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AKLI MOHAND OULHADJ-BOUIRA University

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Mrs. Hadjira AOUADI

Title

Evaluation of certain biological activities and the functional properties of various bee product extracts: Propolis and pollen

Ahead of the Jury:

Last and First name	Grade		
Mr. Farid DAHMOUNE	Professor	Univ. of Bouira	President
Mr. Hocine REMINI	MCB	Univ. of Bouira	Examinator
Mr. Nabil KADRI	Professor	Univ. of Bouira	Supervisor
Mrs. Nourelimane BENZITOUNE	PhD	Univ. of Bouira	Co-supervisor
Mr. Karim TIGHILET	MCB	Univ. of Bouira	Guest of honor

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List of Abbreviations

Abs: Absorbance.

ABTS: 2, 2'azinobis-(3-6 ethyl-benzothiazoline-6-sulfonate).

ANOVA: Analysis of One Variance.

BSA: Bovin Serum Albumin.

C: Concentration.

CFU: Colony Forming Unit.

DNA: Deoxyribonucleic acid.

DPPH: 2,2-diphenyl 1-picrylhydrazyl.

GAE: Gallic acid equivalent.

EC: Emulsifying capacity.

QE: Quercetin equivalent.

Fe^{2+}: Ferrous iron.

Fe^{3+}: Ferric iron.

FRAP: Iron antioxidant reducing power.

FS: Foam stability.

g: Gram.

h: Hour.

H^{+}: Proton.

IC_{50}: 50% inhibitory concentration.

M: Maceration.

M: Molar.

mg: Milligram.

min: Minute.

NaOH: Sodium hydroxide.

OD: Optical Density.

PB: Phosphate Buffer.

pH: Hydrogen potential.

ROS: Reactive oxygen species.

rpm: Rotation per minute.

s: Second.

T: Temperature.

TCA: Trichloroacetic acid.

US: Ultrasound.

UV-VIS: Ultraviolet-visible.

W/V: Weight per volume

µg: Microgram.

µg /mL: Microgram per milliliter.

°C: Degree Celsius.

%: Percentage.

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Introduction

General introduction

Since ancient times, humans have ingeniously exploited natural resources in their quest for sustenance and environmental improvement. Over the centuries, a growing interest in healthy products led to the discovery of the treasures within beehives and the expanded use of apicultural products. Today, numerous studies explore the health benefits and pharmacological properties of these beekeeping products due to their proven effectiveness. These investigations are driving the development of nutraceuticals and functional foods derived from beehive products **(Kebede et al., 2024)**.

In addition to honey, numerous research efforts have focused on other beekeeping products, notably pollen and propolis. These studies have demonstrated their beneficial effects on various pathologies, many of which are induced by oxidative stress. This condition results from an imbalance between oxidants and antioxidants, leading to the excessive production of reactive oxygen species (ROS), which can cause numerous diseases by damaging various molecules in the body **(Fontaine, 2007)**.

Propolis, a waxy substance collected by bees from plants, is used as a sealant to protect their hives and fill cracks. Rich in flavonoids, propolis has the capacity to neutralize free radicals and possesses other therapeutic properties **(Stojanović et al., 2020).**

Another hive product gaining interest in apitherapy is pollen. It is the primary source of protein for bee colonies and is exceptionally rich in proteins, lipids, carbohydrates, and water- and fat-soluble vitamins. Pollen is recognized as a substantial source of antioxidants, essential substances for the protection of the body **(Khalifa et al., 2021)**.

This manuscript is structured into two main parts. The first part, subdivided into two chapters, provides an overview of the characteristics, composition, and general benefits of pollen and propolis. The second chapter delves specifically into the active substances present in these products, exploring their chemical and biological components.

The second part of this work is dedicated to a rigorous experimental approach aimed at evaluating various aspects of beekeeping products, including their antioxidant and antibacterial activities. This section also includes an analysis of the functional properties of proteins present in pollen and propolis. The methodologies used for these evaluations are described in detail, providing a clear understanding of the experimental protocols and analytical techniques

applied. Finally, this section presents the results obtained and offers an in-depth discussion, interpreting the data and comparing it with existing literature to draw relevant conclusions about the beneficial properties of beekeeping products.

Chapter I

Bee products: Propolis and Pollen

I. Bee products

Hive products created by bees include beeswax, royal jelly, and venom synthesized directly, as well as honey, propolis, and pollen collected and transformed. Used for millennia for their nutritional and medicinal benefits, these products are essential in food and healthcare **(Kebede et al., 2024)**.

