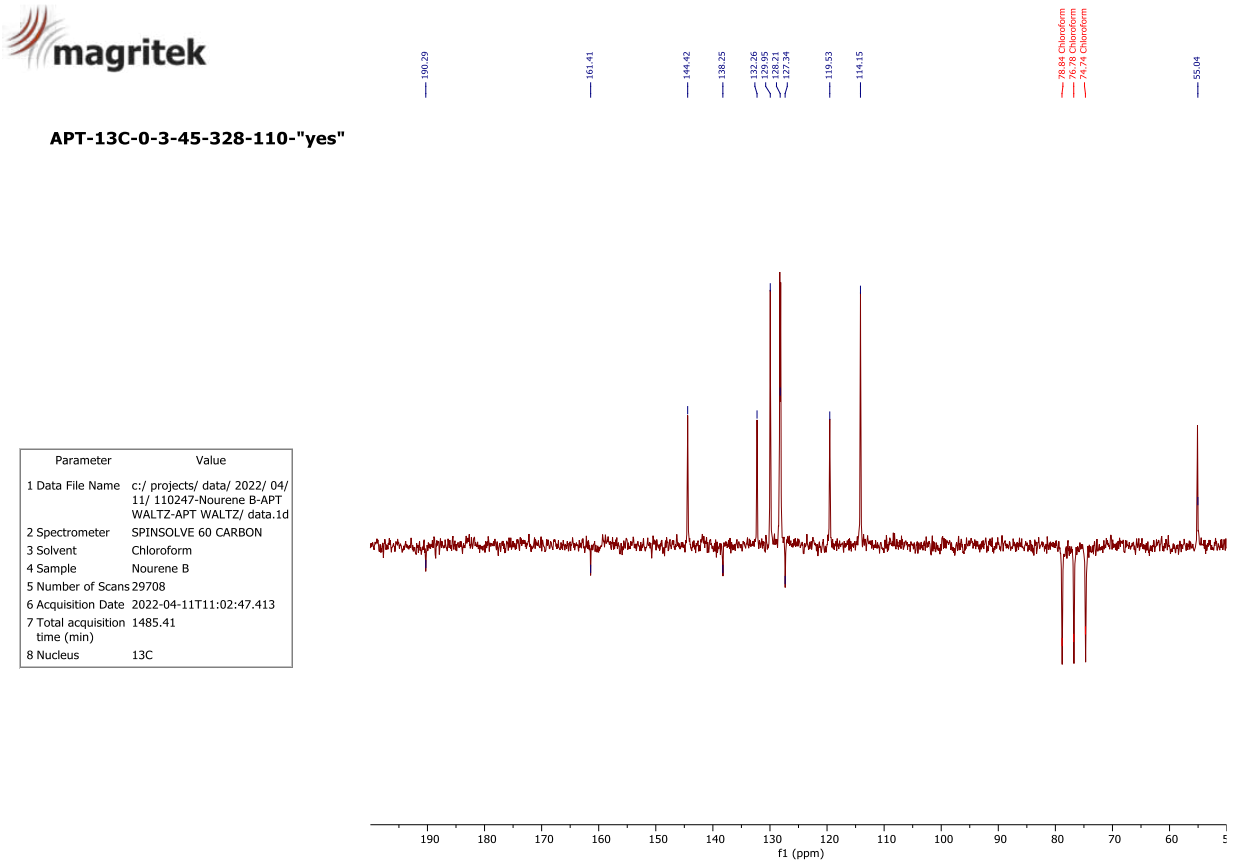
- H^1-NMR Spectra of compounds A and B are identical and show a unique singlet at around 3.8 ppm while we expected to see two singlets for compound A and a complex system for compound B in that range or higher field.





- APT C-13 NMR Spectra of compounds A and B are identical and show a total of 11 signals, far from the expected 14 signals for compound A and 15 signals for compound B.





