People's Democratic Republic of Algeria
Ministry of High Education and Scientific Research
University of Kasdi Merbah Ouargla
Faculty of Mathematic and material sciences
Department of Chemistry

THESIS SUBMITTED TO OBTAIN ACADEMIC MASTER DEGREE IN APPLIED CHEMISTRY

Valorisation of Mermouthia propolis

Presented by:

SAOUD Hibat Errahmane

GUEMMOULA Fatma Zohra

Publicly discussed on **30/05/2024**

In front of the jury members consisting of:

BELFAR Mohamed Lakhdar	**Professor**	**Univ. Ouargla**	**President**
HAMADA Djamila	**Professor**	**Univ. Ouargla**	**Supervisor**
ZAOUI Manel	**Professor**	**Univ. Ouargla**	**Co-Supervisor**
ALLAOUI Messaouda	**Professor**	**Univ. Ouargla**	**Examiner**

Academic year:2023/2024

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ACKNOWLEDGEMENTS

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First of all, I want to thank **Almighty God**, for giving me the strength, the patience, the health and the will to carry out this modest work.

I am profoundly grateful to my professors and teachers in the University of Kasdi Merbah Ouargla for providing me with the resources and environment essential for the completion of my master thesis.

I extend my heartfelt appreciation to my supervisor **Pr HAMADA Djamila** for her continuous guidance, encouragement, and expertise throughout this challenging yet rewarding effort.

I also wish to offer my grattitude to my Co supervisor **Pr ZAOUI Manel**, for contribution and support in our work.

To the chairman of the jury, **Pr BELFAR Mohamed Lakhder**, I thank you for accepting to chair the jury for accomplishing this work.

I would like also to thank **Pr ALLAOUI Messaouda**, for having done me the honor of examining this work.

I would like to express my gratitude for the assistance and motivation provided by my colleagues and friends, particularly **Kaouther** and **Wassila**. Their profound talks and constant backing have been extremely encouraging for me.

Furthermore, I am indebted to my family for their unwavering support, understanding, and belief in my abilities.

Lastly, I express my gratitude to all those whose contributions, whether big or small, have enriched this thesis. This achievement is a testament to the collective effort and dedication of everyone involved in my academic journey.

DEDICATION

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In the name of God, the Most Gracious, the Most Merciful,

To my Dearest Family,

As I stand on the brink of this monumental achievement, I'm filled with thanks for all of you who have been my rock of strength and support throughout my academic journey.

*To my beloved Mother, **Djamila**, and Father, **Mohammed**, your unwavering love, guidance, and sacrifices have been the cornerstone of my success. Your endless support and encouragement have given me the strength to pursue my dreams with determination and resilience.*

*To my dear Sisters, **Ouiam**, **Aya**, and **Ouijdane**, and my Brother, **Azzam**, you are not just siblings but my closest confidants and companions on this journey called life. Your unwavering belief in me, your laughter, and your camaraderie have been my source of joy.*

To my forever supervisor,

***Pr. HAMADA Djamila**, your unwavering role as my esteemed supervisor has been a beacon of light, illuminating my path with knowledge, wisdom, and encouragement. Your mentorship has been invaluable, shaping not only my academic pursuits but also my personal and professional growth.*

*To my dear friend and Partner, **Fatima**,*

Your friendship and constant support have brought me joy and comfort throughout the ups and downs of this journey. Together, we have shared challenges, aspirations, and countless memories that I will never forget. Your friendship is a treasure I hold dear.

*To my right-hand **BOUROUBA Mouad***

Your invaluable support and encouragement throughout my graduation journey, your guidance, and your creativity significantly contributed to the success of my thesis and the dazzling presentation that will always have a lasting impression on both myself and the audience.

To all those who have touched my life in profound ways, whether through a kind word, a listening ear, or a helping hand, I offer my deepest gratitude. This achievement is not just mine but a testament to the collective love, support, and belief that you have showered upon me.

SAOUD Hibat Errahmane

DEDICATION

This work is dedicated to my beautiful family, my safe haven.

My father and my mother, my brothers ***Mohammed*** *and* ***Nassreddin****, and my sisters* ***Malak****,* ***Abir****, and* ***Maha*** *I love you all. May God Almighty protect you and the* ***Guemmoula family****. My deepest gratitude goes out to all my friends and colleagues.*

I am grateful to everyone who has taught me letters and imparted a wealth of knowledge, as well as those who have encouraged me with words and motivated me with smiles.

For all of your help, I am also grateful to those who have been my role models throughout my life.

GUEMMOULA Fatma Zohra

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Abbreviations

M.pro	Mermouthia propolis
UAE	Ultrasound-assisted extraction method
Ext	Extract
TPC	Total Phenolic Content
TFC	Total Flavonoids Content
QE	Quercetin equivalent
GAE	Gallic acid equivalent
LC-MS	Liquid phase chromatography coupled with mass spectrometry
DPPH•	1,1-diphenyl-2-picryl-hydrazyl
TAC	Total antioxidant capacity
HPLC	High-performance liquid chromatography
SPF	Sun Protection Factor
IC_{50}	Concentration Inhibitory at 50%
EC_{50}	Concentration equivalent of the reducing power Mo (VI) at 50 %
Abs	Absorbance
UV/Vis	Ultraviolet –Visible Spectrophotometer
λ_{max}	Maximum absorption wavelength

W	Weight
nm	nanometer
M	Molar
V	Volume
g	gram
mm	millimeter
m	meter

INTRODUCTION

Introduction

Since ancient times, mankind has depended on nature to fulfill their basic needs such as food production, housing construction, clothes manufacture, transportation development, fertilizer production, flavor and aroma formation, and most crucially manufacturing medicines [1].

Traditional medicine has placed substantial focus on natural remedies sourced from plants, microorganisms, and animal products for ages. Presently, these remedies are an invaluable reservoir of new chemical compounds. The authorized pharmaceuticals were composed of synthetic compounds that were either naturally derived or manufactured in imitation of the structures of natural substances.

The studies and actual practice of beekeeping have attracted significant attention from humans due to the exceptional and irreplaceable nature of these organisms [2]. A wide variety of economically valuable products are manufactured by them, including propolis, pollen, honey, bee food, and beeswax. These products are widely utilized in the medical field for protective and supplementary purposes, owing to their high nutritional content. Propolis in particular has gained international recognition [3].

Bees produce propolis, an organic compound, from a variety of plants by fusing wax and saliva with resins extracted from leaves, buds, and plant scratches. It has a rich historical background as a natural remedy for several diseases. Due to its varied biological effects, which are anticancer, antimicrobial, antiparasitic, antiprotozoal, antiviral, antioxidant, anti-inflammatory, immunomodulatory, and organ-protective, Propolis exhibits a multitude of biological and pharmacological properties due to its chemical composition, which includes biologically active compounds like flavonoids, phenolic acids, and polyphenols [4].

This study aimed to evaluate Algerian propolis, a highly valuable product of Algerian bees, with a particular emphasis on propolis from the eastern region of Algeria, known as Tebessa. The purpose was to enhance the profitability of propolis extraction and encourage its broader utilization. The substance's chemical composition and antioxidant activity were

examined. Furthermore, an assessment of the substance's photoprotective properties and efficacy against several kinds of bacteria was performed to determine its antibacterial biological activity

The objectives of this study are

- **Main objective:**
  - ✓ Analyze and evaluate the eastern Algerian propolis in order to identify its chemical compositions.

- **Specific objective:**
  - ✓ Get a thorough knowledge of the main chemical components found in eastern Algerian propolis.
  - ✓ Aim to enhance the utilization of eastern Algerian propolis.

PART ONE

THEORETICAL PART

CHAPTER I

GENERALITIES ABOUT PROPOLIS

1. Introduction

For centuries, natural products have been used for therapeutic purposes. Beehive-derived products have demonstrated significant potential in the field of medicine [5] because of the therapeutic properties of the bioactive chemicals they contain [6]. Bees reside in colonies and have been on earth for about 125 million years. Bees produce various substances, such as beeswax, venom, royal jelly, honey, and propolis, which serve as food, construction materials, and defense mechanisms [7]. The intricate substance known as propolis is produced from plant resins [8]. Traditional medicine has extensively used it, recognizing its beneficial effect on human health since ancient times. The substance has a complex and varied compound, which leads to an extensive variety of biological activities, including antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory, immunomodulatory, and anti-proliferative effects [9].

2. Origin of word propolis

Propolis, a term first introduced by Aristotle (384–322 B.C.) [10], originates from two Greek words: *pro* (meaning before or at the entrance to) and *polis* (meaning the city or hive). Therefore, propolis refers to the "defense of the city or bee colony" [9-11].

3. Definition

Propolis, known as bee glue [8, 9], is a natural resinous substance [11] collected by honeybees from various plants [11, 12]. Bees create propolis by gathering resin from buds, flowers, branches, pollen, and tree exudates [5]. They then combine this resin with beeswax and saliva, which contain enzymes (glucosidase) [11]. Not all bee species produce and use propolis to the same extent. The *Apis mellifera* species has a higher level of production and utilization of propolis compared to other species [9]. The chemical composition of this substance varies depending on the geographic origin, plant sources, and bee species [5].

Figure I.1 raw propolis

4. History of propolis

Bee propolis has a significant historical background, dating back to the same period as honey [10]. Since ancient times, humans have used propolis as a natural cure of particular diseases, as a dietary supplement, and as an essential ingredient of several creams [13].

The first documented utilization of propolis has been dated back to 300 BC [11, 13]. Aristotle's book "HISTORIA ANIMALIUM" has extensive studies on bees in Book V, Chapter XVIII. In this book, he refers to a particular substance as "propolis" [10].

According to the preliminary research, Hippocrates used propolis to heal both external and internal wounds [9].

Propolis has been used by ancient civilizations, including the Egyptians, Romans, Gree ks, Chinese, Indians, and Arabs, due to its numerous benefits and versatile uses [11].
The ancient Egyptians used bee propolis for its antipyretic and antiputrefactive effects [13], as well as for the preservation of corpses through embalming [14]. likewise, the Greeks and Romans used it as an antiseptic, wound-healing agent, and oral disinfectant [13].

Throughout history, propolis has mostly been utilized as a therapeutic solution, however it has also performed a variety of purposes.

5. The source of propolis

Propolis has two sources:

5.1. Internal source

Propolis is a resinous substance produced by bees through the initial digestion of pollen grains in a particular organ found between the honey stomach and the digestive stomach [14].

5.2. External source

Plants typically release sticky resins throughout the budding process and in regions where they have been wounded. These resins have the function of repelling insects, inhibit fungal growth, and protecting against diseases. Bees collect resins from many plants and trees, such as poplar, pine, oak, cottonwood, alder, and birch, and carry them back to the hive. The bees produce propolis in order to protect the colony [14, 15].

6. Harvesting Propolis

6.1. By bees

A team of professional worker bees [14, 16] collect resin from plants and trees as part of their assigned mission. Bees transport the resins to the hive via their hind legs, specifically through their pollen baskets. Propolis is formed by mixing bee saliva, wax, honey, and other substances with resins. The quantity of propolis that can be collected from a single hive varies. Each hive yields bees weighing around 50–150 g per season [15]. The harvesting process is influenced by various factors, with the most significant ones being:

- **Hive location:** Propolis production is higher in colonies situated in lush forest regions in comparison to those in steppe regions [14, 16, 17].
- **Climate:** Bees exhibit heightened propolis gathering process during hot days when temperatures exceed 20°. This process is particularly prominent during the peak heat hours between 10 and 15 after sunrise, when the propolis substances are fluid and easily collectible [14, 16, 17].
- **Types of bees:** The Apis mellifera genus is highly proficient at producing propolis [14, 16, 17].