I.1. Propolis

I.1.1. Definition

The term "propolis" is of Greek origin, where "pro" means "in front" and "polis" means "city," translating to "defensive substance of the hive" **(Hossain et al., 2022).** Propolis is a natural, balsamic, and resinous substance that is highly adhesive and varies in color. It is produced by foraging bees (*Apis mellifera*) from the buds and exudates of plants **(Tosi et al., 2006)**. This substance forms a powerful defense barrier against the development of microorganisms, including, bacteria, viruses, and fungi, inside the hive **(Donadieu, 2008)**.

Figure 1: Propolis.

I.1.2. Origin of propolis produced in Algeria

The origin of Algerian propolis is linked to the diverse variety of plant species present in the region. Depending on these species, Algerian propolis may be derived from pine (*Pinus* species), common in semi-arid areas; oak (*Quercus* species), found in the northeast of the country; chestnut (*Castanea* species); cypress (*Cupressus* species); casuarina; and poplar (*Populus species*). These diverse plant sources contribute to the unique composition and properties of Algerian propolis **(Ferhoum, 2010).**

I.1.3. Propolis variety

There are several types of propolis, and this diversity is due to three main factors: the geographical location of the hive, the plant species available in that region during the season, and the breed of the bee **(Cardinault, Cayeux et al. 2012)**. The major types of propolis by geographical area are summarized in **Table I**.

Table I: The Most Common Types of Propolis **(Cardinault et al., 2012)**.

Type of Propolis	Geographical origin	Botanical Origin
Poplar (Amber to Brown)	Europe, North America, non-tropical regions of Asia, New Zealand	*Populus* spp., mainly *P. nigra* L.
Green from Brazil	Tropical region of Brazil	*Baccharis* spp., mainly *B. dracunculifolia* DC
Birch	Northern Russia	*Betula verrucosa*
Red Propolis	Cuba, Brazil, Mexico	*Dalbergia ecastophyllum*
Red Propolis	Cuba, Venezuela	*Clusia rosea*
Mediterranean	Sicily, Greece, Malta, Crete, Turkey	*Cupressaceae family*
Pacific	Pacific region (Taiwan, Okinawa, Indonesia)	*Macaranga tanarius*

I.1.4. Chemical composition

The botanical origin of propolis is the primary factor influencing its specific composition. Additionally, modifications made by bees' hypopharyngeal secretions contribute unique elements and cause certain transformations, such as the hydrolysis of flavonoid heterosides into aglycones **(Chavda et al., 2023).**

Propolis is generally composed of 50-55% resins and balsams, 30% waxes and fatty acids, 10% essential oils, 5% pollen, and 5% organic and mineral substances. Among these substances, there is a wide variety of flavonoids and other phenolic derivatives, as well as their

esters, volatile aromatic compounds, minerals such as iron, calcium, zinc, copper, and manganese, and vitamins, notably C, E, and those of the B group **(Stojanović et al., 2020).**

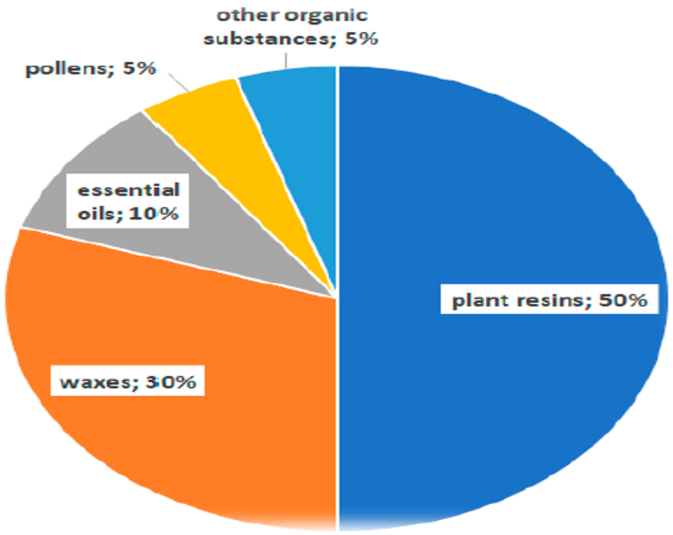

Figure 2: Various components of propolis **(Przybyłek and Karpiński, 2019)**.

I.1.5. Physical properties

Bee propolis exhibits partial solubility in alcohol, acetone, ether, chloroform, benzene, and trichloroethylene. It melts at approximately 70°C and, when gently heated in a water bath, separates into two distinct layers: a viscous layer settling at the bottom and a liquid layer (propolis wax) that floats on the surface, widely used in various beekeeping applications. Propolis has a density of 1.2, which surpasses that of water, indicating its relatively high density compared to other liquids **(Velikova et al., 2000; Pietta et al., 2002)**.