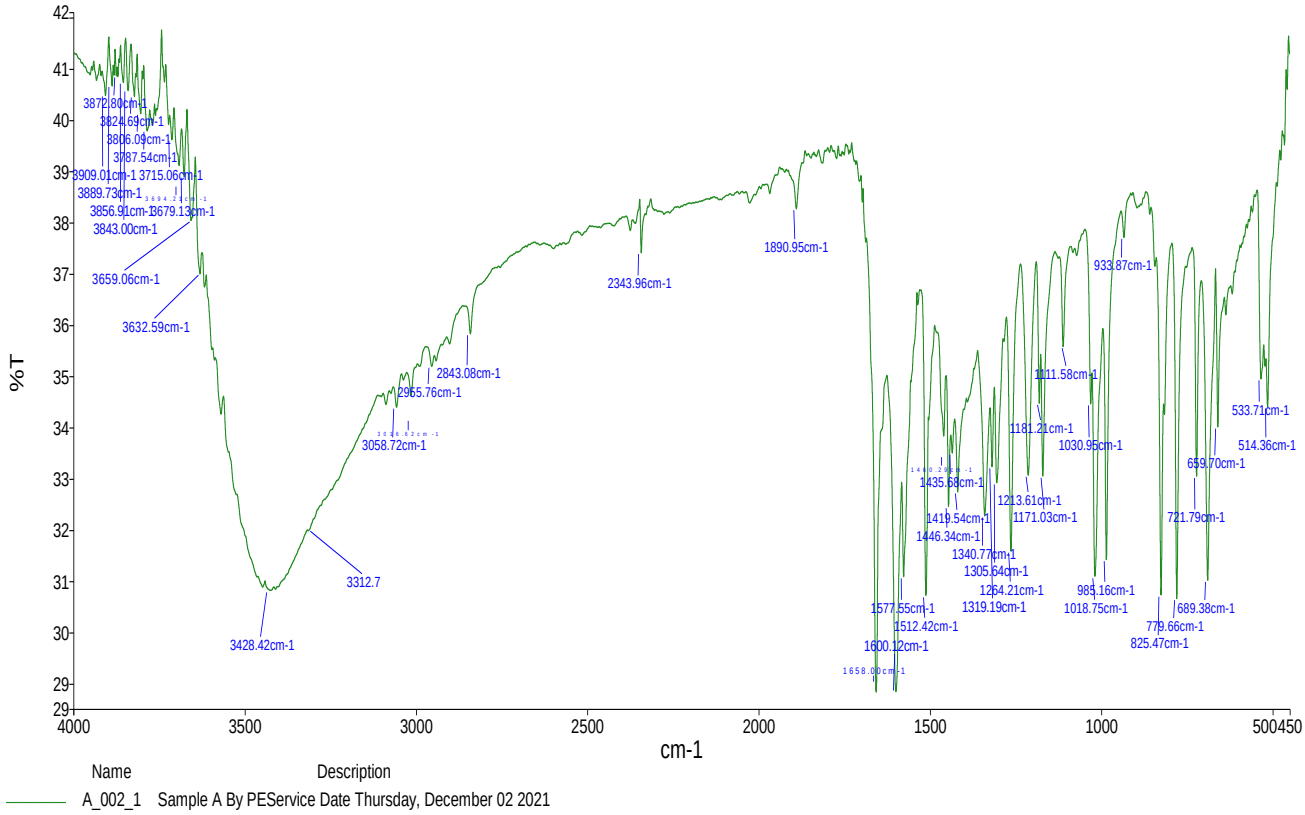
Nevertheless, for the sake of the discussion, we will continue to assume that compounds A and B were different, since at the time of the experimentations, that was our assumption.

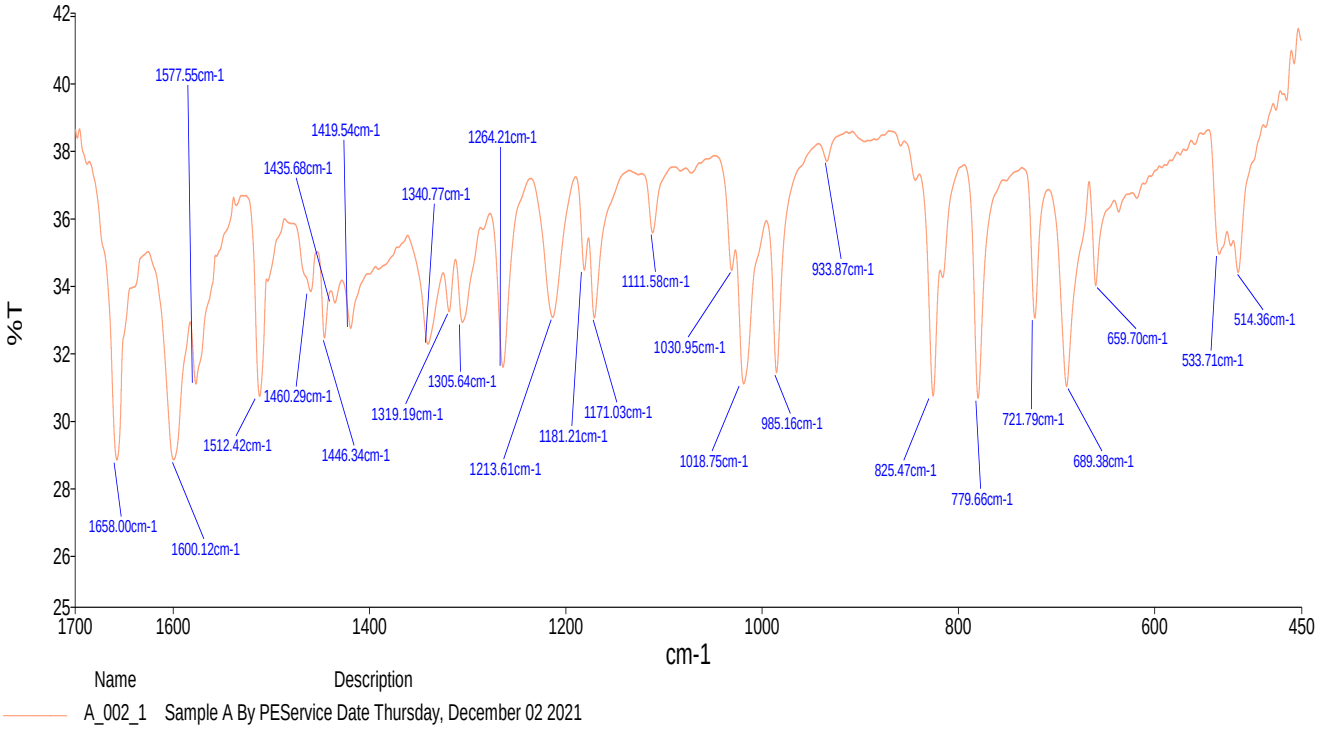
The biological activity of the synthesized compounds A and B was evaluated by screening them against *Escherichia coli, Staphylococcus aereus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonellae typhi, Candida albicans, Rhizopus stolonifer, Aspergillus niger* and *Penicillium notatum,* and the activities were compared with standard drugs, namely gentamicin and Thioconazole for bacteria and fungi respectively. The results revealed that both compounds A and B were good active against gram-positive and negative bacteria and fungi. Compound (A) showed good activity in growth inhibition compared to compound B (see table 4). The growth of *Staphylococcus aereus* (29±1.4) and *Klebsiella pneumonia* (29±1.4) were highly inhibited by Compound A at 100 mg/mL while *Staphylococcus aereus* (25±1.4), *Bacillus subtilis* (25±1.4), and *Klebsiella pneumonia* (29±1.4) were also highly inhibited by Compound B.