- **The harvest season:** often occurs either in early spring or during the autumn months [14, 16, 17]. During this period, the bees are diligently sealing any fissures and gaps in their hive in preparation for the onset of winter conditions [15].

6.2. By humans

Beekeepers employ a variety of methods to gather propolis, including scrapings, which offer a less disruptive the hive. However, propolis gathered by this method may contain impurities such as fragments of wax, wood shavings, deceased bees, and so on. That is why beekeepers utilize a mesh harvest method based on a propolis trap. This contraption is positioned above the hive and consists of numerous small openings that are too narrow for bees to pass through. Consequently, they are compelled to seal these narrow apertures in the trap by utilizing the plant secretions known as propolis. It may take the bees several days or even months to completely fill the trap with propolis [15]. Subsequently, the propolis is extracted from the net, necessitating a cold environment where the propolis is in a solid state and easily crumbles. This facilitates a more efficient harvesting operation [14, 16, 17].

7. Propolis storage

Preserving propolis is a simple process that does not require any specific storage conditions. However, it is advisable to store it in opaque containers that block out light and ensure they are tightly sealed. It is also recommended to keep propolis away from high temperatures. Research has shown that storing propolis for extended periods of time does not diminish the amount of active elements and compounds it contains, nor does it affect its antibacterial effectiveness[14, 16, 17].

Additionally, it is worth noting that by drying propolis at a low temperature under vacuum, we can get a porous powder that exhibits strong antimicrobial properties [14, 16].

8. The use of propolis by bees

Propolis serves as a multifunctional shield for bees, providing physical, chemical, and biological protection. It is utilized for several hive purposes, including [9]:

- Ensure the balance and stability of the hive's internal environment [5].
- Repairing the holes and plugging the cracks in the nest

- Reducing the size of the hive entrance
- Preventing unregulated airflow to the colony
- Maintaining a consistent level of humidity within the hive
- Stabilizing the indoor temperature of the hive
- Providing defense against intruders, parasites, and predators
- Acting as an antiseptic, preventing and inhibiting the growth of harmful microorganisms in the tree cavities [9].

9. The basic composition of raw propolis

Propolis is a plant-derived natural product [18]. The chemical composition of propolis varies depending on where it is collected, the plants used [18, 19], and the season of collection [19], which in turn affects its biological properties [18]. However, the chemical makeup of raw propolis contains fundamental elements that are consistently present in fixed amounts. This is due to the bees, who add some salivary secretions and wax to the resins during the production process [16].

Propolis is mainly composed of the following components:

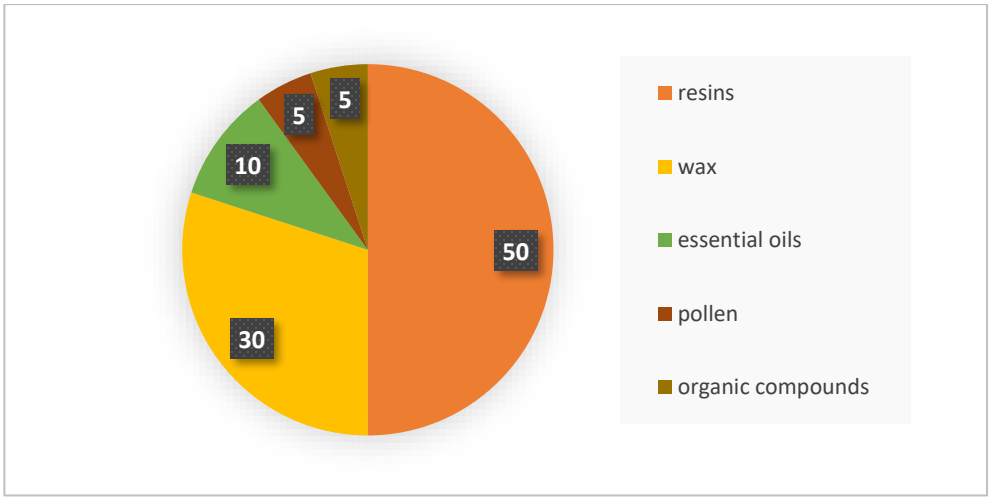

Figure I.2. Composition of propolis

10. Physicochemical properties

The physicochemical characteristics of propolis differ based on its botanical origin and geographical location [14].

Table I.1. Physicochemical properties of propolis

Properties	Propolis
Color	Propolis exhibits a color range that spans from a light-yellow hue to a deep brown [12].
The smell	Characterized by a unique and recognizable scent that combines the aromas of honey, wax, and vanilla. When it is burned, it releases an aroma similar to that of incense [14, 17].
Taste	Pungent and sometimes bitter flavor [16].
Rigidity	Propolis exhibits varied hardness, which is contingent upon the temperature. where: • T=15°C is solid and friable. • T = 30 °C is flexible and adhesive. • T = 60–70 °C fused and becomes a liquid The melting point of the propolis can exceed 100 ° [14, 17].
Solubility	Insoluble in water, but dissolves in organic solvents such as acetone, alcohol, chloroform, benzene and trichloroethylene [14, 16, 17].

11. Biological activity of propolis

Propolis is a compound consisting of a diverse mixture of biologically active compounds [20]. Over 300 chemical compounds have been identified thus far, with the most significant ones being phenols (such as flavonoids, polyphenols, phenolic acids, and other phenolic compounds), esters of phenols, terpenes, terpenoids, steroids, aromatic acids, aromatic esters, aldehydes, alcohols, sugars, sugar alcohols and acids, amino acids, vitamins, fatty acids, hydrocarbons, mineral elements, and alcohols. This is the reason Propolis shows tremendous biological effectiveness [9].

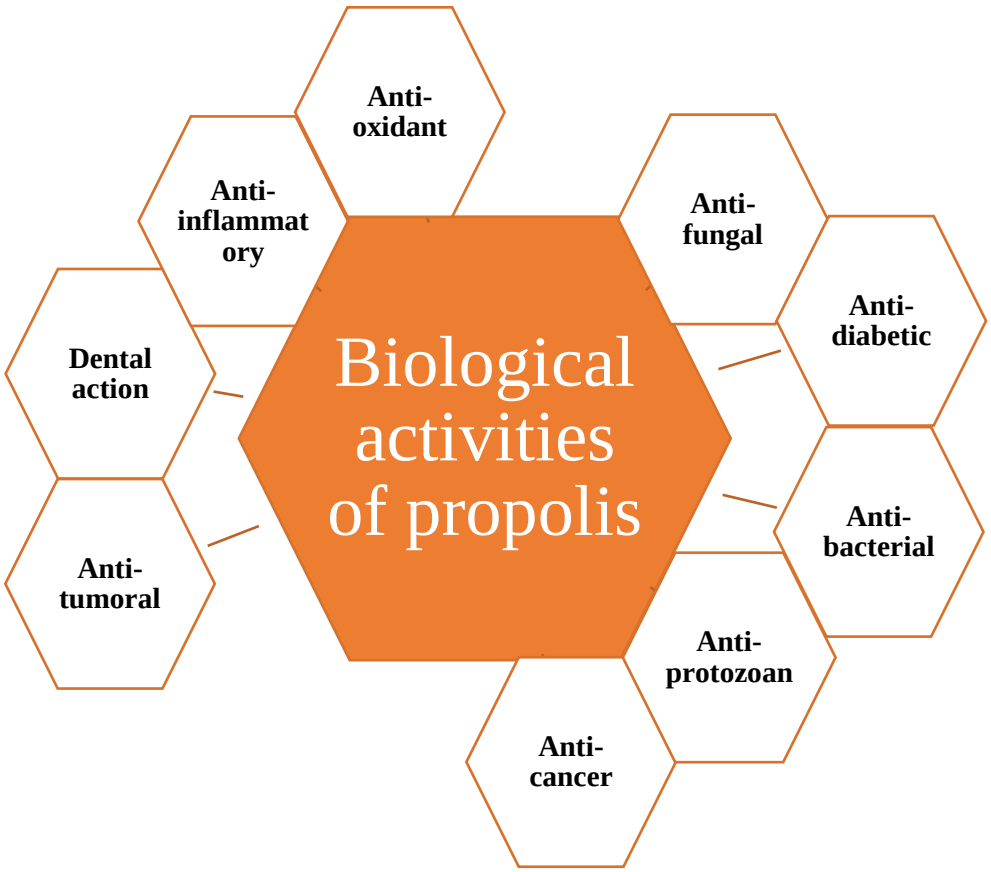

Figure I.3. Biological activities of propolis [21]

CHAPTER II

SECONDARY METABOLITES

1. Introduction

Natural products refer to elements or substances that are extracted from plants. They may take place as either primary or secondary metabolites. These compounds are produced through metabolic processes. It refers to a sequence of biological reactions or transformations that occur inside the cells of an organism. Enzymes mediate these reactions, which might happen through either anabolism or catabolism [22].

Primary metabolites are essential compounds that directly improve the development, growth, and reproduction of living organisms. Primary metabolites encompass carbohydrates, proteins, lipids, oils, and alcohols. Secondary metabolites are organic substances or phytochemicals that do not play a direct role in normal growth, development, or reproduction. They are produced via metabolic pathways in primary metabolism. Their significance stems from their antibacterial properties. They maintain abilities to strengthen the immune system, protect the body from free radicals, eliminate harmful microorganisms, and improve physical well-being. Phenolic compounds, flavonoids, tannins, and alkaloids are considered the most bioactive secondary metabolites [22]. Propolis comes mainly from plants and provides secondary metabolites in its chemical composition.