I.1.6. Organoleptic properties

The organoleptic characteristics such as color, odor, taste, and consistency are listed in the **Table II**.

Table II: Organoleptic characteristics of propolis **(Bankova et al., 1992; Donadieu, 2008).**

Color	Its shade varies depending on its origin, ranging from light yellow to very dark brown, almost black, passing through all shades of brown (brown-green, brown-red, and brown-green).
Consistency	The consistency of propolis changes with temperature: • At 15°C, it is solid. • Around 25 to 45°C, it is soft and malleable. • Above 45°C, it becomes sticky or gooey.

Taste	It often has a pungent flavor and can sometimes be bitter.
Smell	Its scent varies according to its origin, often pleasant and sweet, with hints of honey and wax.

I.1.7. Uses

I.1.7.1. By bees

Bees use propolis to protect their hive from moisture, drafts, and dangers while maintaining hygiene. It seals cracks where microorganisms could develop, and its volatile oils probably serve as an antiseptic and air purifier. Propolis is also used as a construction material to reduce the size of hive entrances and smooth surfaces to facilitate bee traffic. Finally, it is used to embalm the bodies of predators too large for the bees to remove, preventing their decomposition from endangering the hive **(Simone-Finstrom and Spivak, 2010)**.

I.1.7.2. By humans

Propolis finds extensive applications in various fields, including:

I.1.7.2.1. Cosmetics

Propolis and its extracts are frequently incorporated into dermatological and cosmetic products, benefiting from their documented effects on tissue regeneration and renovation (Kebede et al., 2024).

I.1.7.2.2. Medicine

Propolis is used in a range of treatments for cardiovascular issues, respiratory conditions (especially infections), dental care, ulcers, mucous membrane infections, and lesions, as well as in supporting and enhancing the immune system **(Choudhari et al., 2012; Salami et al., 2024).**

I.1.7.2.3. Food industry technology

The use of propolis as a functional food ingredient is increasing as consumers prefer functional foods. They seek fresh, minimally processed foods enriched with natural superfoods or high nutritional value that offer maximum health benefits while retaining organoleptic qualities. For this reason, food industries prefer the application of natural preservatives over

synthetic ones due to their observed carcinogenic and teratogenic tendencies after prolonged consumption **(Irigoiti et al., 2021)**.

In the last two decades, propolis has been considered a natural product that can serve as a functional food ingredient thanks to its rich chemical composition and bio-functionalities such as antibacterial, antioxidant, anti-inflammatory, antiseptic, and anti-ulcer properties. Many food industries use propolis as a microbial inhibitor and antioxidant agent, particularly in bakery products, edible oils, seafood, and animal products. These foods are susceptible to lipid auto-oxidation, which degrades their organoleptic properties. Thus, propolis can serve as a natural food preservative, replacing artificial preservatives to enhance immunity and well-being.

I.1.8. Toxicity

Propolis has very low toxicity. When consumed in reasonable quantities, it poses no danger to humans and animal **(Segueni et al., 2011)**. However, some individuals may develop contact allergies (such as dermatitis or eczema) due to a specific allergen, "3,3-dimethylallyl caffeate." There are no major contraindications for the use of propolis, except for individuals who are allergic or predisposed to allergies. These individuals should use it with caution, avoiding certain routes of administration, particularly inhalation. Those prone to skin allergies should completely avoid its local application **(Gardana and Simonetti, 2011)**.

I.2. Bee pollen

I.2.1. Definition

Bee pollen is a natural substance collected by worker bees from flowering plants. It consists of flower pollen grains, combined with bee salivary secretions, nectar, and occasionally honey. Bees gather pollen during foraging trips to flowers and bring it back to the hive, where it serves as a vital nutritional source for both larvae and adult members of the colony. Beekeepers also harvest bee pollen for its recognized potential health benefits for humans **(Komosinska-Vassev et al., 2015; Denisow and Denisow-Pietrzyk, 2016)**.

Figure 3 : bee pollen.

I.2.2. Different types of pollen

According to some studies, pollen exists in various types, each with compositions that vary based on factors such as plant species, climate, geographical region, harvest period **(Thakur and Nanda, 2020)**, and soil characteristics. Pollen can be broadly categorized into two main groups:

- **Entomophilous Pollen:** These pollens are collected and transported by insects and are considered edible **(Lu et al., 2022)**.
- **Anemophilous Pollen:** These pollens are carried by the wind and are more likely to cause allergies. Bees typically forage flowers containing entomophilous pollen and tend to avoid those with anemophilous pollen. An exception to this pattern is corn, which despite being wind-pollinated (anemophilous) and having low nutritional value, is still foraged by bees. However, intensive corn cultivation can lead to pesticide contamination **(Lu et al., 2022)**.