Toxicity screening of compounds A and B by the brine shrimp lethality test showed that both compounds were non-toxic, having an LC_{50} greater than 1000 µg/mL.

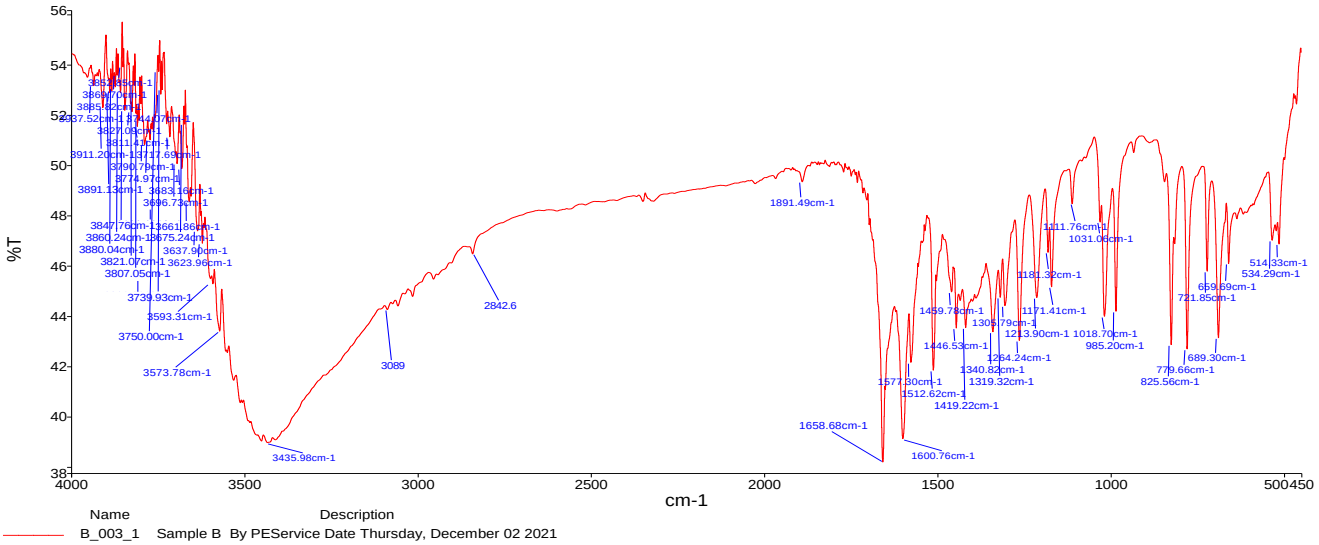
The DPPH method gives the hydrogen ion donating ability of a compound because DPPH is a free radical and becomes paired when it receives a hydrogen atom. The antioxidant activity of compounds A and B compared to the standards (vitamin A and butylated hydroxyanisole) showed moderate antioxidant activity. On the other hand, both Compounds A and B showed good and promising antioxidant activity in the scavenging effects on hydrogen peroxide compared to the standards (vitamin A, vitamin C and BHA). This showed that Compounds A and B can effectively scavenge hydroxyl radicals.