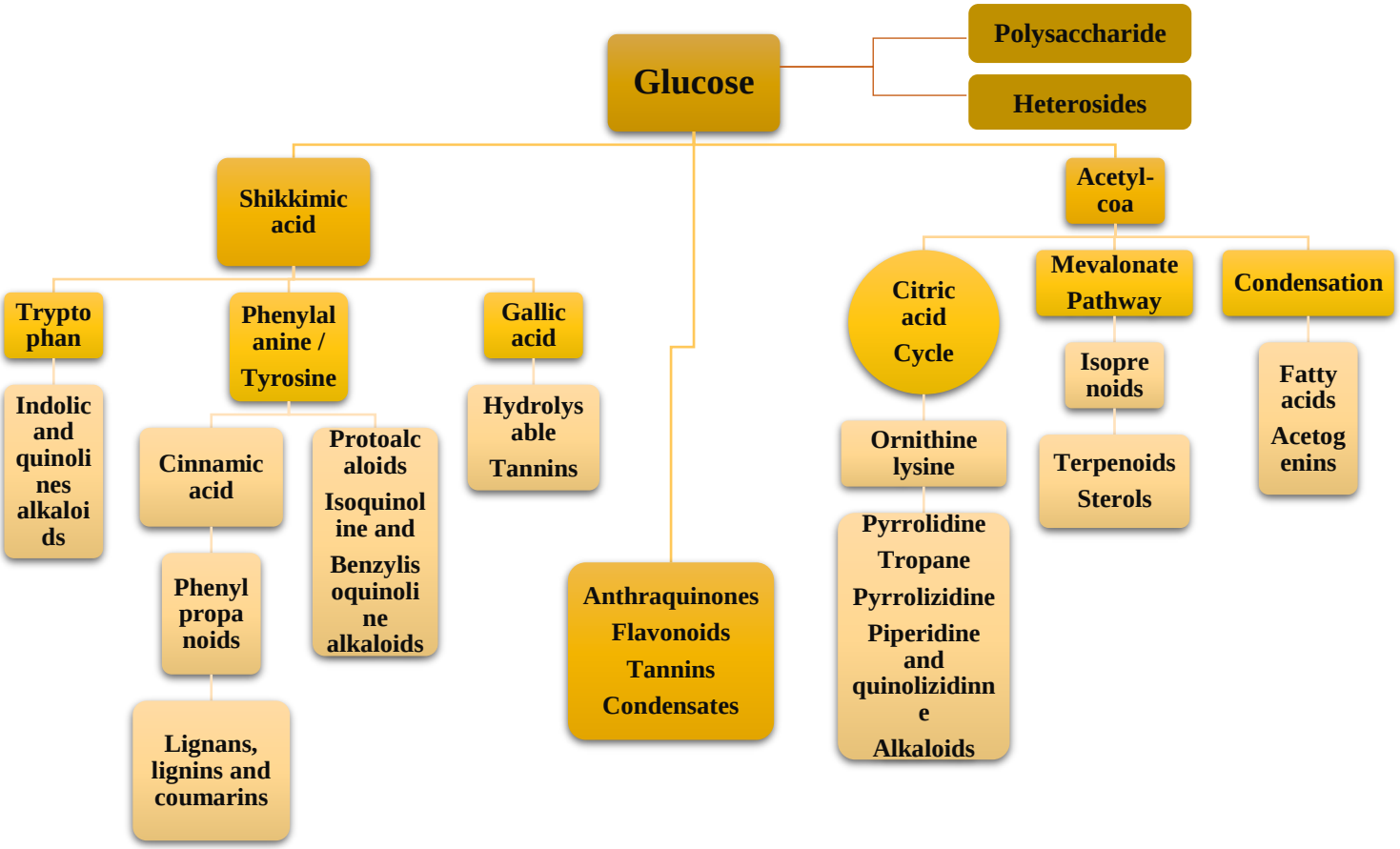

Figure II.1 biosynthesis cycle of the secondary metabolites of medicinal plants

2. phenolic compounds

Plants produce a wide variety of secondary compounds, which are composed of an aromatic cycle containing at least one hydroxyl group [22, 23]. These substances are known as phenolic compounds [22]. Phenolic compounds can be formed in two types: either as simple molecules or as complex polymers with a significant molecular size [23]. They exhibit chemical diversity, with certain ones being soluble in organic solvents, others being soluble in water, and others existing as insoluble polymers [22]. Phenols are characterized as non-nitrogenous compounds that consist of one or more benzene cycles, possess a free hydroxyl group, or are connected to another functional group [24].

3. Classification of phenolic compounds

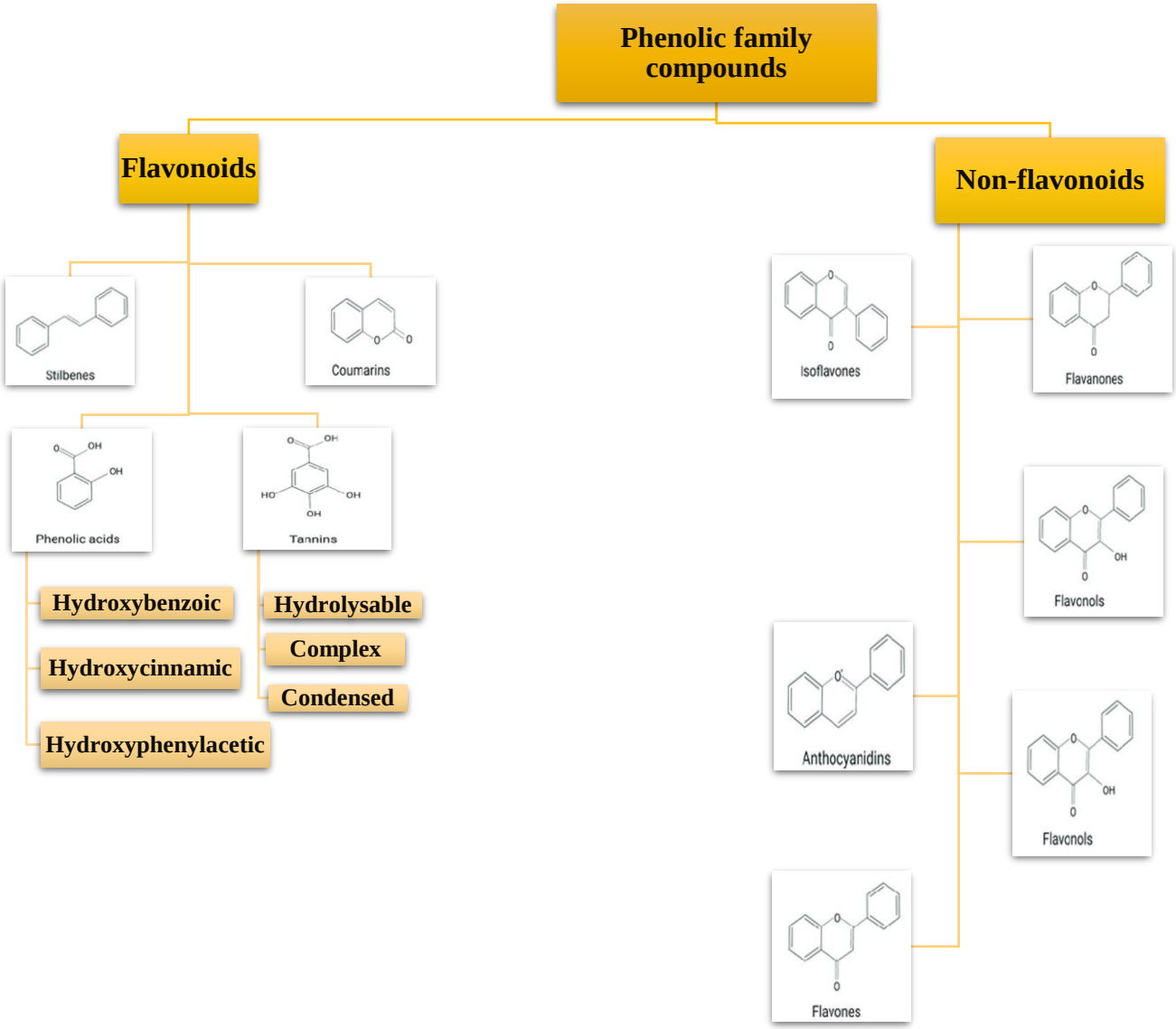

Figure II.2 phenolic family compounds

3.1. Flavonoids

Flavonoids, commonly known as yellow-colored compounds with low molecular masses, are named after the Latin term flavors [24]. They are widespread in nature and perform multiple roles [25]. These compounds have over 600 different structures, all sharing a common flavone nucleus composed of two benzene cycles connected by a pyran cycle containing an oxygen atom [26]. Several compounds exhibit antibacterial properties and are known for their antioxidant effects [25].

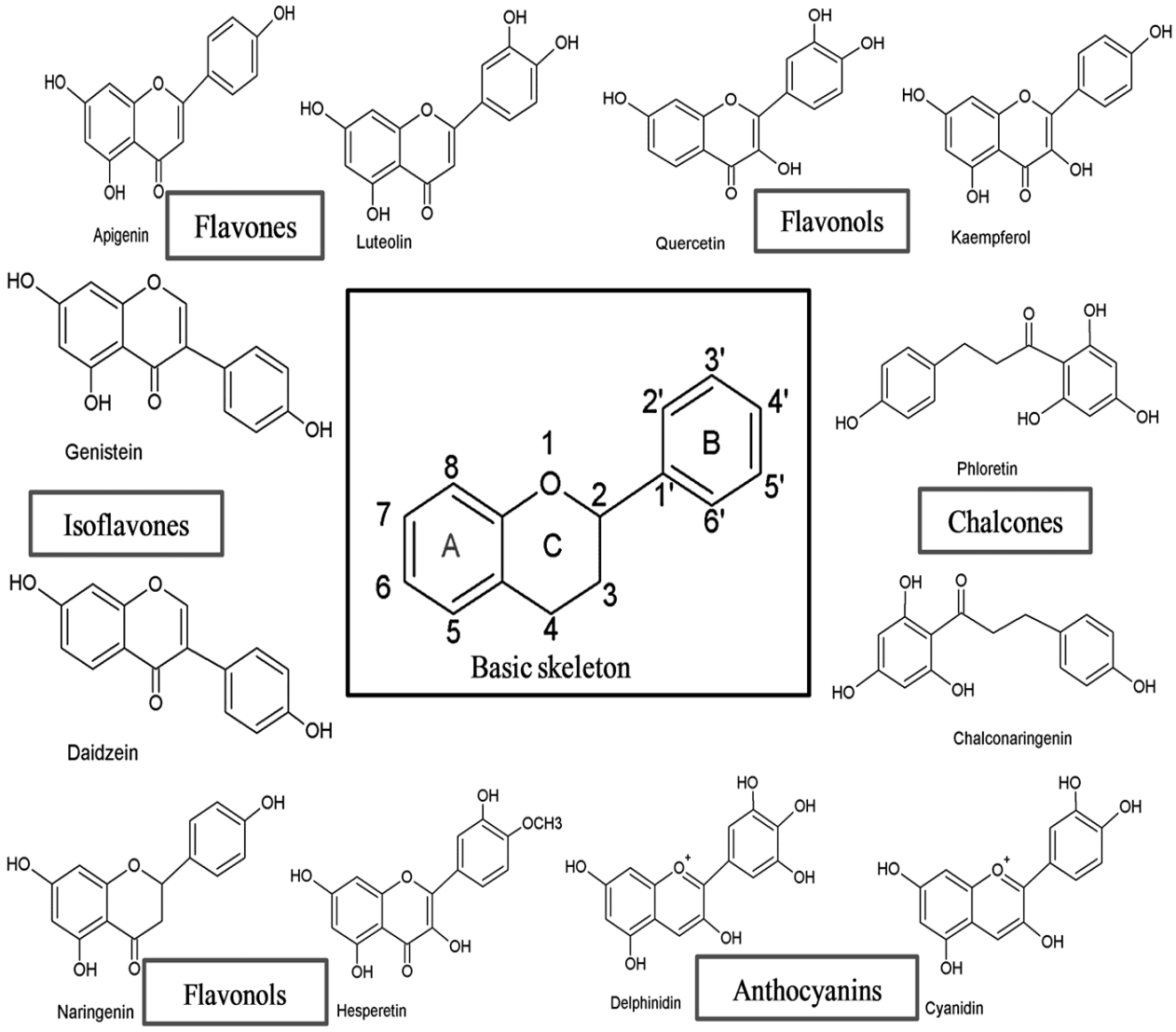

Figure II.3 classifications of flavonoids

3.2. Coumarins

These compounds are formed from two hexagonal cycles, one aromatic and the other heterocyclic, with a C6-C3 [24].

