الجمهورية الجزائرية الديمقراطية الشعبية

Democratic and Popular Republic of Algeria

وزارة التعليم العالي والبحث العلمي

Ministry of Higher Education and Scientific Research

جامعة 8 ماي 1945 قالمة

University 8 May 1945, Guelma

Faculty: Natural and Life Sciences, Earth and Universe Sciences

Field: Natural and Life Sciences

Sector: Biological Sciences

Department: Ecology and Environmental Engineering

Specialty: Applied Microbiology

Master's Thesis

Theme: Antimicrobial activity of Algerian propolis

Master's Student

Taleb Bochra

Committee members

Dr. LAOUABDIA SELLAMI Nadjette, Committee Chair	**Full Professor**	University of 8 May 1945, Guelma
Dr. BOUCHELAGHEM Sarra, Supervisor	**MCB**	University of 8 May 1945, Guelma
Dr. BOUTELDJA Meryem, Examiner	**MCB**	University of 8 May 1945, Guelma

June 2025

Acknowledgments

First and foremost, I am grateful to Allah, the Creator, for giving us the health, willpower, and patience to complete this work.

I would like to express my sincere gratitude and appreciation to Dr. Bouchelaghem Sarra, Lecturer at the University of Guelma, for her invaluable guidance and support throughout this research.

I extend my heartfelt thanks to the committee members, Prof. Laouabdia Sellami Nadjette and Dr. Bouteldja Meryem, for kindly accepting to serve as chair for the committee and examiner for my work, respectively. I am truly grateful for their time and constructive criticism.

I also want to thank all the professors who taught us during my undergraduate studies.

Special thanks to Mr. Salah el Agoune, the Head of the University of Guelma. Deep thanks to Mr. Kamel Rouabhia, the Head of the Department of Ecology and Environmental Engineering, and all the faculty Members for their dedication to the development of the University of Guelma.

Finally, I am immensely grateful for the bacterial strains provided by the Hospital of Ibn Zohr in Guelma, which made this research possible.

Dedication

I am very pleased to dedicate this work to the most precious people in my life.

To my dear father, ***Abd el Hamid*** *and my dear mother,* ***Souhila,*** *for*

Their unconditional support, and for their sincere prayers throughout my academic journey.

To my dear brother and sister, ***Abd Hakim and Zahra,*** *for their encouragement and assistance.*

To my dear grandfather, ***Salah****, who supported me with all love, especially through his prayers.*

To my teachers throughout my academic journey.

To everyone who has supported me, from near or far

أعوذ بالله السميع العليم من الشيطان الرجيم

«وَأَوْحَى رَبُّكَ إِلَى النَّحْلِ أَنِ اتَّخِذِي مِنَ الْجِبَالِ بُيُوتًا وَمِنَ الشَّجَرِ وَمِمَّا يَعْرِشُونَ (68) ثُمَّ كُلِي مِنْ كُلِّ الثَّمَرَاتِ فَاسْلُكِي سُبُلَ رَبِّكِ ذُلُلًا يَخْرُجُ مِنْ بُطُونِهَا شَرَابٌ مُخْتَلِفٌ أَلْوَانُهُ فِيهِ شِفَاء لِلنَّاسِ إِنَّ فِي ذَلِكَ لَآيَةً لِقَوْمٍ يَتَفَكَّرُونَ (69)» سورة النحل الآيتان 68- 69

And your Lord inspired the bees: Make homes in the mountains, the trees, and in what people construct, then eat all the fruits, and go along to pathways of your lord made easy for you. From their bellies comes out a drink of various colors in which there is cure for people. Surely, in that there is a sign for a people who reflect.

Quran 68-69 Surat al-Nahl

Table of Contents

Part Two: Experimental study

Chapter I: Materials and Method

Chapter II: Result and Discussion

List of figures

List of tables

List of abbreviations

°C: Degrees Celsius

µl: Microliter

µm: Micrometer

e. g.: For example

EEP: Ethanolic Extract of Propolis

EEPC1: Ethanolic Extract of propolis the product commercial extraction in water bath

EEPC2: Ethanolic extract of propolis the product commercial extraction in room temperature

EEPR1: Ethanolic Extract of Propolis the Product Raw Extraction in water bath

EEPR2: Ethanolic Extract of Propolis the Product Raw extraxtion in room temperature

GC: Gas chromatography

GM: Growth media

h: Hour

HPLC: High-performance-liquid-chromatography

ISO: International-Organization-for-Standardization

LMIC: Low- and middle-income countries

mg: Milligram

MHA: Mueller-Hinton Agar

MHB: Mueller Hinton Broth

MIC Minimum inhibitory concentration

ml: Milliliters

mm: Millimeters

MRSA: Methicillin-resistant Staphylococcus aureus

Ms: mass spectrometry

NCCLS: National Commite for Clinical Laboratory Standars

NMR: Nuclear-magnetic-resonance

pH: Hydrogen potential

Rbcs: Red blood cells

Spp: Species plural

TLR-2: Toll-like receptor-2

UPEC: Uropathogenic *Escherichia coli*

IHC: International Honey Commission

PCR: Polymerase chain reaction

Introduction

Introduction

Due to technological progress in the medicinal and pharmaceutical fields, multiple research studies have foucsed on the propolis as an alternative and supplemental therapies for the treatment of numerous acute and chronic illnesses. Over the past decade, beekeeping in Algeria has expanded, with notable interest in bee products such as honey, bees wax, royal jelly, bee venom, pollen, and propolis. The propolis has been found to be a promising agent due to the various biological activities. In a number of *in vivo* and *in vitro* studies, it's been highlighted that propolis may reduce symptoms associated with numerous diseases.

Propolis is a sticky, resinous substance secreted by bees from various plant sources, such as bud, flower, and leaf secretions (Simon-Fenström et al., 2017). The bees mix the sap of tree with their salivary secretions and beeswax to produce the propolis. It is also known as bee glue, as bees use it to seal holes in their hives (Martinotti and Ranzato, 2015). Propolis has been described in numerous ancient texts as a wound-healing agent, either alone or in combination with other substances (Martinotti and Ranzato, 2015). Researchers have reported various natural properties of propolis including cytotoxicity and antimicrobial action (Pasupuleti et al., 2017; Oryan et al., 2018; Elkhenany et al., 2019). Propolis has recently been widely used as a dietary supplement to promote human health and prevent disease (Ramos and Miranda, 2007). It has also long been known for its antiviral, anti-inflammatory, and antibacterial properties, as well as its anesthetic, antioxidant, antitumor, anticancer, antifungal, antiprotozoal, hepatotoxic, mutagenic, antiseptic, and cytotoxic properties (Toretti et al., 2013 and Svorsen, 2016). The antibacterial properties of propolis have been documented *in vitro* against numerous Gram-positive and Gram-negative bacteria, due to the synergism of various propolis compounds, particularly the flavonoids galangin and pinocembrin (Przybylek and Karpinski, 2019).

Although numerous studies have documented the antimicrobial activity and chemical composition of propolis collected from various parts of the world, data on Algerian propolis remain limited and insufficient. This study aimed to determine the antibacterial activity of two samples of Algerian propolis against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* using two extraction methods. One sample was harvested from northeastern Algeria (Guelma Mountains), while the other was packaged as a commercial product by a company based in Setif.

Part One

Literature review

Chapter I

General background on propolis

1. Defenition of propolis

Propolis is a natural resinous substance produced by honeybees, specifically bee's worker, from various plant parts, buds, and exudates. The composition of propolis varies depending on its geographic origin, the bee species, and the surrounding vegetation (Marques et al., 2023). The term propolis derived from Greek, where "pro" signifies "at the entrance to" and "polis" means "community" or "city," suggesting this natural product is used in hive defense (Toretti et al., 2013). Another term for propolis is "bee glue". Due to its waxy nature and structural properties, bees utilize propolis in the build and repair of their hives, seal gaps and cracks, smoothing out interior walls, and protect the hive against the predators (Wagh, 2013).

Figure 1: The bee *Apis mellifera* collects resin to produce propolis (Maíra Tomazzoli et al., 2020).

2. History of propolis

Propolis has a long history, having been used by humans for centuries. Historical records indicate its use by ancient civilizations, such as the Egyptians, Persians, and Romans (Trosheva et al., 2007). It has been used as a folk remedy since 300 BC (Toretti et al., 2013). The ancient Egyptians depicted bees involved in propolis production on vases and various decorative objects (Koropatnicki et al., 2013). They were also well aware of propolis's ability to prevent decomposition and used it in the mummification process and to treat several ailments (Wag, 2013). The ancient Egyptians and Jews considered "tzori," the Hebrew term for propolis, a form of medicine (Trosheva et al.,2007). Furthermore, Greek and Roman

physicians, such as Aristotle, Dioscorides, Pliny, and Galen, were aware of the many medicinal benefits of propolis (Toretti et al., 2013). The Inca also used propolis to reduce fever, and the London Pharmacopoeia officially recognized it as a medicinal substance. From the 17th to the 20th century, the use of propolis spread widely in Europe (Toretti et al., 2013).

The Arabs were familiar with propolis. For example, Ibn Sina described two types of wax: pure wax and dark wax, the latter of which he probably meant for propolis. He described the substance as having a pungent odor that caused sneezing and as effective in removing splinters and foreign bodies (Koropatnicki et al., 2013).

3. Origine of propolis

The components of propolis vary from one species to another depending on its source and location. However, many similar components have been found in samples from different locations, while distinct components have been found in samples from specific plant origins (Wagh, 2013). To produce propolis, bees can also utilize substances secreted by plants from their wounds (Toreti et al., 2013).

Propolis has two sources:

- **Plant source:** plant secretions collected by bees; resins secreted by the buds of poplar, pine, birch, chestnut, and maple trees; and lipophilic compounds secreted from plant wounds, resins, or gums (Msrghita et al., 2013).
- **Animal source:** substances secreted by bees (wax and saliva); and incidental substances added during propolis production (pollen, nectar, or honey) (Msrghita et al., 2013). Another influential aspect involves the changes caused by the secretions of the hypopharyngeal gland of bees. These secretions add unique compounds, along with some transformations (such as the decomposition of flavonoid heterosides into aglycones) (Cardeno et al., 2012).

3. 1. Plant source of propolis in the world

Classifying propolis according to its plant sources is an effective method for achieving chemical standardisation, which in turn is essential for guaranteeing the quality and safety required for its commercialisation (Bankova, 2005 and Salatino et al., 2011). Two distinct approaches are utilized to determine the botanical origin of propolis: observations of bee

behavior or the chemical analysis of propolis and plant substances (Bankova et al., 2002). Tropical propolis presents a wholly dissimilar compositional design: the green propolis kind, discovered in Brazil, has its primary plant origin on the leaves of *Baccharis spp.* and chiefly contains prenylated phenylpropanoids (Bankova et al., 2005). Within Venezuela and Cuba, the primary plant origins are the blossom exudates of *Clusia* species, producing a propolis rich in prenylated benzophenones (Bankova et al., 2005). C-prenylflavonoids (or propolins) have been reported in propolis from Pacific islands, where the resin sources are the fruit exudates of the plant Macaranga tanarius (Chen et al., 2003). Plant bud resins from Poplar species are main sources for propolis from temperate regions (Europe, North America, and Asia), but also other species contribute to the composition of propolis from these areas (*Betula sp., Acacia sp., Pinus sp., Salix,* and *Aesculus hippocastanum*) (Groot et al., 2014). Other scientists found that honeybees collect plant material by cutting pieces of vegetative tissues, thus the anatomical traits of plant tissue in the propolis can be used as evidence of propolis origin (Huang et al., 2014).

3.2. Botanical origin of Algerian and Mediterranean propolis

It is worth noting that Mediterranean propolis is the propolis that originated in the countries surrounding the Mediterranean region, such as the coast of southern Europe, the coast of the Levant, and the coast of North Africa (Al-Kanduz et al., 2019). Mediterranean propolis is derived from plants in the cypress family, particularly the tall cypress and the Phoenician juniper. These trees grow in the Mediterranean region, characterized by a temperate climate. (Bankova et al., 2006). A unique type known as Mediterranean propolis has recently been identified. It is characterized by a high content of diterpenes and primarily derived from coniferous species belonging to the Cupressaceae and Pinaceae families, which are widespread in the Mediterranean region. This emerging type of propolis has been the focus of several recent studies, particularly those investigating diterpene-rich samples from Mediterranean countries in North Africa (Konstantia et al., 2016). This type of propolis was first discovered less than ten years ago in Sicily. Since then, it has been found on the island of Crete, southeastern Greece, Malta, and the Adriatic coast of Croatia (Popova et al., 2020). Based on the identified terpenes, the researchers suggested that the source plant was some Conifer species, possibly from the Cupressaceae family, which is abundant in the Mediterranean flora (Popova et al., 2020).

Figure 2: *Cupressus* species (Frezza et al., 2022).

4. Types of propolis

There are three main types of propolis recorded: European propolis, commonly known as poplar propolis, is the most common; then there is green Brazilian propolis, extracted primarily from the resin of the leaves of the *Baccharis draconculifolia* plant, and red Cuban propolis, extracted primarily from the resin of the *Clusia rosea* plant (Otilia, 2022).

Figure 3: Poplar propolis (Jelena suran et al., 2023).

Figure 4: Red and green propolis Brazilian (Adela ramona et al., 2020).

5. Physicochemical characteristic of propolis

The physicochemical properties of propolis are very important for producing a standardized, high-quality product. The diversity of propolis sources significantly affects its composition. Propolis is extracted from a wide variety of trees and shrubs. Each region and each colony seems to have its own preferred source of resin, which explains the great differences in the color, smell, and composition of propolis.

5.1. Physical properties

Propolis demonstrates considerable variation in its physical characteristics, largely influenced by the geographic origin of the hive, the surrounding flora, seasonal plant availability, and the species of bees involved in its production. As a result, its color can range from amber-yellow to dark brown, with occasional green or red hues observed (Cardinault et al., 2012). It emits a characteristic and pleasant aroma, often described as distinctive and agreeable (Wagh, 2013). In terms of consistency, propolis is a lipophilic substance that is typically hard and brittle at ambient temperature. However, upon heating, it transitions into a soft, pliable, and notably adhesive material, exhibiting a sticky texture (Wagh, 2013). Propolis presents a multifaceted composition, thus its direct application is not feasible. For commercial purposes, it's processed through extraction, employing appropriate solvents. A variety of solvents are typically utilized: water, methanol, ethanol, chloroform, dichloromethane, ether, and acetone. Essential antimicrobial agents within propolis tend to dissolve well in water or alcohols, aiding in the separation of undesired substances while retaining the sought-after active elements (Wagh, 2013).

5.2. Chemical compositions of propolis and their bioactive compound

Propolis consists of approximately 50–55% resins and balsams, 30% wax and fatty acids, 10% essential oils, 5% pollen, and 5% organic and mineral components. These components include numerous flavonoids and other phenolic compounds, as well as their esters. It also contains volatile aromatic compounds, minerals such as iron, calcium, zinc, copper, and manganese, as well as vitamins C and E, and B vitamins (Cardeno et al., 2012).