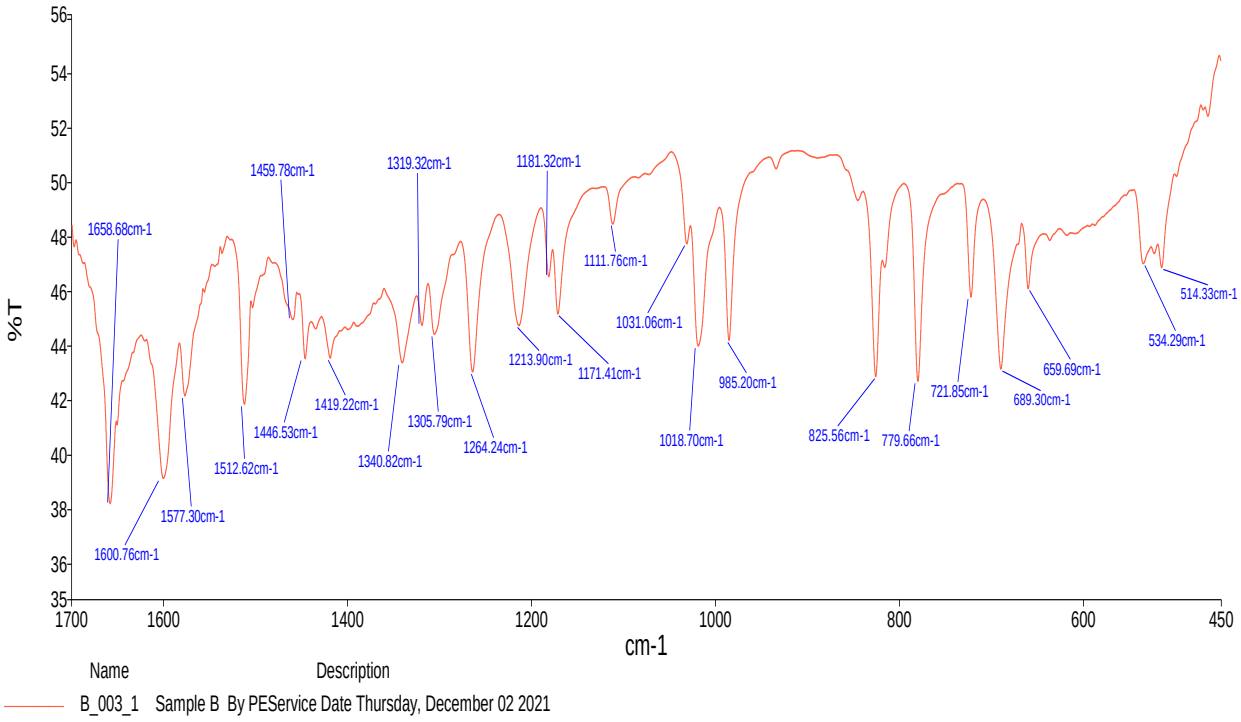
The analysis results for the infrared of compound A