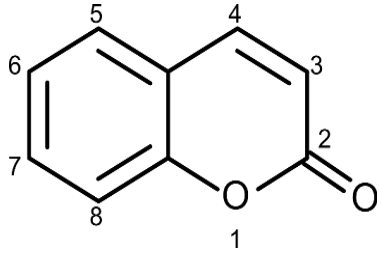

Figure II.4 coumarin structure

3.3. Phenolic acids

Phenolic acids are a type of non-flavonoid polyphenolic compound commonly found in several foods, distinguished by a carboxyl group attached to a benzene cycle. Benzoic and cinnamic acids are the two main phenolic compounds from which they derive. The hydroxybenzoic derivatives include vanillic, syringic, caffeic, ferulic, and sinapic acids [23]. The hydroxycinnamic acids include caffeic, ferulic, sinapic, and p-coumaric acids.

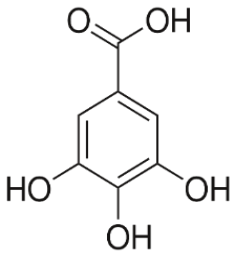

Figure II.5 gallic acid structure

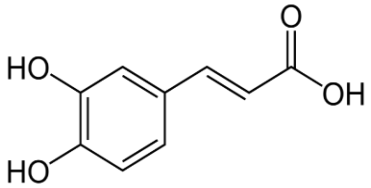

Figure II.6 caffeic acid structure

3.4. Tannins

Are categorized as non-flavonoid phenolic compounds. These compounds contain at least two aromatic cycles in their structure, or even more. Tannins can be divided into two main groups: hydrolysable tannins and non-hydrolysable tannins, also known as condensed tannins [23]. These substances have a high molecular weight [26].

4. The importance of phenolic compounds

- **In the economic field**

  This has significant uses in the food industry as it serves as both an antioxidant and an enzyme inhibitor. In addition, cosmetics utilize it to protect the outer skin from unsafe UV radiation [24].

- **In the medical field**

  Considering its therapeutic properties, this compound holds great importance in the medical and pharmaceutical fields. It has a major effect on living organisms and humans, especially in safeguarding blood vessels, decreasing inflammation, activating enzymes, and inhibiting tumor growth. The presence of hydroxyl groups in phenols enhances their activity as antagonists, making them crucial compounds in free radical inhibition[24] .

5. Terpenes

This is the largest category of secondary products. Terpenoid is their name. Five carbon atoms combine to form the branching carbon skeleton of isopentane, which generates all terpenes. Terpenes are composed of a fundamental building block known as the isoprene unit. These compounds have the ability to decompose at high temperatures, resulting in the formation of isoprene terpene. Indeed, isoprene terpene plays a crucial defensive role in the ecology of plants.

Terpenes have various derivatives, including saponins, steroids, and triterpene glycosides. These compounds are known for their soap-like properties.
Carotenoids, which are derived from terpenes, give rise to the bright yellow, red, and orange pigments noticed in specific plant species, like carrots [22].

Table II.1 types of terpenes

Type of terpenoids	Number of carbon atoms	Number of isoprene units	Example
Monoterpene	10	2	Limonene
Sesquiterpene	15	3	Artemisinin
Diterpene	20	4	Forskolin
Triterpene	30	6	a-amyrin
Tetraterpene	40	8	b-carotene
Polyterpene	several	Several	Rubber

6. Essential oils

Essential oils are volatile and natural chemicals that have an intense fragrance. Aromatic plants produce secondary metabolites. These substances are commonly obtained through steam or hydro-distillation and are well-known for their powerful antiseptic, bactericidal, virucidal, fungicidal, and therapeutic properties. Essential oils play a crucial role in protecting plants in their natural environment [27].

7. Steroids

These steroids are composed of non-flavonoid polyphenolic chemicals which contain a tetracyclic system. Cholesterol serves as a prime illustration of the fundamental structure, but various modifications, particularly to the side chain, play a crucial role in the formation of a

variety of biologically significant organic compounds. In particular, these compounds involve sterols, steroidal saponins, cardioactive glycosides, bile acids, and corticosteroids [28].

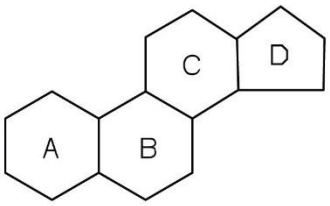

Figure II.7 basic steroid structure

PART TWO

EXPERIMENTAL PART

MATERIALS AND METHODS

1. Raw material:

The focus of our study is a sample of Algerian propolis obtained from the eastern region of Algeria, precisely EL Mermouthia located in Tebessa province. Table III.1 presents the characteristics of the sample.

Table III.1 Presentation of M. propolis

Sample Abbreviation	M. pro
State	Tebessa
region	El Mermouthia
color	Dark brown
Weight (g)	60 g
Propolis harvested	

The raw material (Propolis) was collected from Mermouthia region (34° 19′ 44.7″ N, 7° 44′ 28.4″ E; altitude:34.328750 m) in the Wilaya of Tebessa (southeastern Algeria).

2. Extraction

The extraction technique performed is known as ultrasound-assisted extraction (UAE). For extraction, UAE employs ultrasonic energy through an ultrasonic bath and/or probe [29].

2.1. Preparation of extraction solvent

We obtained bioethanol from the AMETNA company, which was made from date waste, and then distilled it to achieve a high purity of 90%. We prepared a solvent mixture of alcohol and water for the propolis extraction process. The alcohol-water mixture functions as a

polar solvent capable of extracting phenolic compounds. For our extraction method, we used a hydro-ethanol mixture (ethanol/water, 85/15, v/v) as a solvent.

We begin by lowering the alcohol content from 90% to 85%. And that's how we get 1,000 mL of 85% pure alcohol: 950 mL of alcohol mixed with 50 mL of distilled water.

2.2. Preparation of sample

We store the crude propolis at -16 °C for 24 hours before smashing it with an electric blender.

Figure III.1 represents the sample post-grinding.

2.3. Ultrasound-assisted extraction

60 grams of propolis were extracted in a dark room using 600 mL of 85% ethanol in an ultrasonic bath set at 37 kHz for 40 minutes. Next, we filtered the mixture using Whatman filter paper No.1. To get the most bioactive components out of the crude propolis, the solid residue was re-extracted twice under identical conditions. We kept the propolis extracts at 4 °C in the dark until analysis [30-32].

2.4. Evaporation of solvent

The bio ethanolic extract was concentrated using reduced pressure in a rotary evaporator at a temperature of 40 ºC. This process resulted in the production of a non-coherent solid content, which was then prepared for lyophilization [32].

2.5. Drying the extract

Following the complete freezing process in the freezer, drying is performed to remove water from the extract obtained using a lyophilizer for four days, yielding a solid residue ready for use.

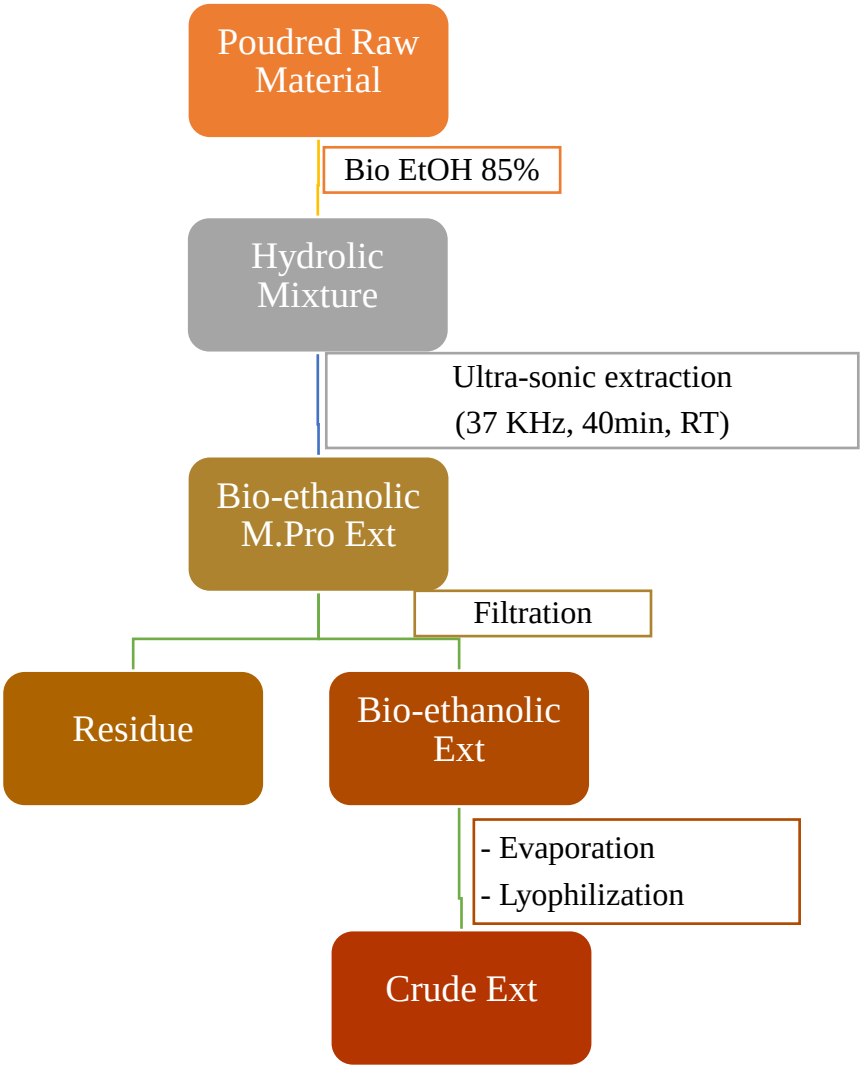

Figure III.1 Extraction protocol of M. propolis.

3. Qualitative and quantitative analysis

3.1. Phytochemical screening

Propolis is rich in bioactive compounds such as phenols, alkaloids, fatty acids, flavonoids, saponins, triterpenes, tannins, volatile oils, and coumarins. The content varies depending on the botanical and geographical origin.

The purpose of qualitative phytochemical screening is to determine the various organic chemical groups that have been found. In the following phytochemical screening, we used an ethanolic extract to qualitatively characterize the different families of secondary metabolites in M. pro, following standard procedures that described by [13, 33-37].

- **Flavonoids test**

Two tests were performed to confirm the result:

- ✓ **First test:** 2ml of ethanolic M. pro extract ,0.5ml of concentrated Hydrochloric acid (HCl) was added and 0.5g fragments of magnesium (Mg), the appearance of a red coloration indicated the presence of flavonoids.
- ✓ **Second test:** 0.5ml of ethanolic M. pro extract is treated with 1%of $AlCl_3$ solution. Yellow color indicates the presence of flavonoids.

- **Tannins test**

0.25mL of ethanolic M.pro extract was combined with 0.25mL of distilled water.To this, 1ml of 1% ferric chloride ($FeCl_3$) solution is added.

A dark green color points to the presence of tannins.