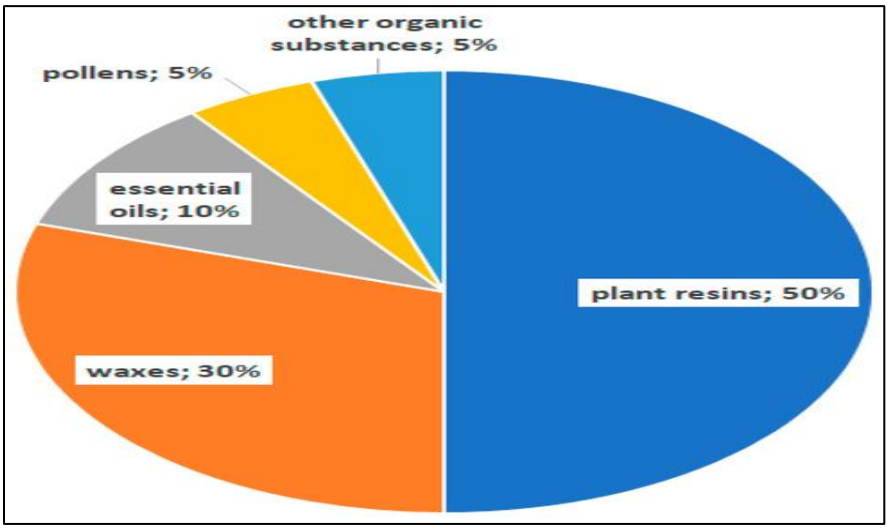

Figure 5: Composition of propolis (Przybylek et al., 2019).

The development of separation and purification methods, such as high-performance liquid chromatography (HPLC), thin-layer chromatography, and gas chromatography (GC), and identification techniques, such as mass spectrometry (MS), nuclear magnetic resonance (NMR), and gas chromatography-mass spectrometry (GC-MS), has facilitated the discovery of novel compounds in propolis for the first time, including flavonoids, terpenes, phenolics and their esters, sugars, hydrocarbons, and mineral elements (Huang et al., 2014).

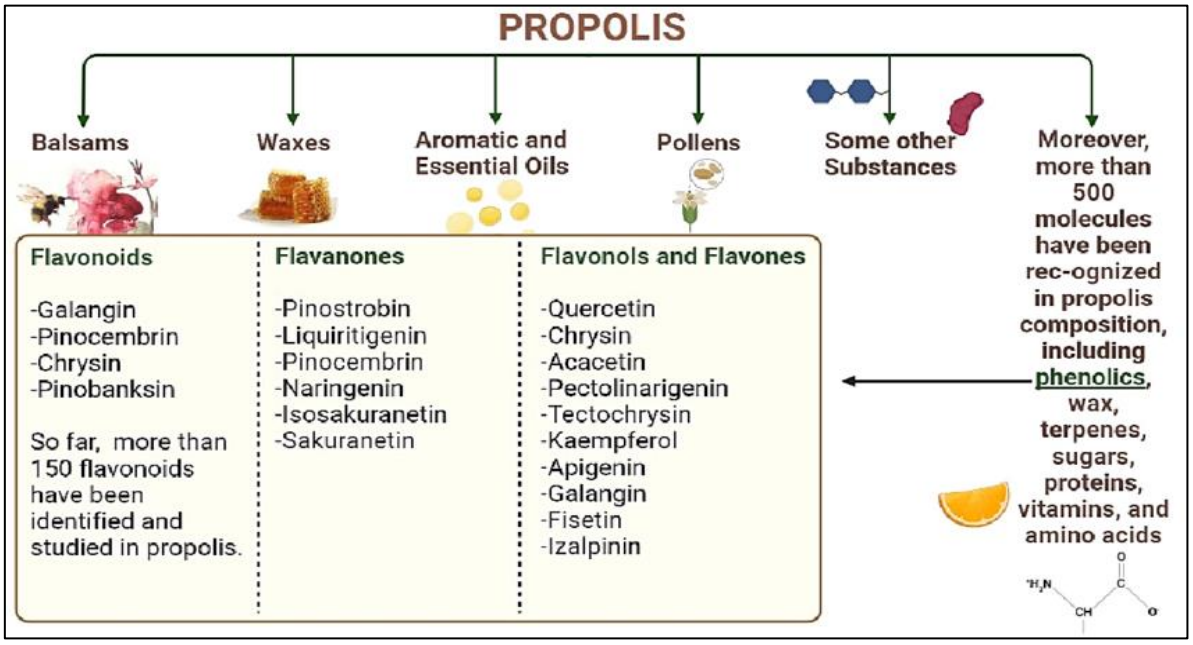

Figure 6: General composition of propolis (Syeda tasmia et al., 2022).

The chemical composition of propolis is directly related to the resins and balsams found in the plants from which it is derived. Extensive research has led to the identification of

more than 500 different chemical components have been found in propolis. In addition to resins, the major chemical groups present in propolis include waxes, polyphenols, and terpenoids (Przybylek and Karpinski, 2019). Several studies indicate that propolis specifically contains phenolic acids, flavonoids, ketones, aldehydes, chalcones, dihydrochalcones, terpenoids, amino acids, aliphatic acids, esters and aromatic acids, carbohydrates, vitamins, minerals, and beeswax (Hussein et al., 2022). Among flavonoids, quercetin and chrysin are the most abundant and are found in the propolis of various bee species. These flavonoids are the active components of the plant resin and have diverse biological effects, such as anticancer, antimicrobial, and anti-inflammatory effects (Zolkivli et al., 2022).

Table 01: Chemical compositions of propolis and their bioactive compounds (Górecka et al., 2022; Abdullah et al., 2020; Ahangari et al., 2018; Maciejewicz et al., 2001)

Chemical Compositions	Bioactive Compounds
Aromatic acids	Benzoic acids, caffeic acid, cinnamic acid, coumaric acid, ferulic acid, and gallic acid
Alcohols	Glycerol, erythritol, α-cedrol, xylitol Caffeic acid phenethyl ester, 2-propenoic acid methyl
Esters	Ester, 4,3-acetyloxycaffeate, 3,4 dimethoxy-trimethylsilyl esters, and 3-methoxy-4-cinnamate
Fatty and aliphatic Acids	Isoferulic acid, glutamic acid, phosphoric acid, malic acid, tartaric acid, propanoic acid, butanedoic acid, and stearic acid
Polyphenols	Flavonoids (e.g., quecentin, islapinin, chrysin ,galangin,alnusin, pinocembrin
Microelements	Aluminium (Al), copper (Cu), magnesium (Mg), zinc (Zn), silicon (Si), tin (Sn), manganese (Mn), nickel (Ni), and chrome (Cr)
Sugars	D-Altrose, d-glucose, maltose, and d-fructos
Vitamins	Vitamin A (retinol), vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (nicotinamide), vitamin B6 (pyridoxine), vitamin B9 (folic acid), vitamin C (ascorbic acid), and vitamin E (tocopherol)
Others	Butane, cyclohexane, cyclopentene, and guanidine

6. Propolis collection and preservation

Propolis is a resinous substance produced by honeybees through the collection of plant exudates, primarily from species such as poplar, cottonwood, alder, and birch, which serve as natural defences against pathogens. These resins are transported to the hive via the pollen baskets on the bees' hind legs and are mixed with bee saliva, wax, and other hive materials to form propolis, which is then used for structural and antimicrobial purposes within the colony (Darvishi et al., 2020). Beekeepers typically harvest propolis either by scraping it from hive structures or using mesh traps. While scraping is more convenient, it may result in higher

contamination, particularly with heavy metals like lead, as flavonoids in propolis can chelate metal ions. Therefore, the mesh collection method is recommended to obtain purer, safer propolis (Sales et al., 2006). After collection, propolis should be stored in airtight containers or bags and frozen to preserve its integrity. Freezing also facilitates its separation from debris, as the material becomes brittle and easier to handle at low temperatures (El-Sakhawy, 2023).

7. Quality criteria for propolis standardization

The chemical complexity and geographical differences of propolis pose significant challenges for its standardization and use in healthcare. To tackle this issue, researchers suggest classifying and standardizing propolis based on its plant origin and related chemical composition. For example, poplar-type propolis, which is the most studied type, has been chemically characterized. Stan and coworkers in 2011 analyzed 56 Romanian samples and recommended tests to aid future standardization efforts. Propolis composition is affected by climate, plant sources, and environmental factors. These quality inconsistencies make it hard to meet global demand and increase the risk of counterfeit products. To support international trade and quality control, we need regulatory frameworks and standards. However, propolis lacks strong evidence for its health claims, and the European Food Safety Authority (EFSA) does not permit it as a major food ingredient (Liu et al., 2025). The International Honey Commission (IHC) has set specific requirements for bioactive compounds in two main types: European poplar propolis (≥21% total phenolic acids, ≥4% flavones, and ≥4% flavonols) and Brazilian green propolis (≥5% total polyphenols, ≥0.5% flavones). Furthermore, the International Organisation for Standardisation (ISO) has issued detailed global standards for propolis quality, testing methods, packaging, and transportation. This standardisation aims to promote the growth and regulation of the propolis industry globally (Liu et al., 2025).

Chapter II

Antimicrobial activities of propolis

1 . Biological activities

Propolis exhibits a wide range of biological activities due to its complex chemical composition, which includes polyphenols, flavonoids, phenolic acids, essential oils, and other bioactive constituents (Bhatti et al., 2024). The ethanolic extracts of propolis have been extensively studied for their antioxidant potential, effects on human red blood cells (RBCs), and their antifungal, antibacterial, and antiviral properties (Woźniak et al., 2022).

1.1. Antibacterial efficacy of propolis

The antimicrobial potential of propolis and several of its bioactive constituents has been extensively documented against a broad range of microorganisms, including bacteria, viruses, fungi, and protozoa (Alotaibi et al., 2019). Numerous studies, including a comprehensive analysis of its efficacy against 600 bacterial strains, have demonstrated that propolis exhibits stronger activity against Gram-positive bacteria than Gram-negative strains (Almuhayawi, 2020). This differential effect is largely attributed to the structural differences in the bacterial cell wall, with the outer membrane of Gram-negative bacteria providing greater resistance to the penetration of antimicrobial agents (Kędzia, 2013). Geographical origin significantly influences the chemical composition and thus the antibacterial potential of propolis (Almuhayawi, 2020). The biological activity of propolis is often associated with its high content of phenolic compounds and flavonoids. However, the mere presence of these compounds does not always correlate directly with antimicrobial efficacy, highlighting the need for further studies to define bioactivity standards (Bhatti et al., 2024). Flavonoids, in particular, are thought to act on multiple bacterial targets (Cushnie and Lamb, 2005). For instance, kaempferide is an active flavonoid component in ethanolic propolis extract, and it has been employed in the treatment of *Staphylococcus aureus* skin infections (Almuhayawi, 2020). It has also shown strong activity against *Enterococcus faecalis*, *Listeria monocytogenes*, and *Staphylococcus saprophyticus* (Almuhayawi, 2020).

Among the most notable antibacterial agents in propolis are prenylated p-coumaric acids, which have demonstrated both antibacterial and cytotoxic properties. Diterpenic acids from Brazilian propolis also exhibit dual antimicrobial and cytotoxic effects (Bankova et al., 2005). Artepillin C, a major phenolic compound, has shown potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (Wojtyczka et al., 2019) and has bacteriostatic effects on Porphyromonas gingivalis, evidenced by membrane blebbing (Veiga

et al., 2017 and Yoshimasu et al., 2018). Other prenyl derivatives such as 3-prenyl-cinnamic acid allyl ester and 2-dimethyl-8-prenylchromene also contribute to antimicrobial activity (Viuda-Martos et al., 2008).

An ethanolic extract of propolis rich in kaempferide, artepillin C, drupanin, and p-coumaric acid displayed both antioxidant and antibacterial activity against *S. aureus, S. saprophyticus, L. monocytogenes,* and *E. faecalis* (Seibert et al., 2009). Further evidence from Chilean propolis research shows that pinocembrin and apigenin exhibit strong antibacterial activity against *Streptococcus mutans,* surpassing even chlorhexidine in efficacy, with minimum inhibitory concentrations (MICs) of 1.4 µg/mL and 1.3 µg/mL, respectively (Veloz et al., 2019). Multiple studies have confirmed the antimicrobial action of pinocembrin against *S. mutans, S. sobrinus, S. aureus, E. faecalis, L. monocytogenes, P. aeruginosa,* and *Klebsiella pneumoniae* (Soromou et al., 2012; Joray et al., 2015; Tasco et al., 2018; Kharsany et al., 2019).

1.2. The antifungal activity of propolis

Ethanolic propolis extract has demonstrated significant antifungal activity, exhibiting fungistatic properties in both dose- and time-dependent manners. The peak inhibitory effect was observed at 12 hours, effectively halting and disrupting the filamentation process of *Candida albicans*. Additionally, the extract caused structural damage to the fungal cell membrane and wall, leading to leakage of intracellular contents. Importantly, no mutagenic effects were observed in the tested propolis extract (Corrêa et al., 2020). The antifungal efficacy of propolis is influenced by its chemical composition, which varies according to its botanical and geographical origin. Numerous studies have confirmed the antifungal properties of propolis samples from different regions, particularly against clinically relevant fungal pathogens.

Algerian ethanolic propolis extracts have shown potent antifungal activity, especially against phytopathogenic fungi. In vitro assays demonstrated inhibition rates of up to 73% against *Fusarium oxysporum* and 69% against *Botrytis cinerea*, highlighting the extract's potential as a natural antifungal agent (Ouahab et al., 2023). In Argentina, (Quiroga et al., 2006) investigated propolis from the northwest region and reported notable antifungal effects. Their study emphasized the potential for developing cost-effective agrochemicals containing propolis extracts or their active constituents, such as pinocembrin and galangin.

Falcão and his coworkers (2014) evaluated Portuguese propolis and its presumed floral sources *Populus canadensis* and *Cistus ladanifer* against *C. albicans*, *Trichophyton rubrum*, and *Aspergillus fumigatus*. While plant extracts showed limited antifungal activity (except moderate inhibition of *T. rubrum*), both propolis samples exhibited substantial antifungal action, with the highest efficacy against *T. rubrum* and the weakest against *A. fumigatus*. Similarly, ethanolic propolis extracts (PEE) from Poland displayed significant activity against *C. albicans*, *C. glabrata*, and *C. krusei* (Szweda et al., 2015). In another study, various organic extracts of French propolis ethanolic (PEE), aqueous (PWE), methanolic, and dichloromethane were assessed for antifungal efficacy. These extracts showed strong activity against *C. albicans* and *C. glabrata*, but relatively weak effects against *A. fumigatus* (Boisard et al., 2015). Brazilian propolis extracts have also demonstrated broad-spectrum antifungal activity. Ethanol extracts were active against multiple *Candida* species, including *C. albicans*, *C. tropicalis*, *C. krusei*, and *C. guilliermondii*. Among these, *C. albicans* was the most sensitive, while *C. guilliermondii* was the most resistant (Ota et al., 2001). Furthermore, both green and red Brazilian propolis samples showed activity against *Trichophyton* spp., which are responsible for dermatophytosis. Red propolis was found to be more effective than the green variant (Siqueira et al., 2009).

1.3. The Antiviral Activity

The creation of new antiviral medications is vital due to several urgent reasons: the worldwide health impact of viral diseases, including COVID-19, AIDS, Ebola, influenza, dengue, and chikungunya, the scarcity of effective antiviral treatments, and the swift occurrence of viral genetic mutations that lead to drug resistance. Viruses are obligate intracellular pathogens, making their control and treatment particularly challenging. Propolis has attracted increasing interest as a natural substance with antiviral potential. Studies have demonstrated its ability to inhibit viral infections in vitro, with its antiviral activity evaluated using various concentrations of propolis applied to infected cell cultures. The effectiveness of propolis in these studies is assessed through a range of techniques, including polymerase chain reaction (PCR), real-time PCR (RT-PCR), cytotoxicity assays, evaluation of cytopathic effects, and neuraminidase inhibition, among others.