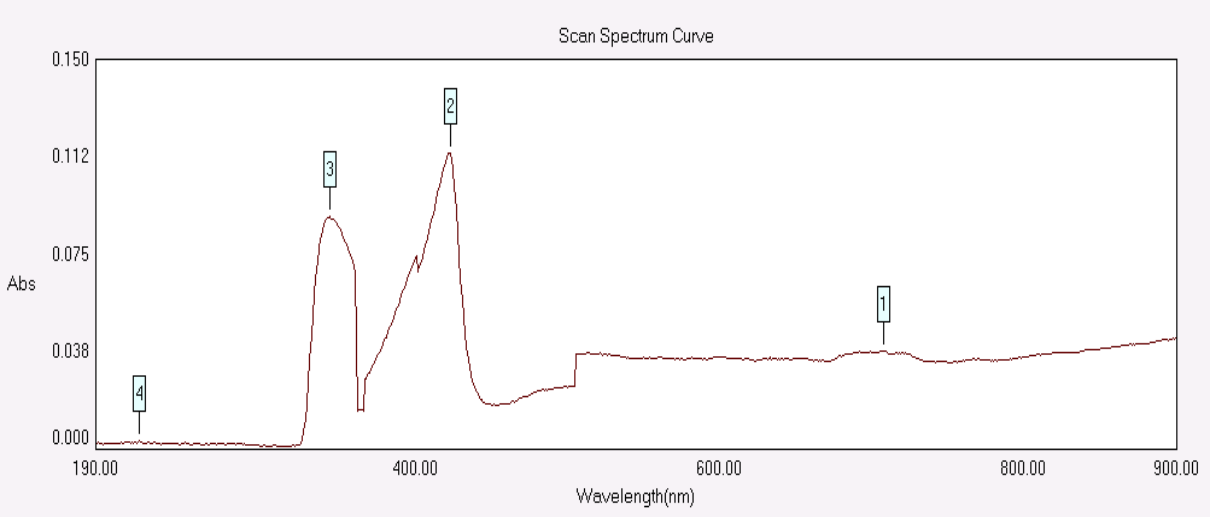


The analysis results for the infrared of compound B



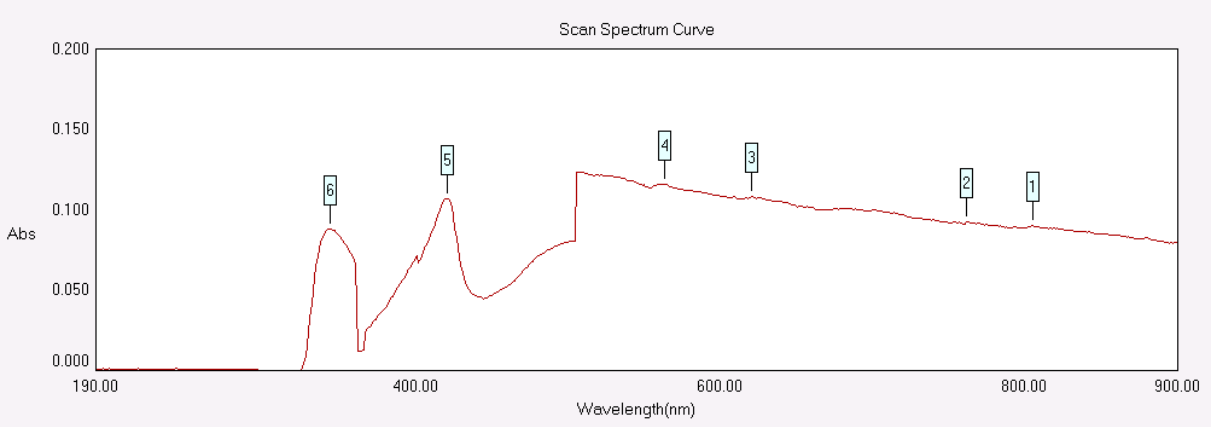


UV-Visible analysis results.



Compound A

No.	P/V	Wavelength(nm)	Abs
1	Peak	708.00	0.038
2	Peak	423.00	0.114
3	Peak	344.00	0.089
4	Peak	219.00	0.003



Compound B

No.	P/V	Wavelength(nm)	Abs
1	Peak	805.00	0.090
2	Peak	762.00	0.092
3	Peak	621.00	0.108
4	Peak	564.00	0.116
5	Peak	421.00	0.107
6	Peak	344.00	0.088

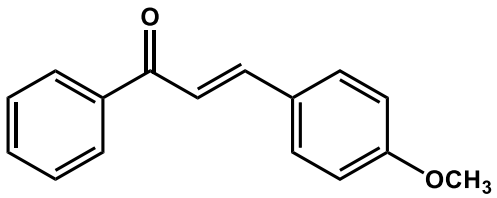
The UV-visible peak shows the wavelength variation and absorbance of compound A and B.

GENERAL CONCLUSION

Mannich bases, N-(1-(4-methoxyphenyl)-3-oxo-3-phenylpropyl) acetamide and 3-(4-methoxyphenyl)-1-phenyl-3-(piperidin-1-yl) propan-1-one were synthesized. The structures and functional groups (C=O, C=C, C-O, N-H, O-H and C-N) were confirmed by UV/visible and infrared spectroscopy. The results obtained from the antimicrobial analysis showed that both compounds A and B possess good antimicrobial activity compared to the standards. Good antioxidant activity was observed by both methods (DPPH and hydrogen peroxide) when activity was compared with vitamin A, C and Butylated Hydroxyl Anisole. Toxicity screening evaluated by Brine shrimp lethality test showed that both compounds A and B were non-toxic with respect to the median lethal concentration. Finally, it can be concluded that the synthesized Mannich bases could serve as leads in the development of new antimicrobial and antioxidant drugs in the pharmaceutical industry.

Note: All the manipulations of our research work were carried out at the Organic Chemistry and Natural Substances Laboratory at the University of Ibadan in Nigeria undertaken within the framework of the Intra-Africa (Academy) project for 6 months of mobility.

After the synthesis of my compounds, we were able to characterize them by spectroscopic methods (infrared and UV-visible) and then we tried to do proton and carbon NMR to confirm the structures of my compounds but unfortunately, we had a resolution problem on the machine. So we decided to go straight to the biological analysis and decided to perform NMR confirmation after returning to Algeria. Unfortunately, NMR analysis of compounds A and B showed proved the originally suggested structures wrong (See pages 16-17). Moreover, the obtained spectra for both A and B were identical, confirming the following structure:



Moreover, re-evaluating the data confirmed this new finding. The very close similarities in all data support even more our new conclusion.

Perspectives

The standards that have been used in our research work on biological activity have proven to be incomplete. It is therefore wise to perform the experiments once again using international standards, namely to use the Maes method for the agar well diffusion when analyzing the antibacterial activity (P Cos, AJ Vlietinck, D Vanden Berghe, L Maes. Journal of Ethnopharmacol., 2006, 106, 290) and the recommendations of the National Clinical Committee Laboratory Standards (NCCLS) in regard to the Agar disc diffusion method for fungi activity evaluation (Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eight Edition, 2003, 23, M2-A8). In this case:

- a Mueller Hinton agar should be used for testing instead of the used Dextrose;
- The adjustment of the inoculum must be done in a more precise way. What we have done was arbitrary and did not follow the general rule used in antimicrobial tests.

Chapter 3 : Experimental Details

1.1. Introduction:

This research work was carried out in the Organic Synthesis Laboratory at the University of Ibadan, Nigeria. This chapter summarizes the methods and materials of synthetic reactions and the procedures for evaluating the biological activity.

1.2. Chemicals:

The chemicals that were used in the course of our research work for the synthesis of the Mannich base are: anisaldehyde, acetamide, acetophenone, piperidine, methanol, ethanol, hexane, ethyl acetate, and sodium hydroxide.

1.3. Device and instrument

The apparatus and instruments that have been used during the research activity are: Round bottom flask, reflux condenser, thermometer, magnetic bar, beakers, syringe, test tube, capillary tube, Buchner funnel, filter paper, aluminum foil, chromatography plate, UV/visible spectrophotometer, Gallenkamp melting point apparatus (Gallenkamp UK) and Weighing balance (Mettler, UK).