- **Phenols test**

2ml of distilled water and 6ml of acetone are added to 0.2ml of the ethanolic M.pro extract. The mixture is heated on a boiling water bath at 60°C for 5 minutes. 2 ml of mixture has been taken then a few drops of a solution containing 10% of ferric chloride ($FeCl_3$) was added. Dark green signified phenolic compound.

- **Saponin test**

5 mL of aqueous M.pro extract was put into a test tube and stirred for a few seconds, tube agitation was stopped and left for 15 min. the appearance of persistent foam indicates the presence of saponins.

- **Proteins test**
  - ✓ **Biuret test:** To 3ml of crude M.pro extract, 1mL of NaOH (4%) and 1mL of $CuSO_4$ (1%) were added. The color change of the solution to purple or pink indicates the presence of the protein.
- **Triterpenes Test**
  - ✓ **Salkowski test:** To 2mL of crud M.pro extract, 5 drops of concentrated sulfuric acid (H_2SO_4) were added. The appearance of a greenish color indicates the presence of triterpenoids.
- **Steroids test**
  - ✓ **Salkowski test:** 2 mL of crude extract was mixed with 2 mL of $CHCl_3$, then 2 mL of concentrated sulfuric acid (H_2SO_4) was added. The appearance of a red coloration indicated the presence of the steroids.
- **Volatile oil test**

We moisten a filter paper with ethanolic M.pro extract then expose it to a UV light. Gray spots appear as evidence of the presence of volatile oils.

- **Coumarins test**

A test tube filled with 2 mL of ethanolic M.pro extract, immersed in a water bath at 65°C, placed a wetted filter paper in NaOH over the tube for 10 minutes, and then the tube was exposed to UV light. The appearance of greenish-yellow dots indicates the presence of coumarins.

The ethanolic M. pro extract was prepared in order to estimate its level of total phenols and flavonoids as well as evaluate its biological activity.

3.2. Dosage of Total Phenolic Content (TPC)

The dosage of total polyphenols in M. pro extract is carried out by Folin-Ciocalteu method [38].

The reagent consists of a mixture of tungstic phospho-acid ($H_3PW_{12}O_{40}$) and molybdic phosphorus ($H_3PMo\ 12O_{40}$), reduced by phenols into a mix of blue oxides of tungsten (W_8O_{23}) and molypdenum (Mo_8O_{23}). The amount of phenolic compounds in the medium affects how blue the color is, with 765 nm being the wavelength at which it absorbs the lightest [39].

Reference standard for calibration curve plotting was aqueous gallic acid solution (0.03-0.3 mg /mL). After adding 0.5 mL of Folin-Ciocalteu reagent (10%) to 0.1 mL of each solution, the mixture was neutralized with 2 mL of Na_2CO_3 solution (20%, w/v) and stored in the dark at room temperature for 30 min.

Blue absorption was measured using a UV/Vis spectrophotometer at 760 nm against a blank.

TPC was determined using linear regression equation from gallic acid standard curve. Calculated as mean ±SD (n=3) and represented as mg gallic acid equivalent GAE /g (Ext) of extract.

3.3. Dosage of Total Flavonoids Content (TFC)

The estimation of the total flavonoid content in the ethanolic M. pro extract is done using the Bahorun method [40].

Ethanolic quercetin solution (0.002 to 0.02 mg/mL) was used as a reference standard for plotting the calibration curve. 1.5 mL of an ethanolic solution of $AlCl_3$ (2%) was added to 1.5 mL of each solution. The mixture was kept in the dark for 30 minutes at room temperature. The reading of the absorbance of each solution was determined at 430 nm against a blank.

The TFC was calculated using a linear regression equation obtained from the standard curve of quercetin. It was calculated as the mean ±SD (n=3) and expressed as the extract's mg quercetin equivalent QE / g(Ext).

3.4. Liquid phase chromatography coupled with mass spectrometry (LC/MS)

Among the various existing chromatographic methods, high-performance liquid chromatography coupled with mass spectrometry has been used to analyze the most active propolis extracts.

The active extracts were analyzed using LC-MS. The HPLC analysis was conducted using the UPLC-ESI-MS-MS Shimadzu 8040 Ultra-High sensitivity with UFMS technology was employed and equipped with binary bump Nexera XR LC-20AD. The column used for chromatographic separation is a Restak Ultra C18 150×4.6 mm, 3µm. The flow rate was 0.2 ml/min.

The mobile phases utilized include a mixture of water and 0.1% formic acid (solvent A) and methanol (solvent B). The gradient used in the experiment is as follows: 0-0.2 min, 98% of A; 0.2-7.5 min, 25% of A; 7.5-12.5 min, 0% of A; 12.5-17 min, 0% of A; 17-18 min, 98% of A; and finally, 18-21 min, 98% of A. The injection volume in the HPLC system is 5 µl. The extract was prepared at a concentration of 1 mg/ml.

4. Evaluation of biological activities

4.1. Antioxidant activity

4.1.1. DPPH• radical scavenging test

Principle

The antioxidant activity of ethanolic M. pro extract was evaluated using the DPPH• (1,1-diphenyl-2-picryl-hydrazyl) method, following the procedure described by Benaissa with slight modifications. This method is commonly employed because to its rapidity, simplicity, and affordability [41]. Antioxidants donate hydrogen atoms, which decrease the amount of purple DPPH radicals and form a yellow molecule [42].

Procedure

1mL of different concentrations of M. propolis extract (diluted in bio-ethanol) and control solution were added to 1 mL of DPPH• (250 µM) solution prepared in ethanol. The mixture was left in the dark for 30 minutes. The disappearance of the purple color of the mixture was compared to the negative control, which contained 1 mL of DPPH solution and 1 mL of

ethanol. The absorbance was measured at 517 nm. The same procedure was repeated, replacing the extract of M. propolis with ascorbic acid as a reference.

The antiradical activity was estimated according to the equation (1) below:

$$antiradical\ activity = \frac{Abs\ 517_{negative\ control} - Abs\ 517_{sample}}{Abs\ 517_{negative\ control}} \times 100$$

The inhibition of DPPH radical was calculated as mean ± SD (n=3). The antioxidant capacity of the extract was expressed as an IC_{50} value, which is defined as the concentration of the substrate that causes the loss of 50% of the activity of DPPH.

4.1.2. Determination of total antioxidant capacity (TAC)

Principle

The total antioxidant capacity of ethanolic M. propolis extract was determined using the phosphomolybdenum method [14] with slight modifications. In this method, molybdenum Mo (VI) in the form of molybdate ion MoO_4^{2-} was changed into molybdenum Mo (V) in the form of MoO_2^{+} when the extract was present. This created a green phosphate complex (Mo (V)) at an acidic pH.

Procedure

0.2 mL of different concentrations of ethanolic M. propolis extract and control solution were mixed with 2 mL of reagent solution (28 mM sodium phosphate, 0.6 M sulfuric acid, and 4 mM ammonium molybdate). The mixture was kept in a water bath at 80 °C for 90 min. After that, it was left to cool at 4 °C. The absorbance was read at 695 nm against a blank (containing all reagents except the M. propolis extract). Ascorbic acid was used as a positive control. The total antioxidant capacity of the extract was expressed as the EC_{50} value, which is defined as the concentration of the substrate that causes the loss of 50% of the reducing power of molybdenum Mo (VI).

4.2. *In vitro* photoprotective activity

The photoprotective activity was evaluated spectrophotometrically by the method of diluted solutions. The dilutions were performed in bioethanol until a concentration of 0.5 and 1

mg/mL was reached. A spectrophotometer (Quimis®) was used, with quartz cuvettes with a 1 cm optical path for the acquisition of the spectra and ethanol as a blank. For maximum absorption wavelength (λ_{max}) determination, spectrophotometric scanning of the ethanolic M. pro extract was performed at wavelengths between 260 and 400 nm, with intervals of 5 nm. The in vitro Sun Protection Factor (SPF) was determined for each concentration by the spectrophotometric method developed by Mansur [43] using Eq. (2).

$$SPF = FC\frac{320}{290}EE(\lambda).I(\lambda).Abs(\lambda) \qquad (2)$$

Where FC = 10 (constant), EE = erythemogenic effect, I = intensity of the sun, and Abs = absorbance of the sample. Absorption readings were performed in the range of 290–320 nm with intervals of 5 nm and added in Eq. (2). The constants EE and I were pre-defined by Mansur, according to Table III.2.

Table III.2 EE and I constants for the calculation of in vitro SPF

λ (nm)	**EE(λ) × I(λ)**
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Σ	1.0000

EE(λ), erythemogenic effect of wavelength radiation; I(λ), sun intensity at wavelength (λ); λ, wavelength (Mansur,1984) [43].

4.3. Antibacterial activity

The antibacterial activity of M. propolis extract was evaluated on 4 strains of bacteria, one in Gram +: *Bacillus subtilis* ATCC6633 and three in Gram - *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, and *Klebsiella pneumoniae* ATCC70603. In this regard, we used the gel diffusion method. This method's principle is to use sterile Wattman paper discs measuring 6 mm in diameter. Each disc is impregnated with 10 microliters of propolis extract at different concentrations (10; 5; 1; 0,5; 0,1; 0.05 mg/mL). These discs are deposited on the surface of a medium flooded by a bacterial suspension. (Figure III.2). We used Mueller-Hinton Agar (MHA) for bacterial strains. After 18 hours at 37°C of bacterial strain incubation, the diameter of the inhibition zone was measured [44].

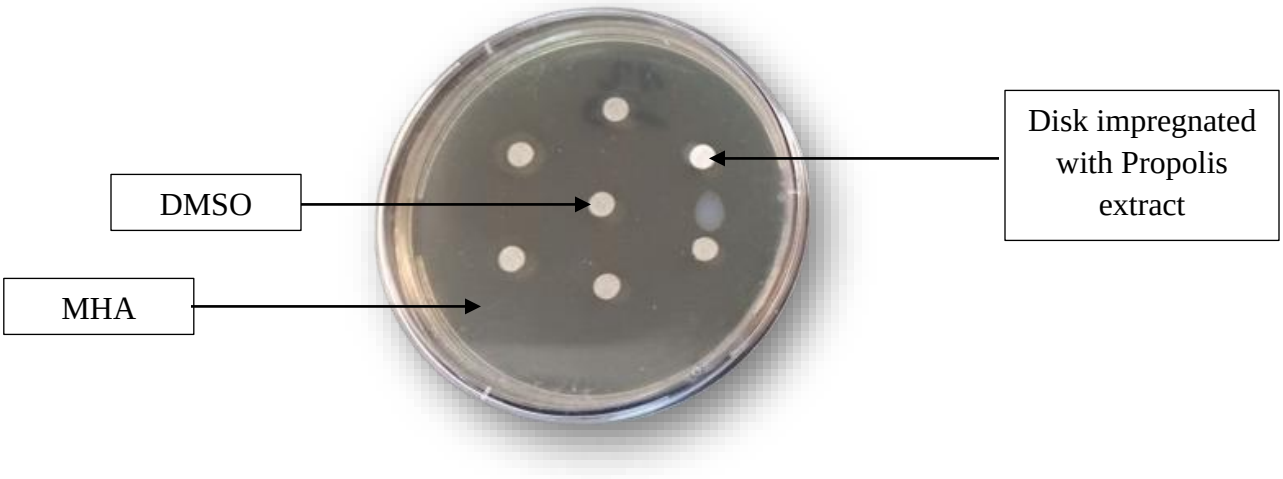

Figure III.2 Method used for antibacterial activity

RESULTS AND DISCUSSION

1. Obtaining the extraction yield

A high yield was achieved using the ultrasound-assisted extraction method.