Research into the antiviral properties of propolis began in the 1960s, and the number of studies has steadily increased since then, reflecting growing interest in natural antivirals as complementary or alternative therapeutic options (Salatino, 2022).

1.4. Antiprotozoan Activity

The antiprotozoal potential of propolis is commonly evaluated through in vitro assays that measure its growth-inhibitory effects on cultured protozoan parasites exposed to varying concentrations of propolis. Several studies have documented the efficacy of European propolis against protozoa responsible for significant human and animal diseases, including trichomoniasis, toxoplasmosis, giardiasis, Chagas disease, leishmaniasis, and malaria. It has been reported against *Giardia lamblia*, *Trichomonas vaginalis*, *Toxoplasma gondii*, *Leishmania donovani*, and *Trypanosoma cruzi*. Moreover, ethanolic extracts of propolis (EEP) have shown inhibitory activity against *Giardia duodenalis* (Dantas et al., 2006). These findings underscore the broad-spectrum antiparasitic potential of propolis and support its further investigation as a natural therapeutic agent against protozoan infections (Dantas et al., 2006).

Table 2: Chemical constituents of propolis that possess known antimicrobial activities.

Chemical compound	Activities	References
Artepillin C	Antimicrobial	(Vică et al., 2023)
Chrysin	Antibacterial ; Antifungal ; Antiviral	(Renuka Mani 2017)
Moronic acid	Anti-HSV	(Kurakawa et al., 1999)
Pinocembrin	Antibacterial ; Antifungal	(Rasul et al., 2013)
ρ-Coumaric acid	Antibacterial	(Chen et al., 2024)
Quercetin	Antimicrobial	(Yang et al., 2020)
Volatile constituents (phenols,esters, terpenoids)	Antibacterial	(Bancova et al., 2014)
2,2-Dimethyl-6-carboxyethyl-2H-1-benzopyran	Antimicrobial	(Toreti et al., 2013)

1.5. Other biological properties

Propolis exhibits a wide range of therapeutic properties, supported by numerous scientific studies. Its strong antioxidant and anti-inflammatory activities, mainly due to the

presence of flavonoids and phenolic compounds, contribute significantly to its immunomodulatory effects by enhancing cytokine production and increasing the expression of immune receptors such as TLR-2 and TLR-4 (ORS et al., 2006; Custa et al., 2005; Sayed et al., 2009; Fischer et al., 2007; Orsatti et al., 2010). These bioactivities make propolis a promising agent in managing neurological disorders like Alzheimer's and Parkinson's diseases, where oxidative stress and inflammation play key roles, thanks to its ability to cross the blood-brain barrier (Nadzirah Zullkiflee et al., 2022). In the gastrointestinal tract**,** propolis has been used to treat conditions such as gastritis, ulcers, mucositis, and colitis due to its bioactive, therapeutic components (Jones et al., 2009). It also shows potential in addressing type 2 diabetes mellitus (T2DM) by reducing insulin resistance, blood glucose levels, and enhancing insulin secretion, particularly through flavonoids like naringin, apigenin, kaempferol, and quercetin (Nadzirah Zullkiflee et al., 2022). In asthma**,** propolis demonstrates anti-allergic, anti-inflammatory, and anti-asthmatic properties by inhibiting the activation of mast cells and basophils (Nakamura et al., 2010). Additionally, in dentistry, it promotes wound healing, supports pulp vitality, and stimulates dentin regeneration (Parolia et al., 2010). In the food industry, propolis is increasingly favored as a natural preservative due to its antimicrobial and antioxidant activities, offering safer alternatives to synthetic preservatives (Sarapa et al., 2025). Lastly, in cosmetics, its inhibition of collagenase activity and ability to protect against UV-induced damage support its use as a natural sunscreen and anti-aging agent (Silva et al., 2020).

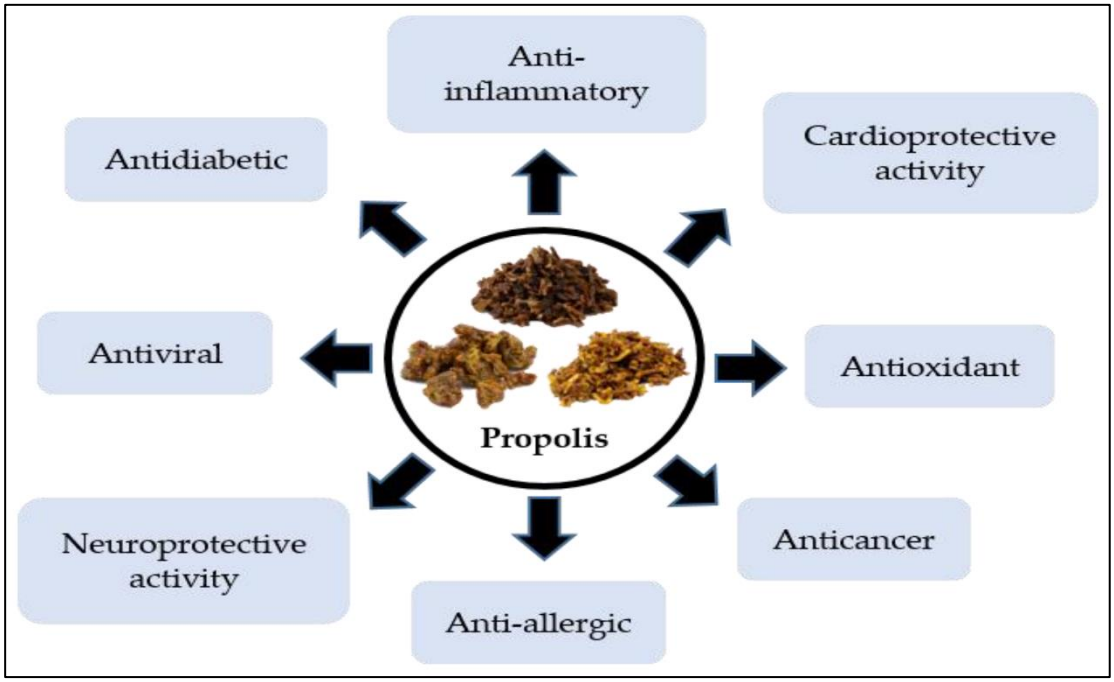

Figure 7: Biological and therapeutic properties of propolis (Nadzirah zullkefliee et al., 2022).

2. The mechanism of action of propolis

Despite its broad therapeutic potential, the clinical application of propolis remains limited due to several factors, including its pungent odor, poor water solubility, instability under physical and chemical conditions, and notably, its low bioavailability. To overcome these limitations, particularly the issue of poor bioavailability, researchers have increasingly employed nanoencapsulation technologies to formulate advanced drug delivery systems. This approach enables the encapsulation of propolis and its bioactive constituents within nanocarriers, offering multiple advantages: protection of active compounds from degradation in the gastrointestinal tract, enhanced stability against light and heat, improved aqueous solubility and bioavailability, as well as controlled release and targeted delivery of the active ingredients (M.Y. Liu et al., 2025).

Propolis has also been shown to impact bacterial cell membrane permeability, potentially disrupting essential membrane functions. This includes a reduction in adenosine triphosphate (ATP) production, which impairs bacterial motility and other energy-dependent processes (Almuhayawi, 2020). One of its major bioactive components, cinnamic acid, exerts antimicrobial effects by inhibiting ATPase activity, interfering with bacterial cell division, and suppressing biofilm formation. These effects result from direct damage to the bacterial cell membrane, which also leads to intracellular pH imbalance and metabolic stress (Almuhayawi, 2020). Additionally, cinnamic acid and its derivatives exhibit anti-quorum sensing activity, further inhibiting bacterial communication and virulence (Przybyłek and Karpiński, 2019). While the exact mechanisms of propolis's biological actions are still not fully elucidated, it is widely accepted that its flavonoid components play a central role. These compounds interact with various bacterial targets, disrupting critical cellular functions. Specifically, the B-ring of flavonoids has been suggested to inhibit the synthesis of nucleic acids, thereby affecting bacterial replication and viability (Olegário et al., 2019).

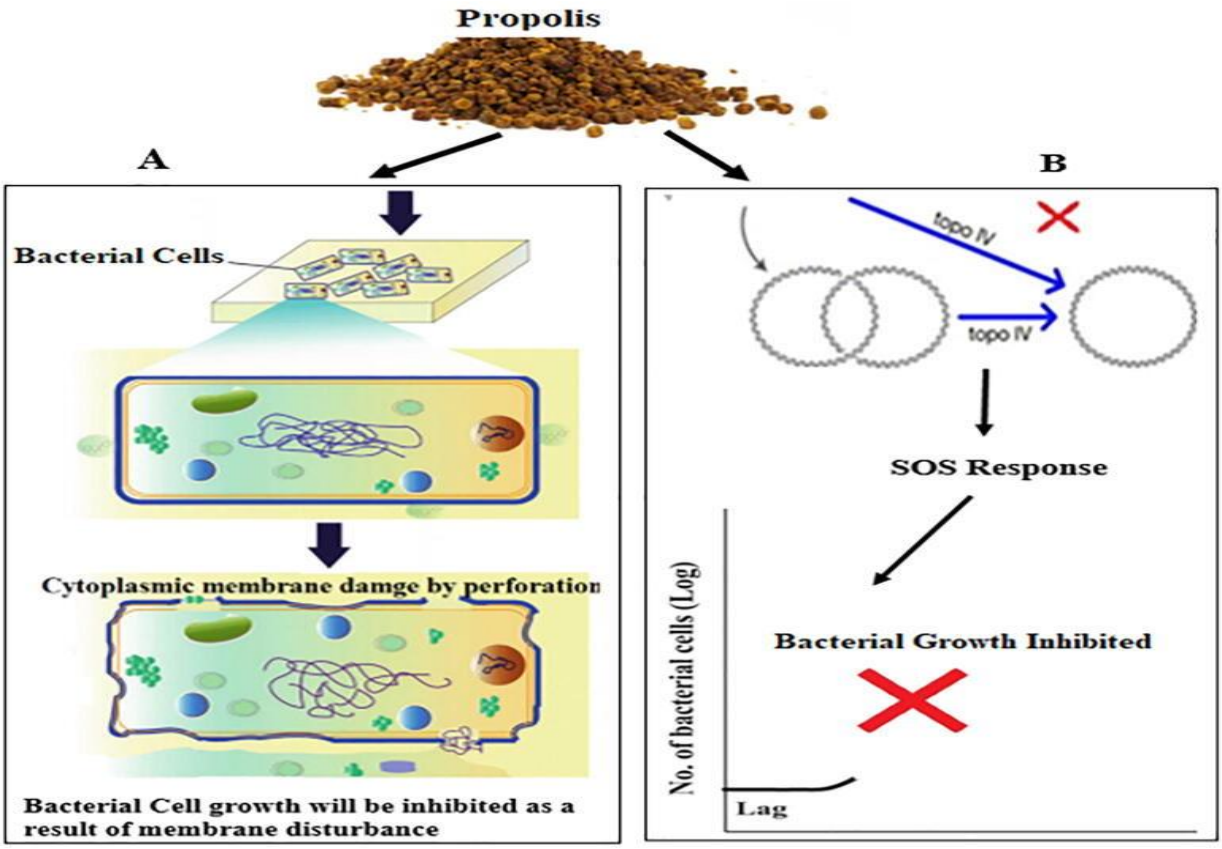

Figure 8: Mechanism action of propolis as anti-bacterial agent.

(A) The active components of propolis attached on the cytoplasmic membrane of bacterial cell and then the structural integrity has been damaged leading to perforation of the membrane where the cytoplasmic content expelled outside and leading to cell death (B) flavonoids may result in the inhibition of topoisomerase IV-dependent deactivation activity and lead to (associated cellular response and is known as SOS 'save our ship' or 'save our souls') growth inhibition of bacterial cells (Almuhayawi, 2020).

3. The toxic effects and allergic reactions caused by propolis

While propolis contains various potentially harmful components, many studies have found that, at commonly used concentrations, it does not cause significant toxicity in tissues or organs. The literature reviewed indicates no major toxic effects, suggesting that propolis is generally well-accepted (Mendonça et al., 2013). However, it is crucial to remember that propolis is a recognized sensitizer and can trigger allergic reactions in people with hypersensitivity. Consequently, it is not recommended for individuals with a history of allergies or predispositions to allergies. Among the constituents that could be toxic in propolis are benzyl benzoate, which may excessively stimulate the central nervous system, resulting in symptoms like dizziness and seizures, and benzoic acid, which can react with vitamin C to produce benzene, a carcinogen linked to DNA damage and chromosomal abnormalities.

Another component, phenol, has been associated with toxic impacts on the heart, liver, kidneys, and lungs when exposure occurs at high levels or over long durations. These toxic compounds are mainly metabolized in the liver, and both acute and chronic exposure can disrupt key metabolic processes, including the management of carbohydrates, lipids, and proteins, as well as enzymatic detoxification and hormonal stability. Despite the inclusion of these compounds, only a few studies have documented toxic effects, implying that the overall toxicity of propolis is low across various geographic sources and collection periods. For example, research on the safety of ethanol extract of Brazilian propolis from Minas Gerais, using Wistar and Catasbyana rat models, showed no significant changes in hematological or biochemical parameters. Histological assessments did not reveal signs of cellular injury, inflammation, hemorrhage, or tissue infiltration in vital organs such as the stomach, lungs, spleen, esophagus, and heart. Moreover, epithelial tissues from the kidneys, liver, and intestines displayed no statistically significant alterations, and the intestinal lining remained intact. Clinical investigations have further substantiated the relative safety of propolis in topical applications. In a study assessing the efficacy and tolerability of a propolis-infused lip balm at concentrations of 0.1%, 0.5%, and 1.0% for treating herpes labialis lesions, all formulations proved effective. Nevertheless, the 1.0% concentration resulted in mild local irritation in some participants, which hindered the healing process of the lesions. Conversely, the 0.5% formulation was better tolerated and did not lead to any adverse local responses, indicating that the potential toxicity of propolis is influenced by dosage (Jautova et al., 2018).

Part Two

Experimental study

Chapter I

Materials and Methods

1. Objectives of the study

The aime of this study is to determine the minimum concentration (MIC) of Algerian propolis extract on three bacterial strains, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, evaluate the impact of the extraction method on the efficacy of the propolis extracts, and compare the effectiveness of raw propolis with that of commercial propolis.

2. Materials

2.1. Chemical components

- Mueller-Hinton Broth (MHB)
- Mueller-Hinton Agar (MHA)
- Distilled water
- Ethanol
- Antibiotic (Cefoxitin 30 µg, Gentamicine 10 µg, Peniciline 10 µg)

2.2. Equipments

- Spectrophotometer
- pHmeter
- Incubator
- Autoclave
- Vortex mixer
- Electric grinder
- Balance

2.3. Tools

- Bishr
- Micropipette
- Petri dish
- Sterile bottle of 100 mL
- Sterile culture tubes

- Sterile Eppendorf tube
- Sterile flasks
- Sterile loop or swab for inoculation
- Sterile pipettes
- Sterile test tubes
- Whatman paper

2.3 Bacterial strain

The three bacterial strains provided by the laboratory of Microbiology at Ibn Zohr Hospital in Guelma. The reference strains are *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923. The overnight cultures incubated in a growth media (GM) for 24 h at 37 °C.