1.4. The synthesis of the Mannich bases

The synthesis of N-(1-(4-methoxyphenyl)-3-oxo-phenylpropyl) acetamide (Compound A).

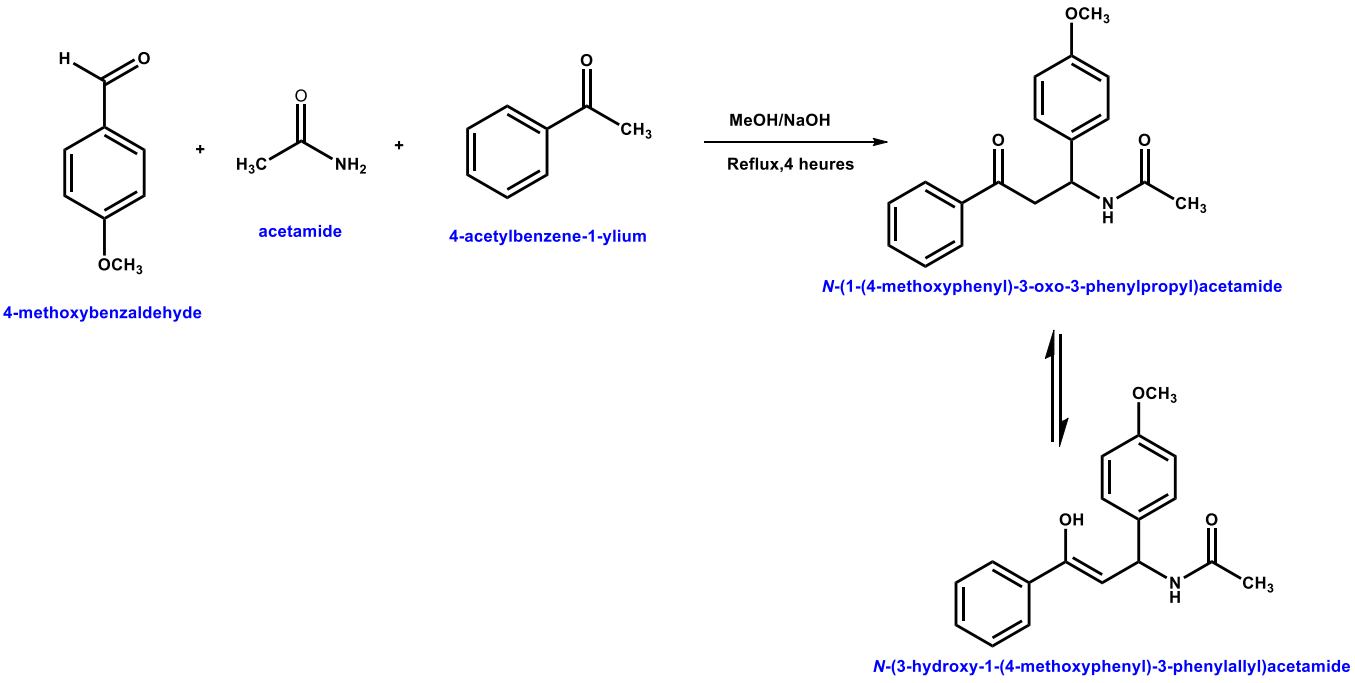


Figure 11 : The synthesis of *N-(1-(4-methoxyphenyl)-3-oxo-phenylpropyl) acetamide (Compound A)*.

A mixture of 1 mmoL anisaldehyde, 1 mmoL acetamide and 1 mmoL acetophone and 0.039 g sodium hydroxide in 50 mL absolute methanol was prepared. The resulting mixture was refluxed for 4 hours

and the resulting mixture was cooled and left at room temperature overnight. The solid that formed was filtered, dried and recrystallized in ethanol.

The synthesis of 3-(4-methoxyphenyl-3-(piperidin-1-yl) propan-1-one (compound B)