The result of M. propolis yield in the UAE (38.62% ± 0.21) was close to those of Funari, Najafi, Paviani and Biscaia which achieved yields of 38.34% ± 2.05, 35% 2.20, 39.45% ≥ 1.20 and 46.00% ≤ 6.00, respectively [45-48]

Based on previous studies, it was found that the propolis from San Jerónimo Tecoalt had the highest yield, ranging from 71.34% to 71.54%. Interestingly, there was no significant difference observed in the extraction method. All in all, the extraction method did not have an impact on the yield of the extracts. However, the place of origin of the propolis did play a role in determining the yield. This could be related to the specific vegetation from which the bees collected the propolis and the harvest season.

2. Qualitative and quantitative analysis

2.1. Phytochemical screening

Phytochemical analysis of propolis extract indicated amount of varied secondary metabolites.

Table IV.1 Chemical screening of propolis

Secondary metabolites	Extract
Flavonoids	+
Tannins	+
Phenols	+
Proteins	-
Steroids	+
Saponins	-
Coumarine	+
Essential oil	+
Terpenoids	+

- : Absence, + : Presence.

The phytochemical screening indicated the existence of phenols, flavonoids, tannins, terpenoids, steroids, essential oil, and coumarines in the ethanolic extract of M. propolis.

The ethanolic M. propolis extract contained high amount of phenols, flavonoids, and tannins. Segueni and al. (2017) and Boulechfar and al. (2021) have corroborated similar results with propolis from Algeria [49, 50]. The ethanolic M. propolis extract was devoid of saponins and proteins.

The high phenolic compounds in propolis extract are what explain why bees utilize it as a conservator to safeguard their hives.

2.2. Total Phenolic Content (TPC)

The TPC of bio-ethanolic M. propolis extract is represented in mg GAE/g ext, which was estimated using the linear regression equation of the gallic acid calibration curve (Figure IV.1).

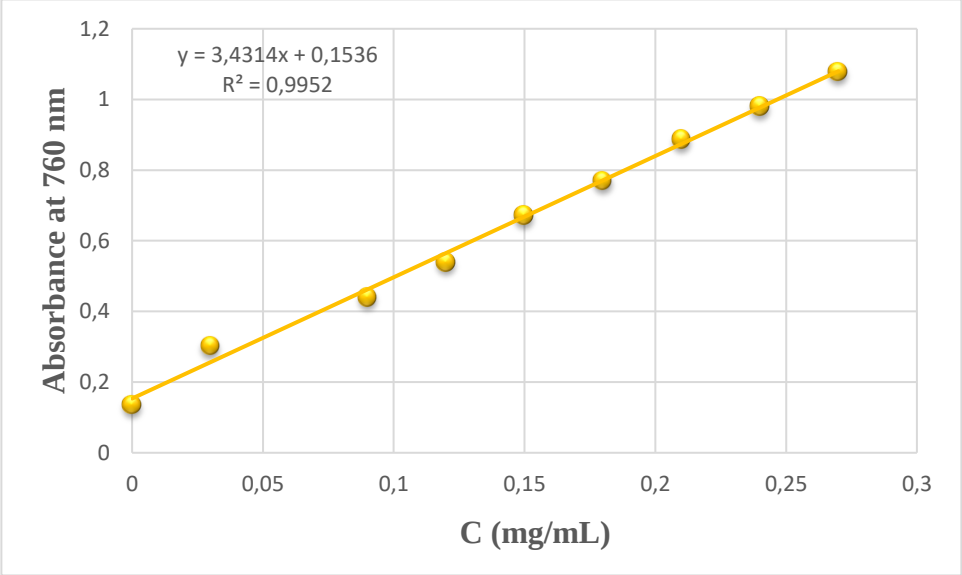

Figure IV.1 Gallic acid calibration curve

Table IV.2 Total phenolic content (TPC) of ethanolic M. Propolis extract

Extract	TPC
Bio-ethanolic M. propolis	34.039 ±2,002

2.3. Total flavonoids content (TFC)

In order to determine the total flavonoid content (TFC) of bio-ethanolic M. propolis extract, a calibration curve was created. The linear regression equation utilized to calculate the TFC in milligrams of quercetin equivalents per extract (mg QE/g Ext) is given in Figure IV.2.

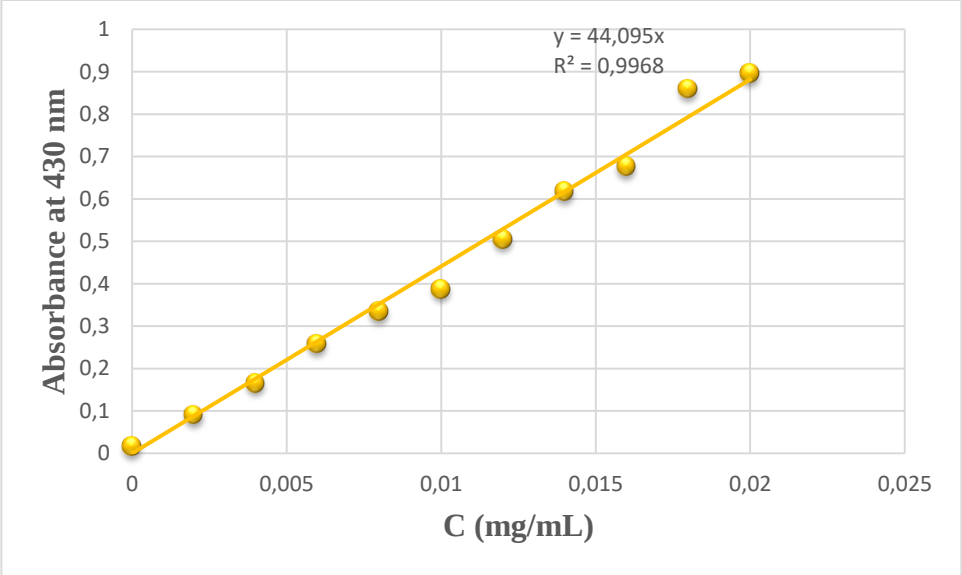

Figure IV.2 Quercetin calibration curve

Table IV.3 Total flavonoid content (TFC) of M. Propolis extract

Extract	TFC
Bio-ethanolic M. propolis	9.221 ±1,052

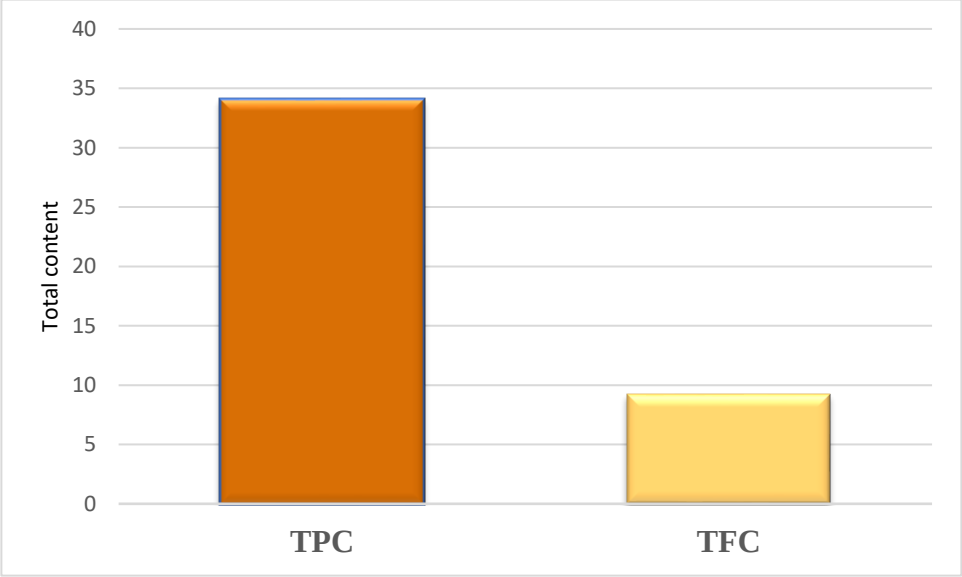

Figure IV.3 Total flavonoid and phenolic content

The results obtained were 34.039 ±2,002mg GAE/g for TPC and 9.221 ±1,052mg QE/g for TFC.

The flavonoid content is consistently lower than that of phenols, as flavonoids are encompassed within phenolic compounds.

The content of El Mermouthia propolis (Tebessa) is greatest in bio-ethanol extract. Compared to other extracts tested by [51], the bio-ethanol extract provides more richness in polyphenols and flavonoids.

Our results align with a comparative analysis carried out by Piccinelli [52] on an extensive variety of Algerian propolis samples. This study listed two distinct types of propolis in Algeria: the first is characterized by a high content of polyphenolic compounds, while the other type is rich in diterpenic compounds.

The results we reached surpass those obtained by Segueni [49] (from 0.81±0,16 to 8.97±0.25 GAE mg/g and from 0.57 ± 0.01 to 3.53±0.84 GAE/g for Beni Belaid collected Propolis)

El Mermouthia propolis contains a significantly greater content of phenolic compounds than multiple eastern propolis varieties studied by Bouaroura [51].

Total flavonoid content detected in Iranian propolis ethanol extracts was 12.2 (± 0,33) mg QE/g of propolis for Tehran, Khorsan's sample [53].

The total flavonoid content of propolis from China ranges from 8.3 (± 3.7) to 18.8 (± 6.6) mg QE/g of raw propolis [54].

The sample value of El Mermouthia (9.221 ±1,052mg QE/g) is similar to that found for propolis in China.

2.4. Analysis of propolis extracts by liquid chromatography coupled with mass spectrometry (LC-MS)

As observed, M. pro showed very interesting antioxidant activity. It was analyzed using liquid chromatography coupled with mass spectrometry. (LC-MS). Twenty-eight (28) compounds

have been used as standards for the identification of phenolic compounds in M. pro extract. Table IV.4 summarizes the LC-MS analysis results.