2.4 Culture medium

Mueller-Hinton Broth (MHB) is commonly used for antimicrobial susceptibility testing due to its homogeneous composition, supporting the growth of a wide range of non-fastidious microorganisms. To prepare MHB, add 27 g of dehydrated MHB powder (Biokar) to one liter of distilled water. A magnetic stirrer was then used to ensure complete dissolution. The solution was autoclaved at 121 °C for 15 minutes. Allow the medium to cool to room temperature before use.

3. The samples of propolis and the extraxction method

Two samples of propolis were used. The raw propolis sample was collected from Guelma's mountains of Maouna (Figure 8) in January of 2025, and the other sample was purchased (Setif, Algeria). They were stored at 4 °C in a dark before use.

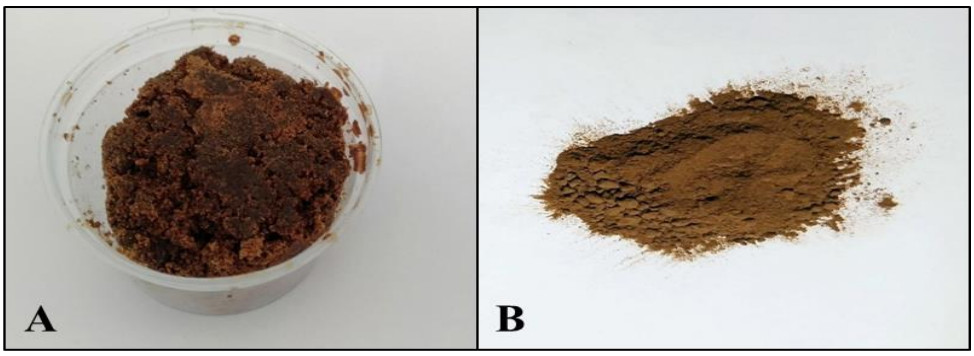

Figure 9: Propolis samples, (a) raw propolis (Guelma), (b) a sample of propolis purchased from (Setif).

Figure 10: The location of the city of Guelma in Algeria.

3.1. Hot extraction method

The samples were ground to a fine powder, and then 5 g of propolis were extracted in 100 mL of ethanol 70% (v/v) in a water bath at 70°C for 30 min. The ethanolic extracts were sterilized through a 0.22 µm pore size filter (Figure 10) to obtain ethanolic extract of commercial propolis (EEPC1) and ethanolic extract of raw propolis (EEPR1). It was stored at 4°C in dark (Alencar et al., 2007).

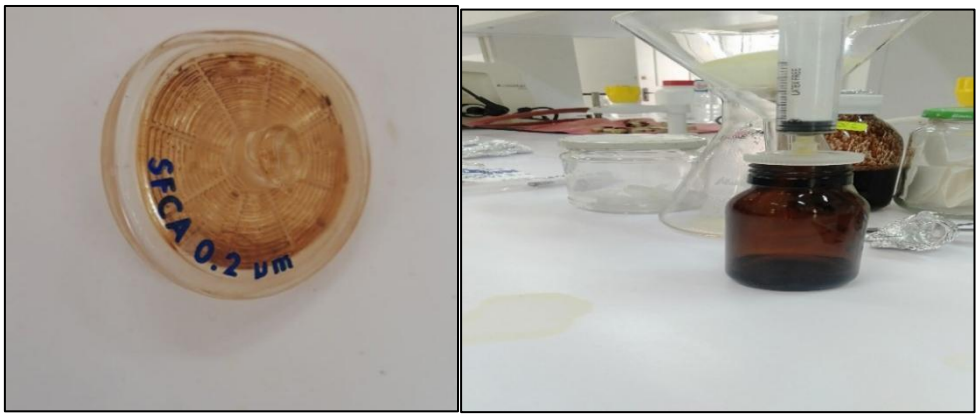

Figure 11: Microfilter 0.2, and filtration process.

3.2. Cold extraction method

The samples were ground to a fine powder, then 5 g of propolis were extracted in 100 mL of 70% (v/v) ethanol. The mixtures were stored in dark at room tempreture for 15 days. The ethanolic extracts were sterilized through a 0.22 µm pore size to obtain EEPC2 and EEPR2. It was stored at 4 °C in dark (Alencar et al., 2007).

4. Spectrophotometric analysis of UV-visible spectra

To distinguish the type of propolis according to the total flavonoid content and scavenging activity (Mărghitaş et al., 2013). The ethanolic extracts of propolis were diluted 10-time in ethanol, and the mixtures were scanned to obtain the absorption spectra at wavelengths between 200 and 500 nm by UV-Vis spectrophotometer (Figure 11) , calibrated for the 70% ethanol solvent.

Figure 12: The spectrophotometer for measuring the UV-vis absorption spectra of the ethanolic extract of propolis (EEP).

5. Antibacterial activity

5.1. The agar diffusion method

To assess the antimicrobial activity of the propolis extract against *S. aureus*, *E. coli*, and *P. aeruginosa*, the disk diffusion method was condected on Mueller Hinton agar (MHA) according to Clinical and Laboratory Standards Institute (CLSI, 2016). Firstfull, a small quantity of the bacterial strains was inoculated into Mueller-Hinton broth and incubated at 37°C for 16 to 18 h. Then, the Mueller-Hinton Agar plates (Figure12) were inoculated with the bacterial suspension, previously diluted and adjusted to a 0.5 McFarland standard (approximately 1×10^8 CFU/mL), using a sterile swab. A sterile paper discs were deeped in the propolis extract for 5 to 10 seconds, and any excess liquid was removed. The discs were then placed on the inoculated agar plates using sterile forceps, ensuring proper spacing. Additionaly, the antibiotic discs were included as positive controls, and ethanol-only discs were used as negative controls. The plates were incubated at 37°C for 24 h. Finaly, the diameters of the inhibition zones surrounding each disc were measured to assess antimicrobial

activity. The results were categorized as sensitive (>18 mm), intermediate (15–18 mm), or resistant (<15 mm).

Figure 13: Mueller-Hinton Agar plate.

5.2. Macrodilution method

The bacterial strains (*S. aureus*, *E. coli*, and *P. aeruginosa*) were tested for their susceptibility to EEP according to CLSI guidelines (2016). Briefly, bacterial suspensions were adjusted to a final concentration of 10^6 colony-forming units (CFU)/mL in Mueller-Hinton Broth (MHB). The suspensions were then mixed in a 1:1 ratio with two-fold serial dilutions of EEP in a final volume of 8 mL, resulting in final EEP concentrations ranging from 3.125 to 25 mg/mL (Figure13). A treatment of the cell suspension with 70% (v/v) ethanol served as the negative control (growth control). Cultures were incubated at 37°C for 24 h, and bacterial growth was assessed by measuring absorbance at 600 nm using a spectrophotometer. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of EEP that resulted in at least 80% inhibition of bacterial growth.

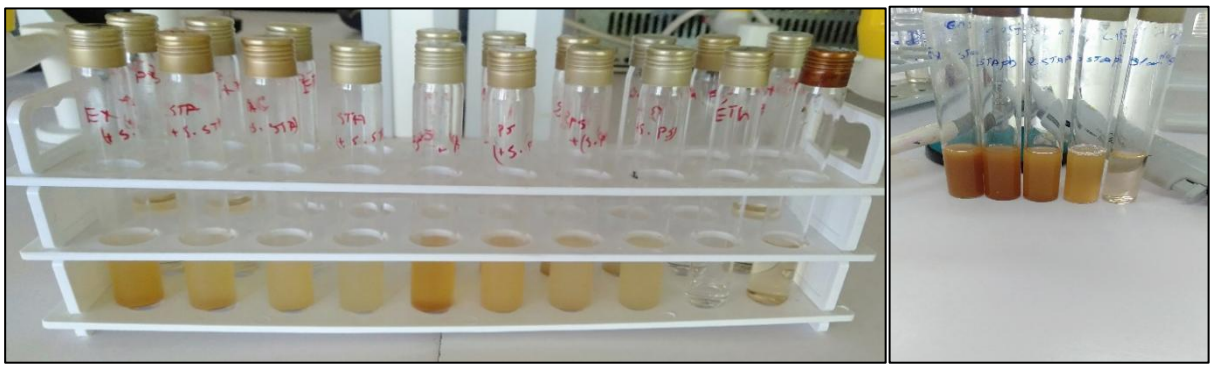

Figure 14: The serial dilution of EEP in MHB.

6. Data analysis

The antibacterial activity assays (Agar diffusion and macrodilution) were conducted in triplicate. The corresponding curves and graphs were plotted using Microsoft Excel 2016.

Chapter II

Results and Discussion

1. Results

1.1. Description of propolis extracts

1.1.1. The propolis extracts color

It is known that the color of propolis varies from one sample to another. The propolis sample from the Guelma Mountains (EEPR) is lighter in color than the propolis sample sold in Setif (EEPC). We also recorded the effect of the extraction method on the color degree. The two samples extracted using the first method, using heat (EEPR1 and EEPC1), were slightly lighter in color than the two samples extracted at room temperature (EEPR2 and EEPC2). We conclude that the extraction method affects the solubility of propolis in the solvent and, consequently, the color of the ethanolic propolis extract.

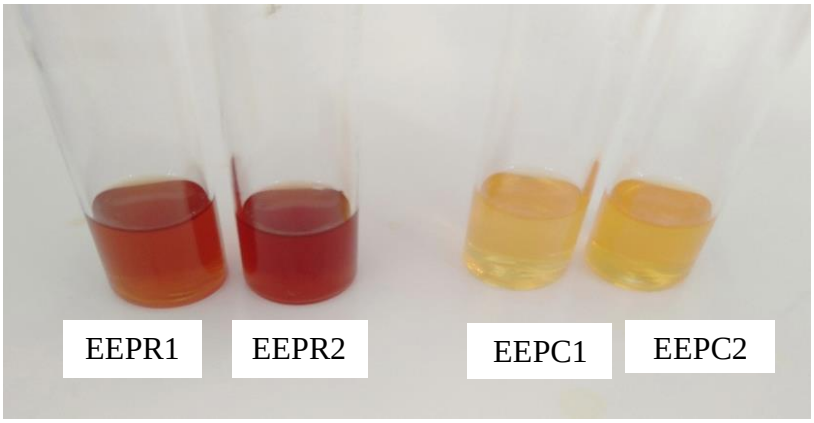

Figure 25: Different colors of two propolis samples.

(EEP: ethanolic extract of propolis, R: raw, C: commercial) extracted by the two methods, 1: hot extraction method, 2: cold extraction method.

1.1.2. Determine the pH of propolis extracts

The pH values of the four ethanolic propolis extracts were determined using pH meter. The EEPC1 and EEPC2 showed a pH value of 6.0±0.2. However, EEPR1 and EEPR2 showed a pH of 5.0±0.2. Regarding acidity, the ethanolic extracts of the commercial propolis samples (EEPC1 and EEPC2) exhibited lower acidity compared to the ethanolic extracts from raw propolis (EEPR1 and EEPR2). Furthermore, the extraction method used did not significantly influence the acidity of the final extracts, as both samples prepared by different methods (1

and 2) within the same propolis type (commercial or raw) displayed only minor variations in pH.

1.1.3. Determine the solubility of each extract

After filtering the ethanolic extracts and drying the ground propolis, the weight of the recovered precipitate was recorded as follows: EEPR1 = 2.9 g, EEPR2 = 3.7 g, EEPC1 = 2.6 g, and EEPC2 = 3.6 g. These values are based on an initial amount of 5 g of propolis dissolved in 100 mL of ethanol. The results suggest that the extraction method influenced the solubility of propolis. In particular, the samples incubated at room temperature for 15 days showed better solubility, with less undissolved material, compared to those extracted at high temperature for 30 min.

1.2. Spectrophotometric analysis of UV-visible spectra

The UV-Vis absorption spectra of Algerian propolis samples showed slight variations between both commercial and raw samples, as well as differences stemming from extraction methods. Raw propolis extracted at 70 °C for 30 minutes (EEPR1) exhibited the highest absorbance, particularly within the 225–250 nm and 300–350 nm ranges, indicating a richer presence of phenolic compounds and flavonoids compared to commercial samples. On the other hand, extraction at room temperature (EEPC2, EEPR2) yielded lower absorbance across most wavelengths. Interestingly, commercial extracts (EEPC1 and EEPC2) displayed slightly higher absorbance at 500 nm. The peaks observed in EEPR1 at 300 and 350 nm, associated with flavonoids, were more pronounced than in the other extracts.

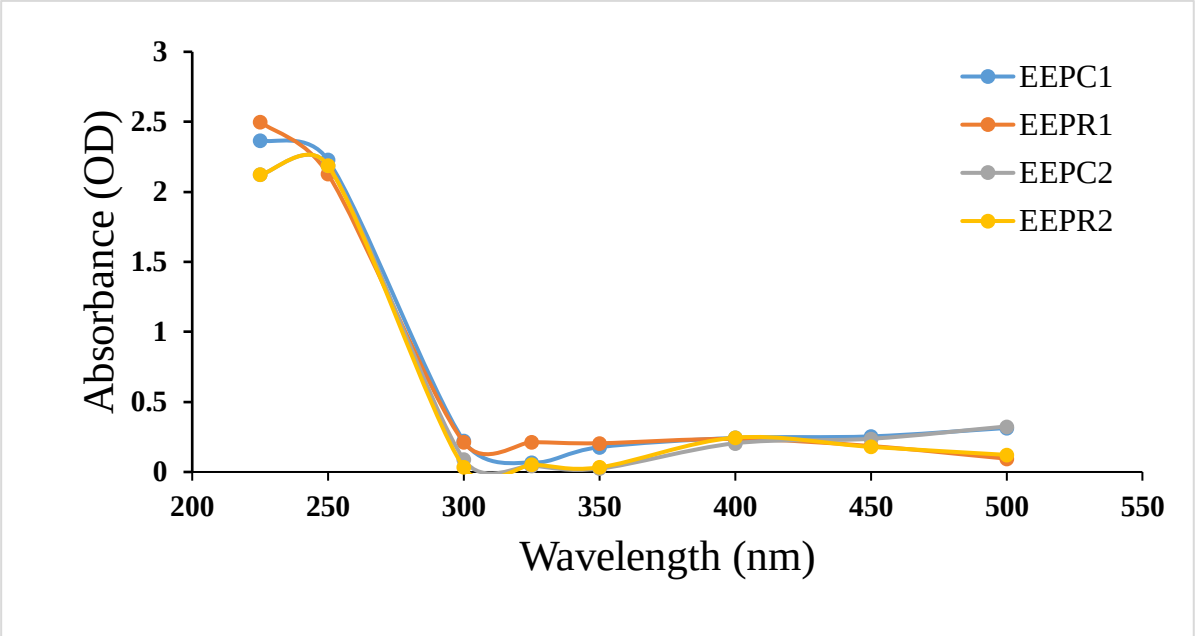

Figure 36 : UV-Vis absorption spectra of the ethanolic extract of Algerian propolis (EEP**)** .

1.3. Antimicrobial activity

1.3.1. Agar diffusion

Agar diffusion test results showed that EEP bacteria exhibited varying antibacterial activity depending on the bacterial strain and extraction method. Crude propolis extracts (EEPR1 and EEPR2) showed significant activity against *S. aureus* (inhibition zones of 14 mm and 12 mm, respectively), while commercial extracts (EEPC1 and EEPC2) showed weaker activity (8 mm and 10 mm, respectively). EEPR1 and EEPR2 showed only slight inhibition against *E. coli* (10 mm and 9 mm), while no inhibition zones were observed for commercial. *P. aeruginosa* was the most resistant strain, with only a weak inhibition zone (8 mm) observed for EEPR1. In comparison, standard antibiotics showed selective activity: penicillin showed activity only against *E. coli* (14 mm), gentamicin was effective against *P. aeruginosa* (18 mm), and cefoxitin showed significant inhibition against *S. aureus* (20 mm). These results indicate that crude propolis, especially when extracted by the cold method, retains greater antibacterial activity than commercial propolis, with *S. aureus* being the most susceptible strain among the tested.