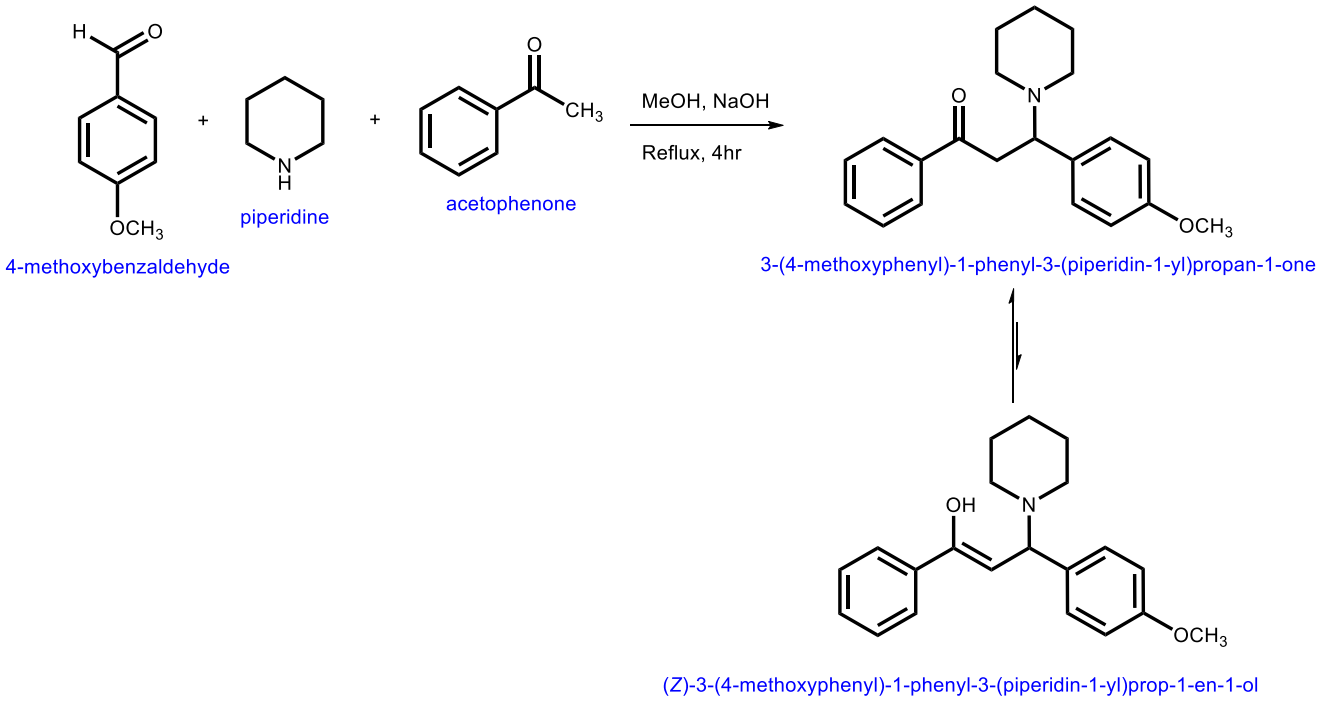


Figure 12 :The synthesis of 3-(4-methoxyphenyl-3-(piperidin-1-yl) propan-1-one (Compound B)

A mixture of 1 mmoL anisaldehyde, 1 mmoL piperidine and 1 mmoL acetophenone and 0.039 g sodium hydroxide in 50 ml absolute methanol was prepared. The resulting mixture was refluxed for 4 hours and the resulting mixture was cooled and left at room temperature overnight. The solid that separated was filtered, dried and recrystallized in ethanol.

1.5. Analysis of the synthesized compounds

The purity of the synthesized compounds was determined by Thin Layer Chromatography and melting point using a Gallenkamp melting apparatus. Spectroscopic analysis based on Infrared and UV/visible was performed to determine the structure of the compounds.

1.5.1. Thin-layer chromatography (TLC)

Thin-layer chromatography is a technique for separating the constituents of a mixture, based on their respective affinities for a stationary phase and a mobile phase.

1.5.2. TLC procedure

This involved preparation of the chromatographic plate, after which a small drop of the solution (sample dissolved in methanol) was applied to the plate of about 6 cm length using a capillary tube. The stationary phase was silica gel precoated on an aluminum sheet (Merck, Germany). Then, an elution vessel was prepared with a 250 mL beaker containing a mixture of two solvents (hexane and ethyl acetate) in specific proportions that was used as mobile phase. The plate was suspended

vertically in the beaker and the eluent (mobile phase) rose along the plate by capillary action. When it reaches almost the top of the plate (2 cm from the edge of the plate), the plate was taken out of the tank, the front line is marked and the eluent is allowed to evaporate. After air drying, UV lamp at 254 nm was used to visualize the spots and the retention factor (Rf) was calculated.

1.5.3. Determination of the melting point

The melting points were determined using a Gallenkamp melting apparatus. Thus, few crystals of the dried compound were packed in a capillary tube which was introduced into the melting apparatus.

1.5.4. Determination of solubility

The crystals were dissolved in different solvents in test tubes and the solubility test was determined at room temperature.

1.5.5. Spectroscopic analysis

The Infrared analysis of the synthesized compounds were recorded in a KBr disc on a Perkin-Elmer FT-IR spectrophotometer in the range 4000-400 cm^{-1}. The vibrational frequencies are given in the spectra.

The Ultraviolet/Visible absorption of the samples was measured using a UVD-2960 spectrometer. The absorption frequencies which are given in Table 1 were taken and used to deduce the structure of the synthesized compounds.

1.6. Study of the biological activity

1.6.1. Analysis of the antimicrobial inhibition

1.6.1.1. Preparation of samples for antimicrobial assay

Exactly 0.5 g of each sample (A or B) were weighed and dissolved in 5 mL of Dimethylsulfoxide (DMSO) to produce a 100 mg/mL concentration, from which 2.5 mL were taken and serially diluted until a concentration of 6.25mg/mL was obtained in the fifth test tube. The sixth test tube contained only the solvent (methanol) as a negative control and the seventh test tube served as a positive control and contained gentamicin (10ug/mL) for bacteria, or Thioconazole (70%) for fungi.