I.2.2. Biochemical composition of pollen

Bee pollen is considered one of the extraordinary superfoods due to its extremely broad range of nutritional compounds. The main chemical substances include proteins, amino acids, carbohydrates, lipids, fatty acids, phenolic compounds, enzymes, coenzymes, vitamins, and bioelements. However, this composition can vary depending on several factors such as the plant source, geographical region, weather conditions, and agricultural practices **(Campos et al., 2008).**

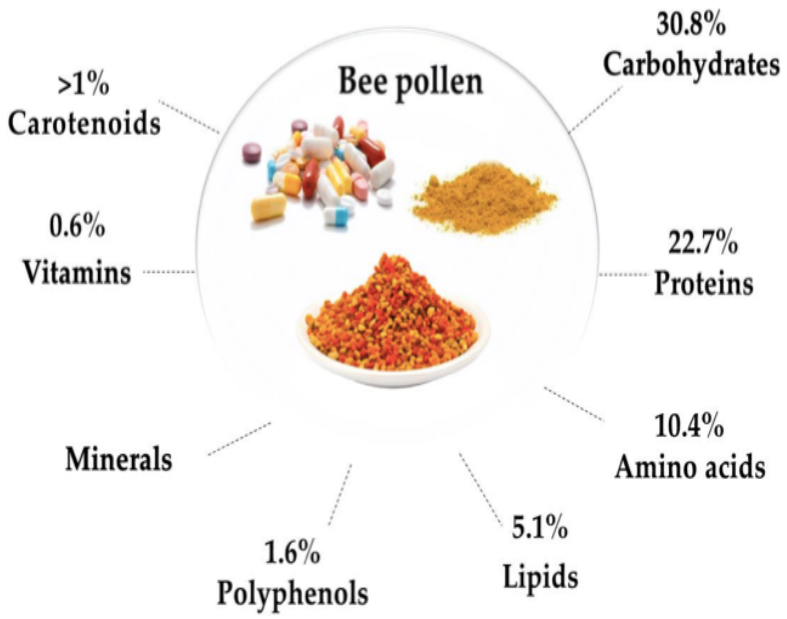

Figure 4: Different components of bee pollen **(Khalifa et al., 2021)**.

I.2.3. Physical properties

Bee pollen pellets typically weigh between 7.5 and 8 mg each. Fresh pollen exhibits diverse shapes including cylindrical, round, triangular, or bell-shaped, whereas dried pollen pellets generally assume a spherical or fusiform shape. The solubility of bee pollen ranges from 84.91% to 87.56%, primarily determined by the nature and composition of proteins and carbohydrates present **(Thakur and Nanda, 2020)**.

I.2.4. Organoleptic properties

The organoleptic characteristics of pollen, such as its color, appearance, odor, size, and taste, vary according to the botanical origin of the plant it comes from **(Siuda et al., 2012). Table III** presents the specific organoleptic properties of bee pollen in detail.

Table III: Organoleptic Properties of Pollen **(Siuda et al., 2012; Thakur and Nanda, 2020)**.

Color	Pollen can exhibit a wide range of colors, influenced by the flowers visited by bees. These shades range from yellow, orange, and even blood red or violet, to greens or even very dark tones, almost black.
Size	The size of pollen granules varies depending on the bee's collection, but on average, the diameter can be estimated at 2.5 mm.

Smell	The smell can be described as "hay-like," varying depending on whether the pollen is fresh or frozen. It is typical of this substance.
Taste	The taste of pollen can be sweet, tangy, bitter, or spicy, with a generally floury texture.
Appearance	Pollen grains show great heterogeneity, with different shapes and sizes.

I.2.5. Benefits of bee pollen

The benefits of bee pollen are extensive and diverse. Serving as a natural source rich in essential nutrients such as proteins, vitamins, minerals, and antioxidants, bee pollen is renowned for its valuable nutritional properties. It supports immune system strength, cardiovascular health, boosts energy levels, enhances vitality, and promotes digestive well-being. Moreover, studies suggest that bee pollen may positively impact skin health, cognitive function, and respiratory well-being. Its consumption is also linked to benefits in weight management, allergy relief, and anti-inflammatory effects **(El Ghouizi et al., 2023)**.