Table 3: Antibacterial activity of ethanolic propolis extracts and standard antibiotics against selected bacterial strains (Agar diffusion method).

Bacterial strains	**EEP R2**	**EEP R1**	**EEP C2**	**EEP C1**	**Ethanol 70%**	**Penicilin 10ug**	**Gentamicin 10 ug**	**Cefoxitin 30 ug**
Escherichia coli	10 mm	9 mm	-	-	-	14 mm	-	*
Pseudomonas aeruginosa	8 mm	-	-	-	-	*	18 mm	-
Staphylococcus aureus	14 mm	12 mm	10 mm	8 mm	-	-	20 mm	-

(-) Absence of inhibition zone, EEP : Ethanolic extraction of propolis, R : Raw propolis, C : commercial propolis,1 : Hot extraction method, 2 : Cold extraction method , *:Not tried.

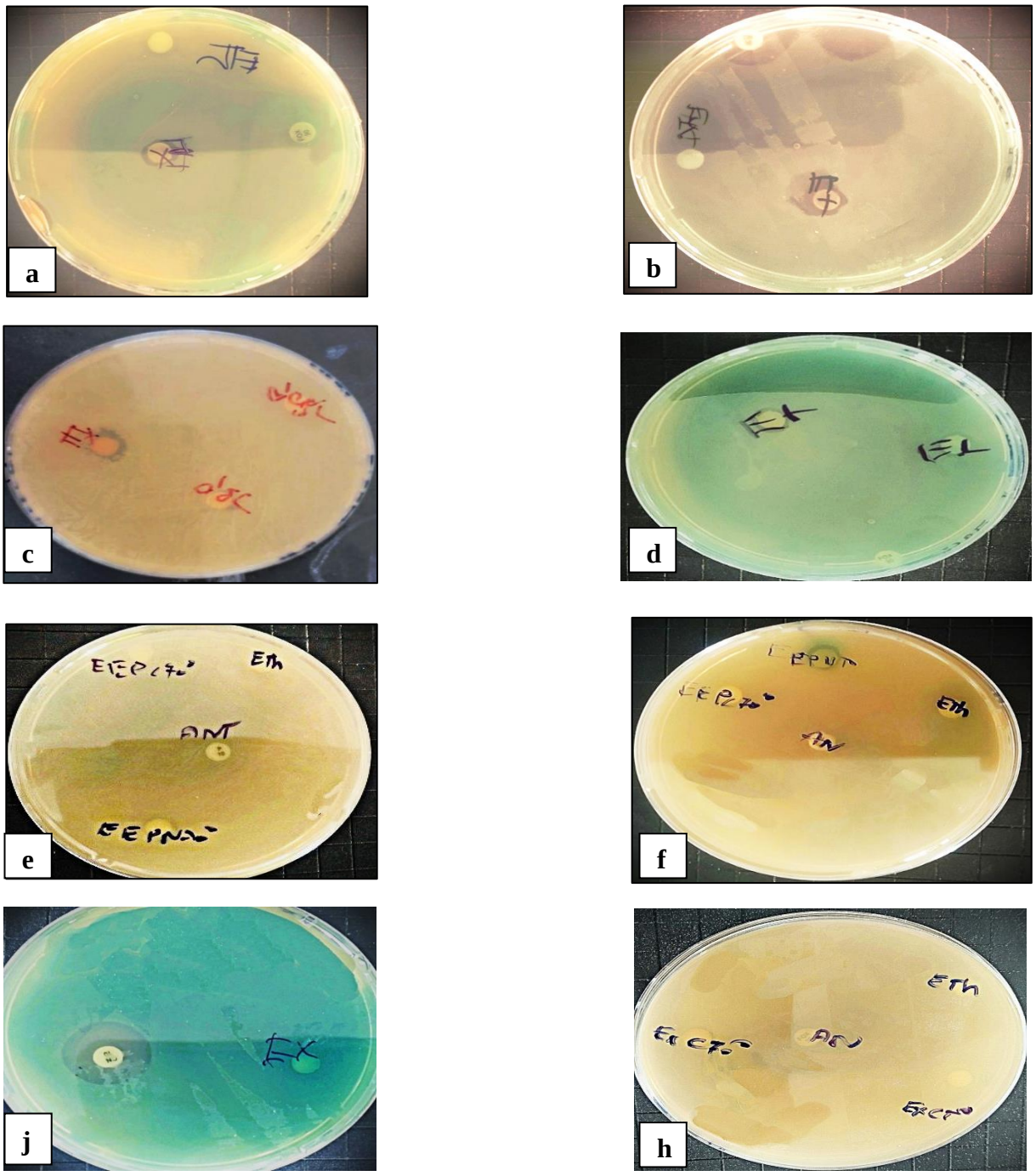

Figure 17 : Zones of inhibtion (Agar diffusion method) provided by : **a/** (EEPR2, Ethanol, Cefoxin) against *Escherichia coli* ATCC 25922 ; **b/** (EEPR2, Gentamicine , Ethanol) against *Staphylococcus aureus* ATCC 25923; **c /** (EEPR1 of different concentration) against *Escherichia coli* ATCC 25922, **d/** (EEPR2 , cefoxin , Ethanol) against *Pseudomonas aeruginosa* ATCC 27853, **e /** (EEPR1 , EEPC1 , penicilin , Ethanol) aganist *Staphylococcus aureus* ATCC 25923, **f** /(EEPC1, EEPC2, cefoxin ,Ethanol) against *Staphylococcus aureus* ATCC 25923, **h/** (EEPR1, Gentamicin) against *Pseudomonas aeruginosa* ATCC 27853, **g/**

(EEPC1, EEPC2,penicilin) against *Escherichia coli* ATCC 25922 . *Escherichia coli* ATCC 25922, **d/** (EEPR2 , cefoxin , Ethanol) against *Pseudomonas aeruginosa* ATCC 27853, **e /** (EEPR1 , EEPC1 , penicilin , Ethanol) aganist *Staphylococcus aureus* ATCC 25923, **f** /(EEPC1, EEPC2, cefoxin ,Ethanol) against *Staphylococcus aureus* ATCC 25923, **h/** (EEPR1, Gentamicin) against *Pseudomonas aeruginosa* ATCC 27853, **g/** (EEPC1, EEPC2,penicilin) against *Escherichia coli* ATCC 25922 .

1.3.2. Broth macrodilution susceptibility

Each of the four propolis extracts showed a minimum inhibitory concentration (MIC_{80}) of 80% effective against bacterial growth, at 25 mg/mL against *S. aureus* (Table 4). In contrast, *E. coli* and *P. aeruginosa* showed significantly lower susceptibility to the same extracts, indicating a higher level of resistance. Notably, all propolis extracts appeared to promote rather than inhibit bacterial growth at lower concentrations, specifically between 1.562 and 12.5 mg/mL. In contrast, EEPR propolis extracts were more effective than EEPC propolis extracts, indicating a clear concentration-dependent inhibitory effect against *S. aureus*.

Table 4: The survival rate of *S. aureus* at 25 mg/mL of EEP.

EEP	EEPC1 (25 mg/mL)	EEPC2 (25 mg/mL)	EEPR1 (25 mg/mL)	EEPR2 (25 mg/mL)
Survival rate (%)	19,2	16,3	15,0	10,3

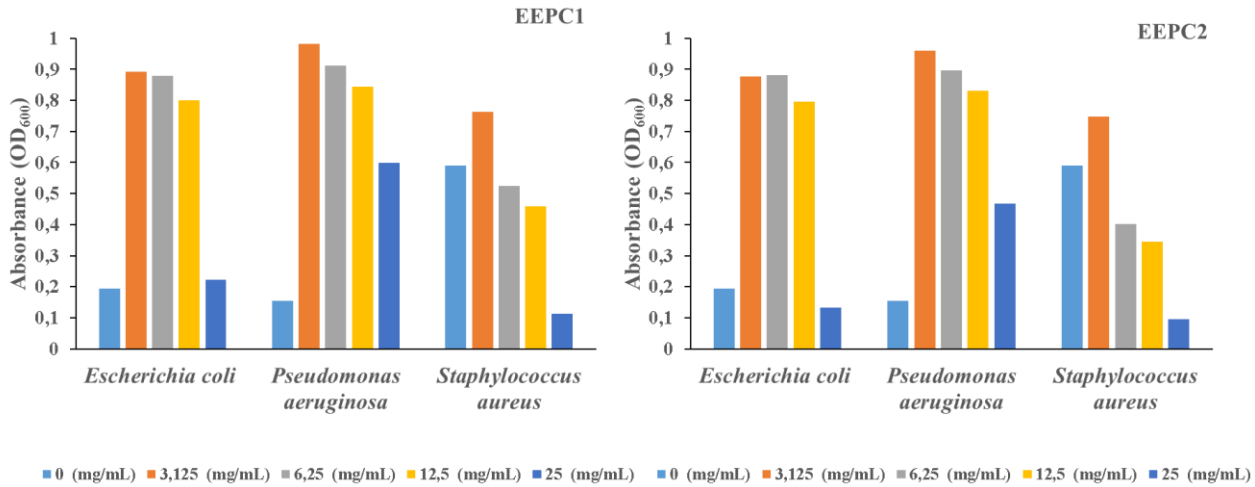

Figure 18: The effect of propolis extract (EEPC1 and EEPC2) at different concentrations against *S. aureus*, *E. coli*, and *P. aeruginosa.*

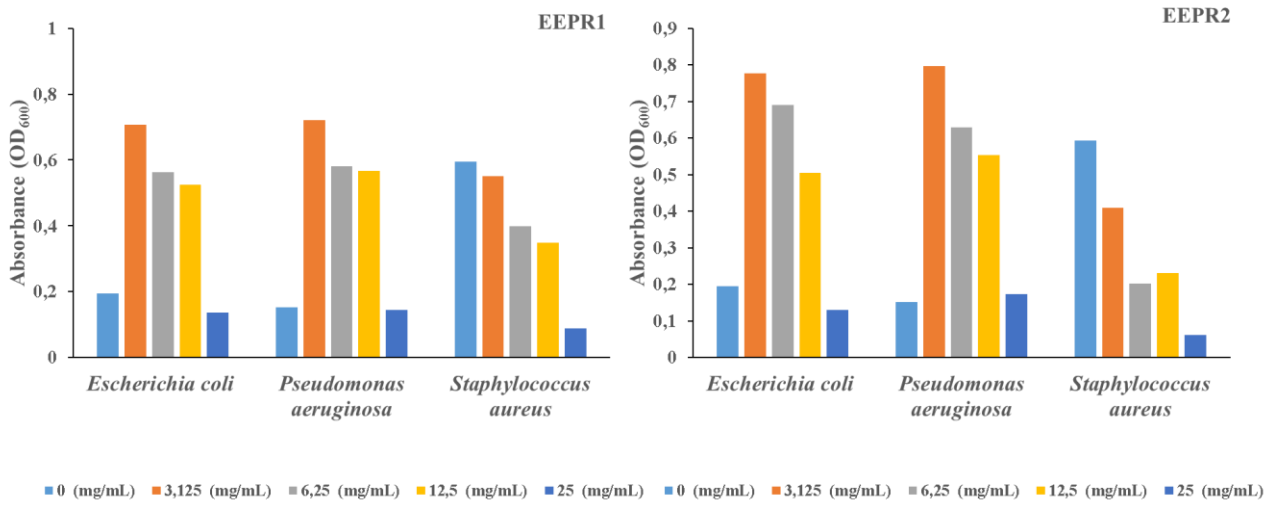

Figure 19: The effect of propolis extract (EEPR1 and EEPR2) at different concentrations against *S. aureus*, *E. coli*, and *P. aeruginosa.*

2. Discussion

2.1. UV-visible spectra

Flavonoids can be considered pigments that strongly absorb ultraviolet light, and therefore, UV-Vis spectroscopy represents the primary method for structural investigation of flavonoids. Flavones and flavonols mostly have two main absorption bands (peaks) in the UV-Vis region. Highly oxygenated flavones and flavonols tend to absorb toward longer wavelengths, shifting the spectrum toward the infrared. Glycosylation or methylation of hydroxyl groups in flavonoids typically results in a chromium-deficient shift in the first band (Zaghad, 2009). Comparing our flavonoid analysis results using UV-Vis spectroscopy with those of Zaghad (2009), our propolis contains the following components: flavones, flavanols, and isovanes. Seguine (2011) analyzed four samples of eastern Algerian propolis using ethanol spectroscopy and found two absorption peaks, one in the second band between λ max= 200 and λ max= 250 nm and the other in the first band between λ max=300 and λ max=350 nm, which correspond to the flavonoid family.

Our results are consistent with this work, showing that the two propolis samples tested using ethanol spectroscopy were rich in flavonoids. The results of the UV-visible spectral analysis of the two propolis EEPRR2 and EEPR1 show a single absorption peak, which corresponds to band I (between λ max = 225nm and λ max =250nm).

2.3. Antimicrobial activity

The zones of inhibition of any good antimicrobial agent vary from one author to another. According to (Pereira et al., 2006), the zone of inhibition should be equal to or greater than 10 mm, according to (Vieira et al., 2001), it is 13 mm, and according to (Kim et al., 2006), it is greater than 6 mm. In all cases, components of propolis extracts can be good antimicrobial agents. The use of EPE in different proportions of ethanol by the disc method shows that the diameter of the zone of inhibition of propolis depends on its solubility in the solvent and, consequently, on its diffusion in the medium. This phenomenon was reported in the work of (Tabera et al., 2000). The results obtained are consistent with those of (Bonvehì and Gutiérrez 2012), who observed inhibition zones of 10 to 16 mm for *S. aureus*.

For Algerian propolis, using ethanolic extracts of it, they showed high antibacterial activity against Gram-positive bacteria, *Staphylococcus aureus* (13.5 mm, inhibition zones), but no effect against Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) (Bouzahouane et al., 2021).

A diffusion assay on agar plates showed that ethanolic extracts of natural and commercial Algerian propolis had varying antibacterial activity depending on the extraction method and bacterial strain. Natural propolis extracted at room temperature (EEPR2) showed the strongest effect, exhibiting a 14 mm inhibition zone against S. aureus, indicating moderate antimicrobial activity according to CLSI standards. All extracts showed weak activity against E. coli and Pseudomonas aeruginosa (8-10), reflecting their known resistance mechanisms. The high activity of EEPR2 may be attributed to the preservation of heat-sensitive bioactive compounds, such as flavonoids and phenolics, which are likely to be degraded at the high temperatures used in other preparations. This is consistent with the UV-vis results, where thermally extracted EEPR1 showed higher absorption but lacked antimicrobial activity against S. aureus, indicating that total phenolic content does not always predict bioactivity. These results highlight the importance of extraction conditions and propolis source on

antimicrobial efficacy, consistent with previous studies (Popova et al., 2007; Bankova et al., 2016; CLSI, 2023).