1.6.1.2. Agar well diffusion: plate method for antibacterial analysis

An overnight culture of each organism was prepared by taking a full loop of the organism from the stock and inoculating each in sterile nutrient broth of 5 mL each incubated for 18 to 24 hours at 37°C. From the overnight culture, 0.1 mL of each organism was taken and placed in 9.9 mL of sterile distilled water to produce 10 mL of a 1:100 (10^{-2}) dilution of the organism.

From the diluted organism (10^{-2}), 0.2 mL were taken in sterile nutrient agar prepared at 45°C, then poured aseptically into sterile Petri dishes, left to solidify for about 45 to 60 minutes. Using a sterile 8 mm diameter corkscrew, the wells were made according to the number of graduated concentrations of the sample, including positive and negative controls. In each well, the different graded concentrations of the sample and the controls were produced; this was done in duplicate. The plates were left on the bench for 2 hours to allow for pre-diffusion. The plates were incubated

vertically in the incubator for 18-24 hours at 37°C. At the end of the incubation, the zones of inhibition formed around the disc were measured with a transparent ruler in millimeters. The experiment was performed in duplicate series and the average reading was taken [17].

1.6.1.3. Agar diffusion: surface plate for fungi

Sterile Sabouraud Dextrose Agar (62 g/L) was prepared accordingly and aseptically poured into the sterile duplicate plates and allowed to cool for 45 minutes. 0.2 mL of 1:100 dilution of the organism was spread on the surface using a sterile spreader to cover the entire agar surface. Wells were made using a sterile 8 mm diameter cork borer. In each well, graded concentrations of the extract were introduced into the wells, including the controls, and the plates were left on the bench for 2 hours to allow for pre-diffusion. The plates were incubated vertically in the incubator for 48 hours at 26-28°C. At the end of the incubation, the clear zone of inhibition was observed and recorded using the same method as for bacteria [17].

1.6.1.4. Observation

The bacteria plates were observed after 24 hours of incubation. It was observed that there were clear zones of inhibition of some plates at higher concentrations and no zone of inhibition at lower concentrations. The fungal plates were also observed after 48 hours of incubation.

1.6.2. Free radical scavenging activities of synthesized compounds

1.6.2.1. DPPH Anti-free radical activity [17]

Antioxidant activity or the ability to scavenge the stable free radical 2,2-diphenyl 1-picrylhydrazyl (DPPH) was determined using the DPPH free radical scavenging activity. Exactly 1.93mg of 2,2-diphenyl 1-picrylhydrazyl radical was weighed and dissolved in 100mL of methanol. To 2 mL of the methanolic solution of DPPH, 1mL of the crystals previously dissolved in methanol and taken from the stock solution was added. The stock solution was prepared by dissolving 2 mg of the crystals in 2 mL of methanol, leading to a concentration of 1 mg/mL. Then, the other concentrations (0.5 and 0.25 mg/mL) were prepared from the latest solution by serial dilutions along with the standards. The mixtures were shaken vigorously and allowed to stand for 10 minutes, after which the absorbance at 517 nm of DPPH was measured. All tests were performed in triplicate for each concentration and the averages of the results were taken.

Antioxidant activity related to the DPPH radical scavenging effect is expressed as percent inhibition (PI) using the following formula:

$$PI=100 \times (A_{DPPH}-A_e)/A_{DPPH}$$

A_{DPPH}: absorbance of DPPH **Ae**: absorbance of the sample

1.6.2.2. Trapping effect on hydrogen peroxide

Spectrophotometric determination of the ability of the synthesized compounds to trap hydrogen peroxide (H_2O_2) was performed at 285 nm by the method of [18]. A 4 mM hydrogen peroxide solution was prepared in phosphate buffered saline (PBS) at pH 7.4. The synthesized compounds at different concentrations (1.0, 0.5 and 0.25 mg/mL) were added to the hydrogen peroxide solution and the decrease in absorbance of H_2O_2 was determined spectrophotometrically at 285 nm, after 10 minutes against the blank solution containing the standards (Vitamin A and C, BHA) in PBS without hydrogen peroxide. All tests were performed in triplicate and averaged results were taken.

1.6.3. Toxicity analysis

1.6.3.1. Brine shrimp lethality test

Toxicity was evaluated using the brine shrimp lethality test [19]. Shrimp eggs (*Artemia salina*) were hatched in seawater for 48 hours at room temperature. The nauplii (harvested shrimp) were attracted to a side of the flasks with a light source. For the preparation of the concentrations, exactly 6 mg of each compound including the standard (Cyclophosphamide) was weighed and added to 6 mL of a solution containing (DMSO and water ,1%). 3 mL of the stock solution was taken and completed with 3 mL of the solution (DMSO + water) to have serial solutions with variable concentrations (1000, 500, 250, 125, and 62.5 mg/mL) and incubated in triplicate flasks with the Artemia larvae. Ten Artemia larvae were placed in each of the triplicate flasks. After 24 hours, the flasks were examined on an illuminated background and the average number of larvae that survived in each flask was determined. The fifty percent larval mortality concentration (LC_{50}) was determined using the Finney computer program.

The percentage of nauplii lethality for each concentration was calculated by the following formula [18].

$$\% \text{ lethality} : 100(N_m)/(Nm+N_v)$$

N_m: number of dead nauplii; **N_v**: number of live nauplii.

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