Table IV.4 LC-MS analysis of propolis extracts

Identified compounds	Chemical formula	Retention Time(min)	Peak area	Identified compounds	Chemical formula	Retention Time(min)	Peak area
Kojic acid	$C_6H_6O_4$	16.197	13716	**Rutin**	$C_{27}H_{30}O_{16}$	9.355	59823
Folic Acid	$C_{19}H_{19}N_7O_6$	12.496	4547	**Sinapic Acid**	$C_{11}H_{12}O_5$	10.142	5197
beta carotene	$C_{40}H_{56}$	13.918	10721	**Syrinjic Acid**	$C_9H_{10}O_5$	7.829	3516
Curcumin	$C_{21}H_{20}O_6$	12.509	13721	**ferulic acid**	$C_{10}H_{10}O_4$	13.346	794130
Cinnamic acid	$C_9H_8O_2$	3.606	696	**naringenin**	$C_{15}H_{12}O_5$	11.119	6148101
Epicatechin	$C_{15}H_{14}O_6$	11.965	27418	**acide picrique**	$C_6H_3N_3O_7$	0.000	ND
thymol	$C_{10}H_{14}O$	11.586	21023	**Chlorogenic Acid**	$C_{16}H_{18}O_9$	6.230	2823
Luteolin	$C_{15}H_{10}O_6$	12.492	68627	**Kampferol**	$C_{15}H_{10}O_6$	12.494	10502
chrysin	$C_{15}H_{10}O_4$	13.108	3892525	**3.5-dihydroxybenzoic Acid**	$C_7H_6O_4$	0.000	ND
Oleanolic Acid	$C_{30}H_{48}O_3$	16.324	10250	**4-mythoxybenzoic Acid**	$C_8H_8O_3$	5.952	2780
Oleauropin	$C_{25}H_{32}O_{13}$	0.000	ND	**Caffeic Acid**	$C_9H_8O_4$	7.560	20828
Quercetin	$C_{15}H_{10}O_7$	11.348	11927	**Vanillic Acid**	$C_8H_8O_4$	0.000	ND
Resveratrol	$C_{14}H_{12}O_3$	16.712	20750	**gallic acid**	$C_7H_6O_5$	16.723	5134
Riboflavin	$C_{17}H_{20}N_4O_6$	14.743	29805589	**p-Coumaric Acid**	$C_9H_8O_3$	8.498	272995

BEMP: Bio-ethanolic Mermouthia Propolis ND: No Detected highest high

The results show that the chemical composition of M. pro extract varies qualitatively and quantitatively. Ethanolic M. propolis extract highly contains riboflavin, naringenin, chrysin, ferulic acid, and p-coumaric acid, with levels of 29805589, 6148101, 3892525, 794130, and 272995 mg/kg of extract, respectively. Moreover, it is worth mentioning that the majority of phenolic compounds (standards) are found in high concentrations.

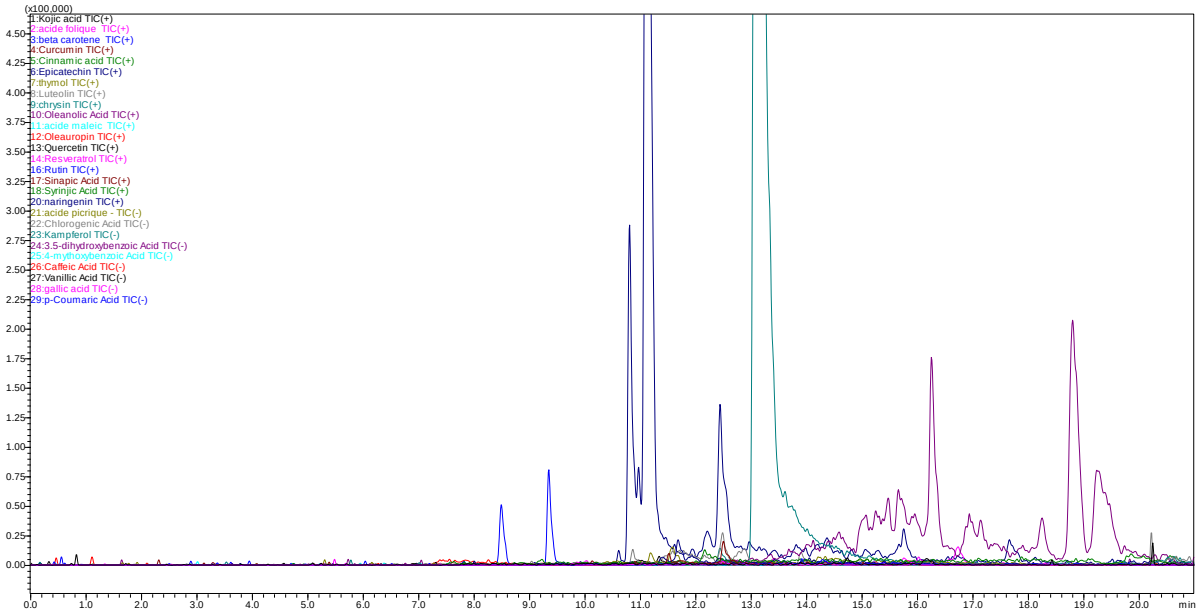

Figure IV.5 Chromatogram of ethanolic M. propolis extract

The chemical composition of the propolis studied indicates that these propolis are of the peuplier type [7]. Demonstrated that propolis containing high levels of phenolic chemicals, including flavones, flavonols, flavanones, and dihydroflavonols, belongs to this particular class of propolis. Our research aligns with the study conducted by Piccinelli [52], which validated the existence of this specific type of propolis in Algeria. LC-MS analyses conducted on various Algerian propolis have detected the presence of chemical markers of this type of propolis.

The major phenolic compounds detected in this study are riboflavin, naringenin, chrysin, ferulic acid, p-coumaric acid, luteolin, rutin, epicatechin, thymol, and caffeic acid. These compounds might have been dependable for the observed antioxidant activity.

The results of our study align with the research conducted by Ahn [54] which proposed that the strong antioxidant properties of Chinese propolis are attributed to the presence of certain components, including caffeic acid, ferulic acid, and caffeic acid phenethyl ester.

Woźniak [55] highlighted the antioxidant characteristics of Polish propolis in a recent study. The authors have shown the significance of incorporating propolis into therapeutic products.

3. Evaluation of biological activities

3.1. Antioxidant activity

We were intrigued by the antioxidant properties of Algerian propolis. Several authors consider this activity to be a global standard for classifying propolis.

The antioxidant impact was evaluated by integrating two complementary methods.

3.1.1. DPPH• radical scavenging test

Spectrophotometry evaluated the extract's anti-radical activity against the DPPH radical at 517 nm after the radical reduced and transitioned from violet to yellow. In this test, the results were compared to the reference standard (ascorbic acid). The IC_{50} of an antioxidant is the concentration required to inhibit 50% of the oxidant involved. The lower the concentration, the higher the antioxidant effect [56]. Figure IV.4 presents the results.

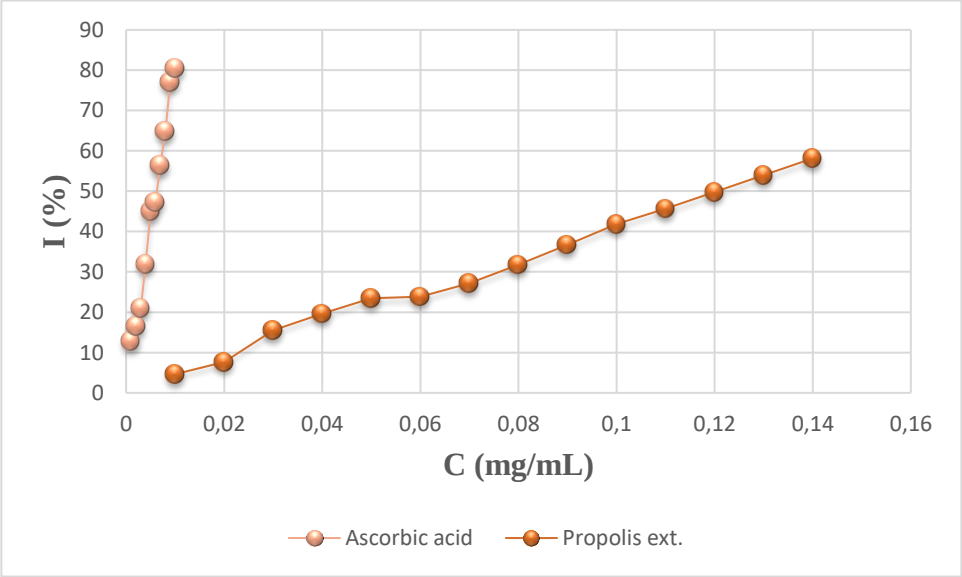

Figure IV.6 DPPH radical inhibition curve by M. propolis extract and ascorbic acid

M. pro extract showed better anti-radical activity (IC_{50} = 0,1204±0,28 mg/mL). However, this activity is not quite as powerful as ascorbic acid (IC_{50} = 0,00609±0.41 mg/mL).

Additionally, it is important to note that the ability to counteract radicals becomes stronger as the concentration increases, which has been observed with the reducing power of ethanol extracts as well as in the study conducted by Leandro [57].

3.1.2. Determination of total antioxidant capacity (TAC)

The result indicates that the M. pro extract has a higher level of activity, with an EC_{50} value of 0,265 mg/mL. Nevertheless, the extract exhibited a notably lower level of anti-radical activity compared to the standard ascorbic acid, as indicated by an EC_{50} 0.082 mg/mL. This result validates the result of the previous DPPH test.

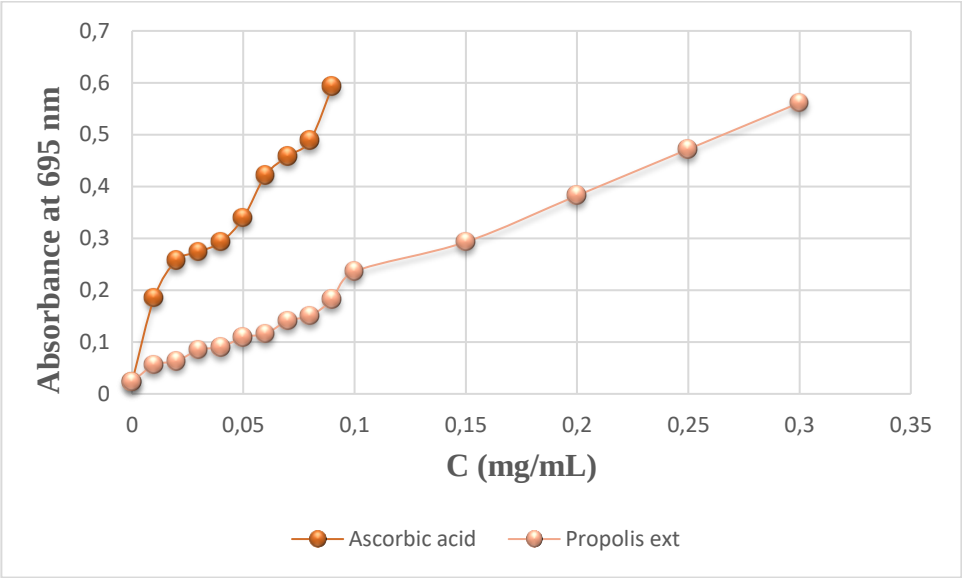

Figure IV.7 TAC curve of M. propolis extract and ascorbic acid

Table IV.5 Results of antioxidant activity

Antioxidant Power (mg/mL)	**Ascorbic acid**	**M.Propolis extract**
Scavenger effect of DPPH (IC_{50})	0,00609±0.41 mg/mL	0,1204±0,28 mg/mL
Total antioxidant capacity (TAC) EC_{50}	0.082 mg/mL	0,265 mg/mL

Through our study of the antioxidant activity of M. propolis extract, we prove that this extract effectively reduces molybdenum Mo (VI) while only slightly inhibits radical DPPH•, when compared to the standard antioxidant, ascorbic acid. Thus, we suggest that the bioactive compounds present in the extract mostly function as hydrogen donors rather than electron donors.