The effectiveness of propolis extracts (EEP) was best compared based on the geographical origin of *S. aureus* as a representative of Gram-positive bacteria and *E. coli* as a representative of Gram-negative strains. For *S. aureus*, the highest EEP activity was observed in Turkey, Taiwan, and Oman, with MIC values of 8, 10 and 81 µg/mL, respectively. The lowest activity was observed in propolis samples from Chile, Australia, and Germany. The MIC values for EEP were 1445, 1200, and 750 µg/mL, respectively. Against *E. coli*, ethanol extracts of propolis from Turkey, Oman, and Slovakia were the most active, with minimum inhibitory concentration (MIC) values of 116, 302, and 510 µg/ml, respectively. Finally, propolis samples from Germany, Korea, and Ireland were the least active, with MIC values ranging from 1,200 to 5,000 µg/ml, While the Italian propolis extract had no effect on *E. coli*.

Despite having the most propolis research, Brazil is midway between Gram-positive and Gram-negative bacteria. Propolis samples from Turkey and Oman were the most active in both cases (Przybyłek et al., 2019).

While the results of the ethanolic extract of Algerian propolis showed the following results: The most sensitive strain in the EAP was *S. aureus*, with the minimum inhibitory concentration (MIC) ranging from 2.5 to 4.30 mg/mL, while the most resistant pathogenic bacteria in all EAP was *E. coli*, with the minimum inhibitory concentration (MIC) ranging from 5.20 to 7.95 mg/mL, according to the propolis extract, and these are results close to our results (Boufadli et al., 2016).

As we observed, each of the four propolis extracts in our experiment showed a minimum inhibitory concentration (MIC_{80}) of 80% against bacterial growth, at a concentration of 25 mg/ml against S. aureus. In contrast, *Escherichia coli* and *Pseudomonas aeruginosa* showed significantly lower susceptibility to the same extracts, indicating a higher level of resistance. Notably, all propolis extracts appeared to promote rather than inhibit bacterial growth at lower concentrations, specifically between 3.125 and 12.5 mg/mL. In contrast, EEPR propolis extracts were more effective than EEPC propolis extracts, indicating a clear concentration-dependent inhibitory effect against *Staphylococcus aureus*.

These results are consistent with the prevailing notion that propolis extracts are generally more effective against Gram-positive bacteria than Gram-negative bacteria (Bankova and Sorcin, 2011). The outer membrane of Gram-negative bacteria likely acts as a permeability barrier, reducing the effectiveness of the bioactive compounds present in propolis (Gorniak et al., 2019). Furthermore, some phenolic components in propolis may act as signaling molecules or metabolic substrates, influencing bacterial growth and biofilm formation (Kakaniova et al., 2020).

However, studies on Algerian propolis must be increased, especially to determine the optimal concentration for the human body, as studies on propolis are limited in Algeria.

Conclusion

Conclusion

Propolis is a natural substance known for its diverse biological properties, including antimicrobial effects and potential medicinal uses. The bioactive compounds present in propolis can vary significantly due to different factors, such as the geographic location, bee species, and the seasons of collection. In the present study, the ethanolic extract of propolis collected from Guelma showed the lowest activity against both Gram-negative bacteria. Moreover, it was found that raw extracts of propolis were more effective than the commercial sample, particularly those that were macerated at room temperature for fifteen days. The antimicrobial activity of the propolis may be attributed to individual compounds or the synergistic effects of multiple ingredients. Hence, the method of extraction can affect the composition and biological activity of the bioactive components present in propolis. Choosing a suitable extraction method is important to replicate the efficacy. In addition, low concentrations of propolis extracts did not inhibit the growth of bacteria, but might even stimulate it. Further research should be conducted on the possible mechanisms of propolis activity on different microorganisms.

Abstracts

Abstract

Propolis is a resinous substance collected by honeybees from plant buds. It is used in the hive to strengthen the structure, seal entrances, and serve as an antiseptic agent.

Due to its therapeutic properties, this study highlighted the antibacterial activity of two samples of Algerian propolis: one harvested from the mountains of Guelma (Maouna) in northeastern Algeria and the other purchased from a pharmacy (produced in Setif city).

Two extraction methods were conducted; one using maceration at 70°C and the other at room temperature, to determine the optimal extraction method that preserves the antibacterial active substances in the ethanolic extract of propolis. Therefore, the propolis was dissolved in 70% ethanol at a ratio of 1:20 (m/v) to find the lowest concentration of ethanolic propolis extract with potential antibacterial activity. The samples were tested to determine their antimicrobial properties against three bacterial strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923, using the agar diffusion method and the macrodilution method.

As a result, the ethanolic extract of raw propolis extracted at room temperature (EEPR2) was more effective, especially against *Staphylococcus aureus*, where an inhibition zone of 14 mm was recorded and a MIC value of 25 mg/mL was measured.

Further evaluation is required to confirm the efficacy of Algerian propolis as an antimicrobial agent for the development of new phytopharmaceutical products that can replace antibiotics.

Keywords: Propolis, Antimicrobial Activity, Bee product, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, Minimum inhibitory concentration (MIC).

ملخص

البروبوليس مادة راتنجية تجمعها نحل العسل من براعم النباتات. تُستخدم في الخلية لتقوية بنيتها، وسد مداخلها و كمادة مطهرة.

ونظرًا لخصائصه العلاجية، سلطت هذه الدراسة الضوء على النشاط المضاد للبكتيريا لعينتين من البروبوليس الجزائري: إحداهما جُمعت من جبال قالمة (ماونة) شمال شرق الجزائر، والأخرى مُشتراة من صيدلية (مُنتجة في مدينة سطيف) وُضعت طريقتان للاستخلاص؛ إحداهما بالنقع عند درجة حرارة 70 درجة مئوية والأخرى في درجة حرارة الغرفة، و ذلك لتحديد طريقة الاستخلاص المثلى التي تحافظ على المواد الفعالة المضادة للبكتيريا في المستخلص الإيثانولي للبروبوليس. ، أُذيب البروبوليس في 70% من الإيثانول بنسبة 1:20 للعثور على أقل تركيز من مستخلص البروبوليس الإيثانولي ذي النشاط المضاد للبكتيريا. تم اختبار العينات لتحديد خصائصها المضادة للميكروبات ضد سلالات ATCC ، الذهبية المكورات العنقودية27853 ATCC ، والزائفة الزنجارية25922 ATCC و الإشريكية القولونية25923 باستخدام طريقتي الانتشار في الأجار والتخفيف الكبير. ونتيجة لذلك، كان المستخلص الإيثانولي للبروبوليس الخام، منطقة تثبيط أكثر فعالية، وخاصة ضد المكورات العنقودية الذهبية (EEPR2) المستخرج في درجة حرارة الغرفة حيث بلغت 14 مم، وقيست قيمة التركيز المثبط الأدنى البالغة 25 ملغ/م.

يلزم إجراء المزيد من التقييم لتأكيد فعالية البروبوليس الجزائري كعامل مضاد للميكروبات، وذلك لتطوير منتجات صيدلانية نباتية جديدة يمكن ان تحل محل المضادات الحيوية .

الكلمات المفتاحية: دنج، نشاط مضاد للميكروبات، منتج ذرية، المكورات العنقودية الذهبية، الإشريكية القولونية، الزائفة الزنجارية، مثبط الحد الأدنى للتركيز .

Résumé

La propolis est une substance résineuse récoltée par les abeilles sur les bourgeons des plantes. Elle est utilisée dans la ruche pour renforcer la structure, sceller les entrées et servir d'agent antiseptique.

Grâce à ses propriétés thérapeutiques, cette étude a mis en évidence l'activité antibactérienne de deux échantillons de propolis algérienne : l'un récolté dans les montagnes de Guelma (Maouna), dans le nord-est de l'Algérie, et l'autre acheté en pharmacie (produit à Sétif).

Deux méthodes d'extraction ont été utilisées, l'une par macération à 70 °C et l'autre à température ambiante, afin de déterminer la méthode d'extraction optimale préservant les principes actifs antibactériens de l'extrait éthanolique de propolis. La propolis a donc été dissoute dans de l'éthanol à 70 % selon un rapport de 1:20 (m/v) afin de trouver la concentration la plus faible d'extrait éthanolique de propolis présentant une activité antibactérienne potentielle. Les échantillons ont été testés afin de déterminer leurs propriétés antimicrobiennes contre trois souches bactériennes : Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 et Staphylococcus aureus ATCC 25923, par la méthode de diffusion en gélose et la méthode de macrodilution.

En conséquence, l'extrait éthanolique de propolis brute extraite à température ambiante (EEPR2) s'est avéré plus efficace, notamment contre Staphylococcus aureus, où une zone d'inhibition de 14 mm a été enregistrée et une CMI de 25 mg/mL a été mesurée.

Des évaluations complémentaires sont nécessaires pour confirmer l'efficacité de la propolis algérienne comme agent antimicrobien pour le développement de nouveaux produits phytopharmaceutiques pouvant remplacer les antibiotiques.

Mots-clés : Propolis, Activité antimicrobienne, Produit apicole, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, Concentration minimale inhibitrice (CMI).

References

References

- Abdullah, N. A., Zullkiflee, N., Zaini, S. N. Z., Taha, H., Hashim, F., & Usman, A. (2020). Phytochemicals, mineral contents, antioxidants, and antimicrobial activities of propolis produced by Brunei stingless bees *Geniotrigona thoracica*, *Heterotrigona itama*, and *Tetrigona binghami*. *Saudi journal of biological sciences*, *27*(11), 2902–2911. https://doi.org/10.1016/j.sjbs.2020.09.014
- Ahangari, Z., Naseri, M., & Vatandoost, F. (2018). Propolis: Chemical Composition and Its Applications in Endodontics. *Iranian endodontic journal*, *13*(3), 285–292. https://doi.org/10.22037/iej.v13i3.20994
- Asma, S. T., Bobiş, O., Bonta, V., Acaroz, U., Shah, S. R. A., Istanbullugil, F. R., & Arslan-Acaroz, D. (2022). General Nutritional Profile of Bee Products and Their Potential Antiviral Properties against Mammalian Viruses. *Nutrients*, *14*(17), 3579. https://doi.org/10.3390/nu14173579
- Airen, B., Sarkar, P. A., Tomar, U., & Bishen, K. A. (2018). Antibacterial effect of propolis derived from tribal region on *Streptococcus mutans* and *Lactobacillus acidophilus* : An *in vitro* study. *Journal of the Indian Society of Pedodontics and Preventive Dentistry*, *36*(1), 48–52. https://doi.org/10.4103/JISPPD.JISPPD_1128_17
- Almeida, EC de, and H. Menezes. (2002). "Anti-inflammatory activity of propolis extracts : a review." Journal of Venomous Animals and Toxins 8.2 : 191-212.
- Almuhayawi MS. 2020. Propolis as a novel antibacterial agent. *Saudi J Biol Sci*. 27(11) :3079-3086. doi:10.1016/j.sjbs.2020.09.016
- Alotaibi, A., Ebiloma, G. U., Williams, R., Alenezi, S., Donachie, A. M., Guillaume, S., Igoli, J. O., Fearnley, J., de Koning, H. P., & Watson, D. G. (2019). European propolis is highly active against trypanosomatids including Crithidia fasciculata. *Scientific reports*, *9*(1), 11364. https://doi.org/10.1038/s41598-019-47840-y
- Anjum, S. I., Ullah, A., Khan, K. A., Attaullah, M., Khan, H., Ali, H., Bashir, M. A., Tahir, M., Ansari, M. J., Ghramh, H. A., Adgaba, N., & Dash, C. K. (2019). Composition and functional properties of propolis (bee glue): A review. *Saudi journal of biological sciences*, *26*(7), 1695–1703. https://doi.org/10.1016/j.sjbs.2018.08.013
- Aryaei, R., & Pakzad, D. P. (2018). Evaluation of the antibacterial activity of Iranian propolis on the strains of Pseudomonas aeruginosa and Staphylococcus

aureus. *Amazonia Investiga*, *7*(13), 430–435. Retrieved from https://amazoniainvestiga.info/index.php/amazonia/article/view/559

- Ayad, A. S., Hébert, M. P. A., Doiron, J. A., Loucif-Ayad, W., Daas, T., Smagghe, G., Alburaki, M., Barnett, D. A., Touaibia, M., & Surette, M. E. (2024). Algerian Propolis from Distinct Geographical Locations : Chemical Profiles, Antioxidant Capacity, Cytotoxicity and Inhibition of 5-Lipoxygenase Product Biosynthesis. *Chemistry & biodiversity*, *21*(4), e202301758. https://doi.org/10.1002/cbdv.202301758
- Alencar, S. M., Oldoni, T. L., Castro, M. L., Cabral, I. S., Costa-Neto, C. M., Cury, J. A., Rosalen, P. L., & Ikegaki, M. (2007). Chemical composition and biological activity of a new type of Brazilian propolis : red propolis. *Journal of ethnopharmacology*, *113*(2), 278–283. https://doi.org/10.1016/j.jep.2007.06.005
- Aguilar Salinas, J L; Pacheco Aguilar, J R; Mayén Hernández, S A; J. Santos Cruz. (2013). International **Journal of Photoenergy ; New York** Vol. 2013, DOI:10.1155/2013/954914
- Andréia Pires DantasI ; Kelly SalomãoI ; Helene Santos BarbosaII ; Solange Lisboa De CastroI. (2006).»The effect of Bulgarian propolis against Trypanosoma cruzi and during its interaction with host cells." Memórias do Instituto Oswaldo Cruz 101 : 207-211.
- Bankova, V., Popova, M., & Trusheva, B. (2014). Propolis volatile compounds : chemical diversity and biological activity: a review. *Chemistry Central journal*, *8*, 28. https://doi.org/10.1186/1752-153X-8-28
- Bankova V. (2005). Chemical diversity of propolis and the problem of standardization. *Journal of ethnopharmacology*, *100*(1-2), 114–117. https://doi.org/10.1016/j.jep.2005.05.004
- Bagno, F. F., Godói, L. C., Figueiredo, M. M., Sérgio, S. A. R., Moraes, T. F. S., Salazar, N. C., Kim, Y. C., Reyes-Sandoval, A., & da Fonseca, F. G. (2020). Chikungunya E2 Protein Produced in *E. coli* and HEK293-T Cells-Comparison of Their Performances in ELISA. *Viruses*, *12*(9), 939. https://doi.org/10.3390/v12090939
- Bankova, V., Popova, M., Bogdanov, S. & Sabatini, A. (2002). Chemical Composition of European Propolis : Expected and Unexpected Results. *Zeitschrift für Naturforschung C*, *57*(5-6), 530-533. https://doi.org/10.1515/znc-2002-5-622

- Boufadi Yasmina Mokhtaria, Sobhi Jalal, Nive Jan, Anvers Pierre Van, Riazi Ali, 27 octobre 2016 dans IFST, Effets antimicrobiens de six extraits de propolis algérienne,, https://doi.org/10.1111/ijfs.13247
- Becerra, Thalia B.Roger D. Calla-Poma Margarita F. Requena-Mendizabal
- and Pablo A. Millones-Gómez, (2019). "Antibacterial effect of Peruvian propolis collected during different seasons on the growth of Streptococcus mutans." The Open Dentistry Journal 13.1.
- BOUZAHOUANE, H., Ayari, A. ., Guehria, I. ., & Riah, O. . (2021). The PROPOLIS : ANTIMICROBIAL ACTIVITY AND CHEMICAL COMPOSITION ANALYSIS : Properties of propolis. *Journal of Microbiology, Biotechnology and Food Sciences*, *10*(6), e3211. https://doi.org/10.15414/jmbfs.3211
- Bhatti, Neelam, Younis Ahmad Hajam, Saresh Mushtaq, Lovepreet Kaur, Rajesh Kumar & Seema Rai. (2024). "A review on dynamic pharmacological potency and multifaceted biological activities of propolis." Discover Sustainability 5.1 : 185
- Bobiş, O. Plants : Sources of Diversity in Propolis Properties. Plants 2022, 11, 2298. https://doi.org/10.3390/plants11172298
- Boisard, S., Le Ray, A. M., Landreau, A., Kempf, M., Cassisa, V., Flurin, C., & Richomme, P. (2015). Antifungal and antibacterial metabolites from a French poplar type propolis. *Evidence-based complementary and alternative medicine : eCAM*, *2015*, 319240. https://doi.org/10.1155/2015/319240
- Bankova V, Popova M, Trusheva B (2006) Plant sources of propolis : an update from a chemist's point of view. Nat Prod Commun 1(11) :1023–1028. https://doi.org/10.1177/1934578X0600101118
- BUCIO-VILLALOBOS, Carlos Manuel and MARTINEZ-JAIME, Oscar Alejandro. 2017. Antibacterial activity of aqueous extract of propolis from Irapuato, Guanajuato, Mexico. Agron. Mesoam [online]. vol.28, n.1, pp.223-227. ISSN 2215-3608. http://dx.doi.org/10.15517/am.v28i1.24253.
- Bonvehí, J. S., & Gutiérrez, A. L. (2012). The antimicrobial effects of propolis collected in different regions in the Basque Country (Northern Spain). *World journal of microbiology & biotechnology*, *28*(4), 1351–1358. https://doi.org/10.1007/s11274-011-0932-y

- Cardinault, N., Cayeux, M.O. & Percie du Sert, P. La propolis. (2012), origine, composition et propriétés. Phytothérapie 10, 298–304. https://doi.org/10.1007/s10298-012-0733-y
- Chen F, Zhang X, Wang J, Wang F, Mao J. P-coumaric Acid. (2024) : Advances in Pharmacological Research Based on Oxidative Stress. Curr Top Med Chem. 24(5) :416-436. doi : 10.2174/0115680266276823231230183519. PMID : 38279744.
- Charchar, N., elafri, a, Rais, R., & Ismahen, H. (2020). Land Application of Sewage Sludge : Physiological and Biochemical Response of the Rio Grande Tomato, *Journal of, Bioresource, Management, 7* (2). DOI : https://doi.org/10.35691/JBM.0202.0126
- Corrêa, J. L., Veiga, F. F., Jarros, I. C., Costa, M. I., Castilho, P. F., de Oliveira, K. M. P., Rosseto, H. C., Bruschi, M. L., Svidzinski, T. I. E., & Negri, M. (2020). Propolis extract has bioactivity on the wall and cell membrane of Candida albicans. *Journal of ethnopharmacology*, *256*, 112791. https://doi.org/10.1016/j.jep.2020.112791
- Cushnie, T. P., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International journal of antimicrobial agents*, *26*(5), 343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002
- De Groot AC, Popova MP, Bankova VS. (2014) An update on the constituents of poplar-type propolis. Wapserveen, The Netherlands : acdegroot publishing, 2014, 11 pages. ISBN/EAN : 978-90-813233-0-7.
- Darvishi N, Yousefinejad V, Akbari ME, Abdi M, Moradi N, Darvishi S, Mehrabi Y, ,Ghaderi E, Jamshidi-Naaeini Y, Ghaderi B, Davoodi SH.(2020). Antioxidant and anti-inflammatory effects of oral propolis in breast cancer patients treated with chemotherapy: a randomized controlled trial. Journal of herbal medicine, https://doi.org/10.1016/j.hermed.2020.100385.
- David Guillermo Piedrahita Márquez, Marcelo Maraschin, Eva Regina Oliveira, Lady Viviana Camargo Ovalle, Consuelo Díaz-Moreno, Héctor Suárez-Mahecha, (2023).Metabolomic Analysis and Antioxidant Potential of Tropical Propolis Nonpolar Extracts from Colombia. Journal of Food, Article ID 9489176, 16 pageshttps://doi.org/10.1155/2023/9489176
- Diggle, S. P., & Whiteley, M. (2020). Microbe Profile: *Pseudomonas aeruginosa*: opportunistic pathogen and lab rat. *Microbiology (Reading, England)*, *166*(1), 30–33. https://doi.org/10.1099/mic.0.000860

- Elkhenany H, El-Badri N, Dhar M. 2019 Jul, Green propolis extract promotes in vitro proliferation, differentiation, and migration of bone marrow stromal cells. Biomed Pharmacother. 115 :108861. doi : 10.1016/j.biopha.2019.108861. Epub 2019 Apr 18. PMID : 31005795.
- Elliud Muli, John Maingi, january 2007, Antibacterial activity of Apis mellifera L. Propolis collected in three regions of Kenya, Journal of Venomous Animals and Toxins, DOI:10.1590/S1678-91992007000300008
- El-Guendouz S, Lyoussi B, Miguel MG (2019) Insight on propolis from mediterranean countries : chemical composition, biological activities and application fields. Chem Biodivers 16(7):1–35. https://doi.org/10.1002/cbdv.201900094
- Ebadi, Zeynab, Ainaz Khodanazary, Seyyed Mehdi Hosseini,Nasim Zanguee. (2019)."The shelf life extension of refrigerated Nemipterus japonicus fillets by chitosan coating incorporated with propolis extract." *International Journal of Biological Macromolecules* 139 : 94-102.
- Falcão, S. I., Vale, N., Cos, P., Gomes, P., Freire, C., Maes, L., & Vilas-Boas, M. (2014). In vitro evaluation of Portuguese propolis and floral sources for antiprotozoal, antibacterial and antifungal activity. *Phytotherapy research : PTR*, *28*(3), 437–443. https://doi.org/10.1002/ptr.5013
- Frezza, C., De Vita, D., Sciubba, F., Toniolo, C., Tomassini, L., Nicoletti, M., Franceschin, M., Guiso, M., Bianco, A., Serafini, M., & Foddai, S. (2022). There Is Not Only *Cupressus sempervirens* L.: A Review on the Phytochemistry and Bioactivities of the Other *Cupressus* L. Species. *Applied Sciences*, *12*(14), 7353. https://doi.org/10.3390/app12147353
- Gargouri, Wafa, Sandra M. Osés b , Miguel A. Fernández-Muiño b ,M. Teresa Sancho b, Nabil Kechaou a. (2019). "Evaluation of bioactive compounds and biological activities of Tunisian propolis." *Lwt* 111: 328-336.
- Gonsales, G. Z. ; Orsi R. O.I; Fernandes Júnior A.II; Rodrigues P.I; Funari S. R. C.I.12 (2006). "Antibacterial activity of propolis collected in different regions of Brazil." *Journal of Venomous Animals and Toxins Including Tropical Diseases* : 276-284.
- Heleno, S. A., Ferreira, I. C., Esteves, A. P., Ćirić, A., Glamočlija, J., Martins, A., Soković, M., & Queiroz, M. J. (2013). Antimicrobial and demelanizing activity of Ganoderma lucidum extract, p-hydroxybenzoic and cinnamic acids and their synthetic

acetylated glucuronide methyl esters. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, *58*, 95–100. https://doi.org/10.1016/j.fct.2013.04.025

- Hernández Tasco AJ, Ramírez Rueda RY, Alvarez CJ, Sartori FT, Sacilotto ACBC, Ito IY, Vichnewski W, Salvador MJ. (2020). Antibacterial and antifungal properties of crude extracts and isolated compound from *Lychnophora markgravii*. Nat Prod Res. 34(6) :863-867. doi :10.1080/14786419.2018.1503263. Epub 2018 Nov 16. PMID : 30445853.
- Jautová, J., Zelenková, H., Drotarová, K., Nejdková, A., Grünwaldová, B., & Hladiková, M. (2019). Lip creams with propolis special extract GH 2002 0.5% versus aciclovir 5.0% for herpes labialis (vesicular stage) : Randomized, controlled double-blind study. Lippencreme mit 0,5 % Propolis-Spezialextrakt GH 2002 versus 5 % Aciclovir bei Herpes labialis (Bläschenstadium) : Randomisierte, kontrollierte Doppelblindstudie. *Wiener medizinische Wochenschrift (1946)*, *169*(7-8), 193–201. https://doi.org/10.1007/s10354-018-0667-6
- Kujumgiev, A., Tsvetkova, I., Serkedjieva, Y., Bankova, V., Christov, R., & Popov, S. (1999). Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. *Journal of ethnopharmacology*, *64*(3), 235–240. https://doi.org/10.1016/s0378-8741(98)00131-7
- Konstantia Graikou, Milena Popova, Olga Gortzi, Vassya Bankova, Ioanna Chinou, January 2016 Characterization and biological evaluation of selected Mediterranean propolis samples. Is it a new type?, https://doi.org/10.1016/j.lwt.2015.08.025
- Krell, R. (1996) Value-Added Products from Beekeeping. FAO Agricultural Services Bulletin, 124, Food and Agriculture Organization of the United Nations, Rome, Italy.
- Kurek-Górecka, A., Keskin, Ş., Bobis, O., Felitti, R., Górecki, M., Otręba, M., Stojko, J., Olczyk, P., Kolayli, S., & Rzepecka-Stojko, A. (2022). Comparison of the Antioxidant Activity of Propolis Samples from Different Geographical Regions. *Plants (Basel, Switzerland)*, *11*(9), 1203. https://doi.org/10.3390/plants11091203
- Kurokawa, M., Basnet, P., Ohsugi, M., Hozumi, T., Kadota, S., Namba, T., Kawana, T., & Shiraki, K. (1999). Anti-herpes simplex virus activity of moronic acid purified from Rhus javanica in vitro and in vivo. *The Journal of pharmacology and experimental therapeutics*, *289*(1), 72–78.

- Kim, S., Kubec, R., & Musah, R. A. (2006). Antibacterial and antifungal activity of sulfur-containing compounds from Petiveria alliacea L. J. Ethnopharmacol., 104(1-2): 188-92.https://doi.org/10.1016/j.jep.2005.08.072
- Karen Veasey - Updated July 9, 2024, What to Know About Staphylococcus Aureus Infection, Medically Reviewed by Darragh O'Carroll, MD - Written
- Kuropatnicki, A. K., Szliszka, E., & Krol, W. (2013). Historical aspects of propolis research in modern times. *Evidence-based complementary and alternative medicine : eCAM*, *2013*, 964149. https://doi.org/10.1155/2013/964149
- Laura Stan, Liviu Al. Mărghitaş, Daniel Dezmirean, 2011, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca - 400372 Cluj-Napoca, Calea Manastur 3-5, Romania, Quality Criteria for Propolis Standardization,al./Scientific Papers: Animal Science and Biotechnologies, 44 (2).
- Lavigne, J. P., Ranfaing, J., Dunyach-Rémy, C., & Sotto, A. (2020). Synergistic Effect of Propolis and Antibiotics on Uropathogenic *Escherichia coli*. *Antibiotics (Basel, Switzerland)*, *9*(11), 739. https://doi.org/10.3390/antibiotics9110739
- Moise AR, Bobiş O , 2020. *Baccharis dracunculifolia* and *Dalbergia ecastophyllum*, Main Plant Sources for Bioactive Properties in Green and Red Brazilian Propolis. Plants (Basel). 9(11) :1619. doi : 10.3390/plants9111619. PMID : 33233429 ; PMCID : PMC7700410.
- Liviu Al MsrghitaG, Daniel S. Dezmirean, and Otilia BobiG, 2013, Important Developments in Romanian Propolis Research, http://dx.doi.org/10.1155/2013/159392.
- Liu M, Li X, Chen H, Pana F, Zhenga X, Battinob M, Tiana W, Peng W. 2024, Propolis as a promising functional ingredient : A comprehensive review on extraction, bioactive properties, bioavailability, and industrial applications. Food Science and Human Wellness, https://doi.org/10.26599/FSHW.2024.9250236.
- Mani, R., & Natesan, V. (2018). Chrysin : Sources, beneficial pharmacological activities, and molecular mechanism of action. *Phytochemistry*, *145*, 187–196. https://doi.org/10.1016/j.phytochem.2017.09.016
- Martinotti S, Ranzato E. Propolis , 2015 Jul 22 , a new frontier for wound healing ? Burns Trauma. 3:9. doi : 10.1186/s41038-015-0010-z. PMID : 27574655 ; PMCID : PMC4964312.

- Maíra Maciel Tomazzoli, Amélia Regina Somensi Zeggio, Remi Dal Pai Neto, Leandro Specht, January 2020, Botanical source investigation and evaluation of the effect of seasonality on Brazilian propolis from Apis mellifera L. Scientia Agricola 77(6), DOI : 10.1590/1678-992x-2018-0258.
- Izabel C.G. Mendonça1 ; Michelle L.B.B. Medeiros1 ; Roberta A.P.M. Penteado1 ; Abhishek Parolia2 ; Isabel C. C.M. Porto. (2013), "An overview of the toxic effects and allergic reactions caused by propolis." drugs 4.7. http://pharmacologyonline.silae.it ISSN : 1827-8620
- Idrees, M., Sawant, S., Karodia, N., & Rahman, A. (2021). *Staphylococcus aureus* Biofilm: Morphology, Genetics, Pathogenesis and Treatment Strategies. *International journal of environmental research and public health*, *18*(14), 7602. https://doi.org/10.3390/ijerph18147602
- Mohamed El-Sakhawy*,(2023) Propolis Harvesting and Extraction, Egyptian Journal of Chemistry 2022, DOI : 10.21608/EJCHEM.2022.122043.5469
- Minogue TD, Daligault HA, Davenport KW, Bishop-Lilly KA, Broomall SM, Bruce DC, Chain PS, Chertkov O, Coyne SR, Freitas T, Frey KG, Gibbons HS, Jaissle J, Redden CL, Rosenzweig CN, Xu Y, Johnson SL. Complete Genome Assembly of Escherichia coli ATCC 25922, a Serotype O6 Reference Strain. Genome Announc. 2014 Sep 25;2(5):e00969-14. doi: 10.1128/genomeA.00969-14. PMID: 25291776; PMCID: PMC4175212.
- Mohammed Saad Almuhayawi, (2020) Propolis as a novel antibacterial agent, November, Saudi Journal of Biological Sciences 27(11) :3079-3086, DOI : 10.1016/j.sjbs.2020.09.016
- Minogue TD, Daligault HA, Davenport KW, et al. Complete Genome Assembly of Escherichia coli ATCC 25922, a Serotype O6 Reference Strain. *Genome Announc*. 2014 ; 2(5):e00969-14. Published 2014 Sep 25. doi:10.1128/genomeA.00969-14
- Meto, A., Colombari, B., Meto, A., Boaretto, G., Pinetti, D., Marchetti, L., Benvenuti, S., Pellati, F., & Blasi, E. (2020). Propolis Affects *Pseudomonas aeruginosa* Growth, Biofilm Formation, eDNA Release and Phenazine Production : Potential Involvement of Polyphenols. *Microorganisms*, *8*(2), 243. https://doi.org/10.3390/microorganisms8020243