4. *In vitro* photoprotective activity

The SPF was determined by applying the Mansur method. An ultraviolet spectrophotometer was used to carry out the test. The absorbance values obtained were inserted into Equation (1) to calculate the SPF values, which are presented in Table IV.6.

Table IV.6 SPF values calculated from BEMP extract in different concentrations

Concentration (mg/mL)	SPF 85% BEMP extract
0.5	36.6323
1	60.7239

BEMP: Bio-Ethanolic Mermouthia Propollis

As observed in Table IV.6 the ethanolic M.pro extract provides exceptional sun protection.

5. Antimicrobial activity

We measured the antibacterial activity of M. propolis extract against four (4) bacterial strains, one in Gram +: *Bacillus subtilis* ATCC6633 and three in Gram - *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, and *Klebsiella pneumoniae* ATCC70603.This activity was carried out utilizing the gel diffusion technique. Table IV.7 illustrates the results.

Table IV.7 M. Propolis inhibition diameters for the studied strains.

		E.coli	*Psm*	*Ks*	*Bc*
M.pro	C(mg/mL)	Medium(mm)	Medium(mm)	Medium(mm)	Medium(mm)
	10	10	8.6	8.2	10.2
	5	9.9	7.6	5.93	5.2
	1	3.9	6.1	-	4.9
	0.5	3.6	-	-	6.6
	0.1	2.2	-	-	3.6
	0.05	1.7	-	-	3.6

E. coli: Escherichia coli, Psm: Pseudomonas aeruginosa, Ks: Klebsiella pneumoniae, Bc: Bacillus subtilis

Based on the results presented in Table IV.7, it can be assumed that M. pro sample exhibited an effective antibacterial activity (as determined by the diameter of the inhibitory zones). The extract of M. pro had the greatest efficacy against *Bacillus subtilis*, whereas its effectiveness was least evident against *Klebsiella pneumoniae*. However, it shown significant efficacy against *E. coli*, despite being a gram-negative bacterium.

The precise mechanisms by which propolis inhibits or delays the penetration of Gram-negative bacteria remain unidentified; however, the outer membrane (which consist of phospholipids, proteins, and lipopolysaccharide structure) might be responsible for their low sensitivity [58].

In addition, it has been found that M. propolis extract exhibits significant potential as a substitute for antibacterial compounds, as indicated by studies carried out [53, 59-61].

In summary, these results provide evidence that M. propolis has the potential to serve as an effective natural food preservative in the food industry as well as a possible solution for interacting with bacterial infections caused by drug-resistant microorganisms.

In order to use propolis into food and medicinal microbiology, additional research is required to examine its constituents and understand its mechanisms.

CONCLUSION

Conclusion

The present study aimed to evaluate propolis from El Mermouthia (Tebbesa), an eastern Algerian region. The study achieved its technical approach through three complementary parts: phytochemical study, biological activities, and composition.

By employing a hydroethanol solvent system for ultrasonic extraction of the phytochemical part, we have achieved a better yield. Furthermore, the phytochemical screening tests revealed few cumarins and high levels of phenols, flavonoids, and tannins.

We used LC-MS to determine the chemical composition of propolis extract, identifying flavonoids (e.g., flavonol, flavone), sterols, and phenolic compounds. We identified phenolic acids (ferulic acid and gallic acid) and flavonoids (chrysin, naringenin and kaempferol) using high-performance liquid chromatography (HPLC).

To optimize the performance of M. propolis extract, several biological activities were assessed.

Studies have indicated that the geographical origin of a propolis sample influences its biological effect. Indeed, M. pro extract has been shown to have the best antioxidant effect and the best inhibitory effect. In addition, M. pro extract showed varying effectiveness against the bacterial strains tested, with a considerable effect against gram-positive bacteria (*Bacillis*).

Overall, these results validated the most significant applications of propolis as a therapeutic agent.

Further investigation into this study could be facilitated by the following:

- ✓ Perform an additional experimental study *in vivo* to corroborate the results that we achieved *in vitro*.
- ✓ Identify extra bioactive molecules by employing alternative molecular identification methods, such as Nuclear Magnetic Resonance (NMR).

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ABSTRACTS

Abstracts

Abstract: Propolis, a natural substance collected by bees from different plants and trees, has several advantageous characteristics, such as antibacterial, anti-inflammatory, antioxidant, and other therapeutic benefits. This study aimed to analyze the chemical composition of propolis from Mermouthia, which was sourced in the arid region of eastern Algeria. The analysis was carried out using liquid chromatography coupled with mass spectrometry (LC-MS). Additionally, the study assessed the total phenolic and flavonoid content of the propolis, evaluated its antioxidant and antibacterial activities, and determined its sun protection factor. The phytochemical screening performed have detected phenols, flavonoids, tannins, sterols, and triterpenes in M. propolis, as well as coumarins and essential oils. The analysis revealed a significant content of phenolic and flavonoid compounds, which was confirmed by the Folin Ciocalteu and aluminum chloride tests, respectively.

The most significant components of M. propolis, as determined by LC-MS analysis, were riboflavin, naringenin, chrysin, ferulic acid, and p-coumaric acid. The antioxidant activity of M. propolis was measured using the reducing power of molybdenum Mo (VI) and diphenyl-1,1-picrirylhydrazil (DPPH) tests. Propolis exhibited a substantial level of antioxidant activity when compared to ascorbic acid. Furthermore, M. propolis demonstrated an extraordinary level of UV protection, thus confirming its effectiveness as an antioxidant.

Propolis shows significant potential as a natural alternative for food preservation and healthcare.

Key words: Propolis, antioxidant activity, antibacterial activity, LC-MS, phytochemical screening, SPF, phenolic compounds

الملخص: البروبوليس مادة طبيعية يجمعها النحل من نباتات وأشجار مختلفة، تتميز بخصائص عدة، فهو مضادة للبكتيريا، مضاد للإلتهاب، ومضاد للأكسدة، وغير ذلك من المنافع العلاجية. والهدف من هذه الدراسة هو معرفة التركيب الكيميائي لبروبوليس منطقة المرموثية، المصنفة ضمن المناطق القاحلة في شرق الجزائر. أُجري التحليل باستخدام الكروماثوغرافيا السائلة بالاقتران مع مطيافية الكتلة .(LC-MS) وبالإضافة إلى ذلك، قيّمت الدراسة مجموع محتوى البروبوليس من الفينول والفلافونويد، وقيّمت أنشطتها المضادة للأكسدة والمضادة للبكتيريا، وحددت عامل حمايتها من الشمس. وقد كشفت الاختبارات الكيميائية الأولية عن وجود متعددات الفينول، والفلافونويدات، والتانينات، والستيرولات، والتريتربينات، وكذلك الكومارينات والزيوت الأساسية. وكشفت التحاليل عن تركيز كبير في مركبات متعددات الفينول والفلافونويد، وهذا ما أكده اختباري Folin Ciocalteuوكلوريد الألومنيوم على التوالي. وكانت أهم مكونات بروبوليس المرموثية، كما حددها تحليل-LC MS، هي ريبوفلافين، ونارينجينين، والكريسين، وحامض فيروليك، وحامض الكوماريك. وقد قيست القدرة المضادة للأكسدة للبروبوليس باستخدام اختباري: كسح الجذر الحر (DPPH) و القدرة الكلية المضادة لأكسدة molybdenum Mo (VI) حيث أظهر البروبوليس مستوى كبيراً من النشاط المضاد للأكسدة عند مقارنته بحمض الأسكوربيك. وعلاوة على ذلك، أظهر الروبوليس مستوى استثنائياً من الحماية للأشعة فوق البنفسجية، مما يؤكد فعاليته كمضاد للأكسدة. هذا ما يؤكد إمكانية استعماله كبديل طبيعي للحفاظ على الأغذية والرعاية الصحية.

الكلمات المفتاحية: بروبوليس، النشاط المضاد للأكسدة، النشاط المضاد للبكتيريا، LC-MS، الفحص الفيتوكيميائي، SPF، المركبات الفينولية.

Résumé : La propolis, une substance naturelle recueillie par les abeilles à partir de différentes plantes et arbres, a plusieurs caractéristiques avantageuses, telles que antibactériennes, anti-inflammatoires, antioxydantes et autres avantages thérapeutiques. Cette étude visait à analyser la composition chimique de la propolis provenant de Mermouthia, qui a été obtenue dans la région aride de l'est de l'Algérie. L'analyse a été effectuée à l'aide de la chromatographie liquide couplée à la spectrométrie de masse (LC-MS). En outre, l'étude a évalué la teneur totale en phénols et en flavonoïdes de la propolis, évaluée ses activités antioxydantes et antibactériennes, et déterminé son facteur de protection solaire. Les tests phytochimiques effectués ont détecté des phénols, des flavonoïdes, des tanins, des stérols et des triterpènes dans M. propolis, ainsi que des cumarines et des huiles essentielles. L'analyse a révélé un taux significatif de composés phénoliques et de flavonoïdes, ce qui a été confirmé par les tests de Folin Ciocalteu et de chlorure d'aluminium, respectivement. Les composants les plus importants de M. propolis, déterminés par l'analyse LC-MS, étaient la riboflavin, la naringenine, la chrysine, l'acide ferulique et l'acide p-coumarique. L'activité antioxydante de M. propolis a été mesurée en utilisant la puissance réductrice du molybdène Mo (VI) et du diphényl-1,1-picrirylhydrazil (DPPH). La propolis a montré un niveau substantiel d'activité antioxydante par rapport à l'acide ascorbique. En outre, M. propolis a démontré un niveau extraordinaire de protection UV, confirmant ainsi son efficacité en tant qu'antioxydant. La propolis présente un potentiel considérable en tant qu'alternative naturelle à la conservation des aliments et aux soins de santé.

Mots-clés: Propolis, activité antioxydante, activité antibactérienne, LC-MS, test phytochimique, SPF, composés phénoliques.

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Valorisation of Mermouthia propolis

Thesis submitted to obtain a Master degree in applied chemistry

ABSTRACT

Propolis, a natural substance collected by bees from different plants and trees, has several advantageous characteristics, such as antibacterial, anti-inflammatory, antioxidant, and other therapeutic benefits. This study aimed to analyze the chemical composition of propolis from Mermouthia, which was sourced in the arid region of eastern Algeria. The analysis was carried out using liquid chromatography coupled with mass spectrometry (LC-MS). Additionally, the study assessed the total phenolic and flavonoid content of the propolis, evaluated its antioxidant and antibacterial activities, and determined its sun protection factor. The phytochemical screening performed have detected phenols, flavonoids, tannins, sterols, and triterpenes in M. propolis, as well as coumarins and essential oils. The analysis revealed a significant content of phenolic and flavonoid compounds, which was confirmed by the Folin Ciocalteu and aluminum chloride tests, respectively.

The most significant components of M. propolis, as determined by LC-MS analysis, were riboflavin, naringenin, chrysin, ferulic acid, and p-coumaric acid. The antioxidant activity of M. propolis was measured using the reducing power of molybdenum Mo (VI) and diphenyl-1,1-picrirylhydrazil (DPPH) tests. Propolis exhibited a substantial level of antioxidant activity when compared to ascorbic acid. Furthermore, M. propolis demonstrated an extraordinary level of UV protection, thus confirming its effectiveness as an antioxidant.

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Academic year: 2023/2024