- Mueller M, Tainter CR.(2023) Escherichia coli Infection. StatPearls. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK564298/
- Mărghitaş, L. A., Dezmirean, D. S., & Bobiş, O. (2013). Important developments in romanian propolis research. *Evidence-based complementary and alternative medicine : eCAM*, *2013*, 159392. https://doi.org/10.1155/2013/159392
- Nakamura, R., Nakamura, R., Watanabe, K., Oka, K., Ohta, S., Mishima, S., & Teshima, R. (2010). Effects of propolis from different areas on mast cell degranulation and identification of the effective components in propolis. *International immunopharmacology*, *10*(9), 1107–1112. https://doi.org/10.1016/j.intimp.2010.06.013
- Nieva Moreno, M.I., Isla, M.I., Cudmani, N.G., Vattuone, M.A., Sampietro, A.R. (1999) ,Screening of antibacterial activity of Amaicha del Valle (Tucuman, Argentina), propolis. *Journal of Ethnopharmacology* **68**, 97 102.DOI : 10.1016/s0378-8741(99)00051-3
- Olegário, L. S., Andrade, J. K. S., Andrade, G. R. S., Denadai, M., Cavalcanti, R. L., da Silva, M. A. A. P., & Narain, N. (2019). Chemical characterization of four Brazilian brown propolis: An insight in tracking of its geographical location of production and quality control. *Food research international (Ottawa, Ont.)*, *123*, 481–502. https://doi.org/10.1016/j.foodres.2019.04.004
- Orsatti, C. L., Missima, F., Pagliarone, A. C., Bachiega, T. F., Búfalo, M. C., Araújo, J. P., Jr, & Sforcin, J. M. (2010). Propolis immunomodulatory action in vivo on Toll-like receptors 2 and 4 expression and on pro-inflammatory cytokines production in mice. *Phytotherapy research : PTR*, *24*(8), 1141–1146. https://doi.org/10.1002/ptr.3086
- Oryan, A., Alemzadeh, E., & Moshiri, A. (2018). Potential role of propolis in wound healing: Biological properties and therapeutic activities. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, *98*, 469–483. https://doi.org/10.1016/j.biopha.2017.12.069
- Ota, C., Unterkircher, C., Fantinato, V., & Shimizu, M. T. (2001). Antifungal activity of propolis on different species of Candida. *Mycoses*, *44*(9-10), 375–378. https://doi.org/10.1046/j.1439-0507.2001.00671.x

- Pasupuleti, V. R., Sammugam, L., Ramesh, N., & Gan, S. H. (2017). Honey, Propolis, and Royal Jelly: A Comprehensive Review of Their Biological Actions and Health Benefits. *Oxidative medicine and cellular longevity*, *2017*, 1259510. https://doi.org/10.1155/2017/1259510
- Przybyłek, I., & Karpiński, T. M. (2019). Antibacterial Properties of Propolis. *Molecules (Basel, Switzerland)*, *24*(11), 2047. https://doi.org/10.3390/molecules24112047
- Prytzyk, E., Dantas, A. P., Salomão, K., Pereira, A. S., Bankova, V. S., De Castro, S. L., & Neto, F. R. (2003). Flavonoids and trypanocidal activity of Bulgarian propolis. *Journal of ethnopharmacology*, *88*(2-3), 189–193. https://doi.org/10.1016/s0378-8741(03)00210-1
- Pereira, S. L., Cardoso, J. R. V., De Medeiros, P. L., Pereira, R. M. L., De Menezes, V. L. L., Satiro, H. X., & De Oliveira, E. L. (2006). Antimicrobial activity of Indigofera suffruticosa. Evid-Based Complementary Altern. Med., 3(2): 261-265.https://doi.org/10.1093/ecam/nel010
- Popova M, Trusheva B, Cutajar S, Antonova D, Mifsud D, Farrugia C, Bankova V. 2012 May. Identification of the plant origin of the botanical biomarkers of Mediterranean type propolis. Nat Prod Commun.; 7(5) :569-70. PMID : 22799077.
- Pobiega, K.; Kraśniewska, K.; Przybył, J.L.; Bączek, K.; Żubernik, J.; Witrowa-Rajchert, D.; Gniewosz, M. **2019.** Growth Biocontrol of Foodborne Pathogens and Spoilage Microorganisms of Food by Polish Propolis Extracts. *Molecules* , *24*, 2965. https://doi.org/10.3390/molecules24162965
- Quiroga, E. N., Sampietro, D. A., Soberón, J. R., Sgariglia, M. A., & Vattuone, M. A. (2006). Propolis from the northwest of Argentina as a source of antifungal principles. *Journal of applied microbiology*, *101*(1), 103–110. https://doi.org/10.1111/j.1365-2672.2006.02904.x
- Ramos, A.F.N. and Miranda, J.L. (2007) Propolis : A Review of Its Anti-Inflammatory and Healing Actions. Journal of Venomous Animals and Toxins Including, Tropical Diseases 13,697-710. http://dx.doi.org/10.1590/S1678-91992007000400002
- Rasul, A., Millimouno, F. M., Ali Eltayb, W., Ali, M., Li, J., & Li, X. (2013). Pinocembrin : a novel natural compound with versatile pharmacological and

biological activities. *BioMed research international*, *2013*, 379850. https://doi.org/10.1155/2013/379850

- Salatino, A., Fernandes-Silva, C. C., Righi, A. A., & Salatino, M. L. (2011). Propolis research and the chemistry of plant products. *Natural product reports*, *28*(5), 925–936. https://doi.org/10.1039/c0np00072h
- Sales, A., Alvarez, A., Areal, M. R., Maldonado, L., Marchisio, P., Rodríguez, M., & Bedascarrasbure, E. (2006). The effect of different propolis harvest methods on its lead contents determined by ET AAS and UV-visS. *Journal of hazardous materials*, *137*(3), 1352–1356. https://doi.org/10.1016/j.jhazmat.2006.05.026
- Sarapa, A., Peter, A., Büttner, A., & Loos, H. (2025). Organoleptic and chemical properties of propolis : a review. European Food Research and Technology. https://doi.org/10.1007/s00217-025-04708-y
- Seibert, J. B., Bautista-Silva, J. P., Amparo, T. R., Petit, A., Pervier, P., Dos Santos Almeida, J. C., Azevedo, M. C., Silveira, B. M., Brandão, G. C., de Souza, G. H. B., de Medeiros Teixeira, L. F., & Dos Santos, O. D. H. (2019). Development of propolis nanoemulsion with antioxidant and antimicrobial activity for use as a potential natural preservative. *Food chemistry*, *287*, 61–67. https://doi.org/10.1016/j.foodchem.2019.02.078
- Sforcin, J. M., & Bankova, V. (2011). Propolis : is there a potential for the development of new drugs?. *Journal of ethnopharmacology*, *133*(2), 253–260. https://doi.org/10.1016/j.jep.2010.10.032
- Sforcin J. M. (2016). Biological Properties and Therapeutic Applications of Propolis. *Phytotherapy research : PTR*, *30*(6), 894–905. https://doi.org/10.1002/ptr.5605
- Silva MV, de Moura Jr NG, Motoyama AB, Ferreira VM. (2020). A review of the potential therapeutic and cosmetic use of propolis in topical formulations. J Appl Pharm Sci.10(1) :131–141.
- Simone-Finstrom, M., Borba, R. S., Wilson, M., & Spivak, M. (2017). Propolis Counteracts Some Threats to Honey Bee Health. *Insects*, *8*(2), 46. https://doi.org/10.3390/insects8020046
- Soromou, L. W., Chu, X., Jiang, L., Wei, M., Huo, M., Chen, N., Guan, S., Yang, X., Chen, C., Feng, H., & Deng, X. (2012). In vitro and in vivo protection provided by pinocembrin against lipopolysaccharide-induced inflammatory

responses. *International immunopharmacology, 14*(1), 66–74. https://doi.org/10.1016/j.intimp.2012.06.009

- Szweda, Piotr Katarzyna Gucwa, Ewelina Kurzyk, Ewa Romanowska.(2014). "Essential oils, silver nanoparticles and propolis as alternative agents against fluconazole resistant Candida albicans, Candida glabrata and Candida krusei clinical isolates." Indian journal of microbiology 55 (2015) : 175-183.
- Šuran, J., Radić, B., Trevisan-Silva, D., Cindrić, M., & Hozić, A. (2024). First Proteome Analysis of Poplar-Type Propolis. *Plant foods for human nutrition (Dordrecht, Netherlands), 79*(1), 83–89. https://doi.org/10.1007/s11130-023-01127-w
- Serra Bonvehí, J., & Ventura Coll, F. (2000). Study on propolis quality from China and Uruguay. *Zeitschrift fur Naturforschung. C, Journal of biosciences*, *55*(9-10), 778–784. https://doi.org/10.1515/znc-2000-9-1017
- Segueni, N. (2011). Contribution à l'étude de la composition chimique et des propriétés biologiques de la propolis. Thèse de doctorat, Université Mentouri de Constantine : 299 p.
- Tatiane Luiza Cadorin Oldoni,Ingridy S.R. Cabral,Marisa Aparecida Bismara Regitano D'Arce, PedroR osalen.(2011).Isolation and analysis of bioactive isoflavonoids and chalcone from a new type of Brazilian propolis, , Separation and Purification Technology 77(2):208-213, DOI:10.1016/j.seppur.2010.12.007
- Toreti VC, Sato HH, Pastore GM, Park YK. (2013). Recent progress of propolis for its biological and chemical compositions and its botanical origin. Evid Based Complement Alternat Med. 2013 :697390. doi : 10.1155/2013/697390. Epub 2013 Apr 30. PMID : 23737843 ; PMCID : PMC3657397.
- Tabera, A., Bedascarrasbure, E., Maldonado, L., Alvarez, A., & Van Der Horst, A. (2000). Actividad antibacteriana de propóleos argentinos enfrentados a Staphylococcus aureus. Congreso Internacional de propóleos. Bueonos-Aires, Argentina : p. 97.
- Trusheva, B., Trunkova, D., & Bankova, V. (2007). Different extraction methods of biologically active components from propolis: a preliminary study. *Chemistry Central journal*, *1*, 13. https://doi.org/10.1186/1752-153X-1-13
- Tegos G., Stermitz F.R., Lomovskaya O., Lewis K., 2002. Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. Antimicrob. Agents Chemother. 46, 3133—3141.

- Uzel, A., Sorkun, K., Onçağ, O., Cogŭlu, D., Gençay, O., & Salih, B. (2005). Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. *Microbiological research*, *160*(2), 189–195. https://doi.org/10.1016/j.micres.2005.01.002
- Vasconcelos, N. G., Croda, J., & Simionatto, S. (2018). Antibacterial mechanisms of cinnamon and its constituents: A review. *Microbial pathogenesis*, *120*, 198–203. https://doi.org/10.1016/j.micpath.2018.04.036
- Bankova, Vassya. 2009. Chemical diversity of propolis makes it a valuable source of new biologically active compounds. Journal of ApiProduct and ApiMedical Science 1: 23–28. doi:10.3896/ibra.4.01.2.01.
- Veiga, R. S., De Mendonça, S., Mendes, P. B., Paulino, N., Mimica, M. J., Lagareiro Netto, A. A., Lira, I. S., López, B. G., Negrão, V., & Marcucci, M. C. (2017). Artepillin C and phenolic compounds responsible for antimicrobial and antioxidant activity of green propolis and Baccharis dracunculifolia DC. *Journal of applied microbiology*, *122*(4), 911–920. https://doi.org/10.1111/jam.13400
- Veloz, J. J., Alvear, M., & Salazar, L. A. (2019). Evaluation of Alternative Methods to Assess the Biological Properties of Propolis on Metabolic Activity and Biofilm Formation in *Streptococcus mutans*. *Evidence-based complementary and alternative medicine : eCAM*, *2019*, 1524195. https://doi.org/10.1155/2019/1524195
- Vieira, R. H., Rodrigues, D. P., Gonçalves, F. A., Menezes, F. G., Aragão, J. S., & Sousa, O. V. (2001). Microbicidal effect of medicinal plant extracts (Psidium guajava Linn. and Carica papaya Linn.) upon bacteria isolated from fish muscle and known to induce diarrhea in children. *Revista do Instituto de Medicina Tropical de Sao Paulo*, *43*(3), 145–148. https://doi.org/10.1590/s0036-46652001000300005
- Vică, M. L., Glevitzky, M., Heghedűş-Mîndru, R. C., Dumitrel, G. A., Heghedűş-Mîndru, G., Popa, M., Faur, D. M., Bâlici, Ș., & Teodoru, C. A. (2023). Phyto-Inhibitory and Antimicrobial Activity of Brown Propolis from Romania. *Antibiotics (Basel, Switzerland)*, *12*(6), 1015. https://doi.org/10.3390/antibiotics12061015
- Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., & Pérez-Alvarez, J. A. (2008). Functional properties of honey, propolis, and royal jelly. *Journal of food science*, *73*(9), R117–R124. https://doi.org/10.1111/j.1750-3841.2008.00966.x

- W. Maciejewicz,M. Daniewski, K. Bal,W. Markowski.(2001).GC-MS identification of the flavonoid aglycones isolated from propolis,Chromatographia 53(5):343-346, DOI:10.1007/BF02490438
- Wagh VD. (2013).Propolis : a wonder bees product and its pharmacological potentials. Adv Pharmacol Sci. 2013 :308249. doi : 10.1155/2013/308249. Epub 2013 Dec 9. PMID : 24382957 ; PMCID : PMC3872021.
- Wieslawa Maciejewicz. (2006).Isolation of flavonoid aglycones from propolis by a column chromatography method and their identification by GC-MC and TLC method, Journal of Liquid Chromatography & Related Technologies, 24(8)(8):1171-1179, DOI:10.1081/JLC-100103439
- Woźniak, M., Sip, A., Mrówczyńska, L., Broniarczyk, J., Waśkiewicz, A., & Ratajczak, I. (2022). Biological Activity and Chemical Composition of Propolis from Various Regions of Poland. *Molecules (Basel, Switzerland)*, *28*(1), 141. https://doi.org/10.3390/molecules28010141
- Wilson, M. G., & Pandey, S. (2023). Pseudomonas aeruginosa. In *StatPearls*. StatPearls Publishing.
- Yang, D., Wang, T., Long, M., & Li, P. (2020). Quercetin : Its Main Pharmacological Activity and Potential Application in Clinical Medicine. *Oxidative medicine and cellular longevity*, *2020*, 8825387. https://doi.org/10.1155/2020/8825387
- Yıldırım, Hatice Kalkan. (2022). "Assessment of propolis treated by different extraction methods." *Brazilian Archives of Biology and Technology* 65): e22210251.
- Yilmaz, S., Sova, M., & Ergün, S. (2018). Antimicrobial activity of trans-cinnamic acid and commonly used antibiotics against important fish pathogens and nonpathogenic isolates. *Journal of applied microbiology*, *125*(6), 10.1111/jam.14097. https://doi.org/10.1111/jam.14097
- Yoshimasu, Y., Ikeda, T., Sakai, N., Yagi, A., Hirayama, S., Morinaga, Y., Furukawa, S., & Nakao, R. (2018). Rapid Bactericidal Action of Propolis against Porphyromonas gingivalis. *Journal of dental research*, *97*(8), 928–936. https://doi.org/10.1177/0022034518758034
- Yvette Brazier. December 11, 2017 .What to Know About E. coli Infections, Medical Review by Susan Falk, MD, F.A.C.P.
- Yue-Wen Chen,L.-C. Lu,Cheng-Chun Chou.(2003).Antibacterial and DPPH free radical-scavenging activities of the ethanol extract of propolis collected in

Taiwan,December ,Journal of Food and Drug Analysis 11(4):277-282, DOI:10.38212/2224-6614.2676

- Zullkiflee, N., Taha, H., & Usman, A. (2022). Propolis: Its Role and Efficacy in Human Health and Diseases. *Molecules (Basel, Switzerland)*, *27*(18), 6120. https://doi.org/10.3390/molecules27186120
- Zeghad, N. (2009). Etude du contenu polyphénolique de deux plantes médicinales d'intérêt économique (Thymus vulgaris, Rosmarinus officinalis) et évaluation de leur activité antibactérienne. Mémoire de Magister, Université de Constantine.