Exploring the composition, properties, and benefits of bee pollen and propolis underscores their significant role in nutrition and health. Subsequent chapters will delve deeper into the specific active substances found in these products and their biological implications, followed by rigorous experimental analyses assessing their antioxidant and antibacterial activities.

I.2.6. Toxicity

Pollen is known to cause allergies, particularly those from grasses, willow, linden, poplar, or birch, as they are anemophilous and suspended in the air. However, bee-collected pollen can even be consumed by allergic individuals, as it is transformed by the insect through its saliva, which destroys the majority of allergens. Additionally, it can be used in the treatment of allergies by inhibiting histamine release through flavonoids and stimulating the immune system thanks to the presence of copper, zinc, vitamins A and E, selenium, arginine, and leucine **(Blanc, 2010)**.

Chapter II

Bioactive Substances and Bioactivities

II.1. Primary Metabolites

Primary metabolites, including amino acids, lipids, sugars, and nucleic acids, are essential for the physiological functions of organisms, such as growth, reproduction, and cell division **(Silori et al., 2019)**.

Numerous studies have extensively detailed the chemical composition of pollen and propolis.

II.1.1. Proteins

Pollen is rich in high-quality proteins, averaging between 10 and 40 grams of protein per 100 grams of pollen, and includes 10.4% essential amino acids such as methionine, lysine, threonine, histidine, leucine, isoleucine, valine, phenylalanine, and tryptophan. These essential amino acids are vital for life, as our bodies cannot synthesize them and must obtain them through our diet. Additionally, pollen contains significant amounts of nucleic acids, particularly ribonucleic acid (RNA) **(Campos et al., 2008; Franko, 2020)**.

In contrast, propolis has a lower content of proteins and amino acids. The amino acids present in propolis include aspartic acid, glutamic acid, alanine, arginine, cystine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine **(Donadieu, 2008)**.

II.1.2. Carbohydrates

Carbohydrates are one of the main components of pollen, with a rate ranging from 13% to 55%. They mainly consist of polysaccharides and cell wall materials **(Thakur and Nanda, 2020)**. Propolis contains a very low carbohydrate content, including glucose, fructose, and sucrose **(Liolios et al., 2018)**.

II.1.3. Lipids

Lipids constitute approximately 5.1% of pollen, including essential fatty acids such as linoleic, γ-linolenic, and arachidic acids, which make up 0.4% of the total. Phospholipids account for 1.5%, while phytosterols, particularly β-sitosterol, are present at 1.1%. In contrast, propolis is primarily composed of lipids, with a significant presence of terpenoids like farnesol and lipids derived from wax **(Donadieu, 2008).**

II.2. Minerals and vitamins

Pollen is rich in various vitamins, mainly B vitamins (B1, B2, B5, B6, B8, B9, and B12), as well as vitamin C and carotenoids, which are precursors of vitamin A. It also contains vitamins D and E in smaller quantities. Additionally, it has several minerals and trace elements such as potassium, magnesium, calcium, copper, iron, silicon, phosphorus, sulfur, chlorine, manganese, selenium, and zinc **(Mărgăoan et al., 2012; Komosinska-Vassev et al., 2015; Franko, 2020).**

Propolis, on the other hand, is rich in health-beneficial minerals and vitamins. It contains calcium, magnesium, iron, manganese, zinc, copper, boron, barium, aluminum, nickel, and silica in significant amounts. These minerals are essential for various physiological processes such as bone formation, muscle contraction, and nerve transmission. In terms of vitamins, propolis is a good source of provitamin A (beta-carotene), B vitamins (B1, B2, B6, niacin), vitamin C, and vitamin E **(Zulhendri et al., 2021)**.

II.3. Secondary metabolites

Secondary metabolites found in beekeeping products such as pollen and propolis are bioactive compounds produced by bees or collected from surrounding plants. Unlike primary metabolites, these compounds are not essential for survival but play a crucial role in long-term health and survival **(Soto et al., 2011)**. Here is an overview of the main secondary metabolites found in these apicultural products.

II.3.1. Phenolic compounds

II.3.1.1. Phenolic acids and derivatives

The phenolic composition of bee pollen and propolis varies depending on geographic regions and botanical sources, comprising a diverse range of phenolic acids such as chlorogenic acid, ferulic acid, caffeic acid, gallic acid, vanillic acid, syringic acid, and *p*-coumaric acid **(Stojanović et al., 2020; Thakur and Nanda, 2020)**.

II.3.1.2. Flavonoids

Flavonoids, phenolic compounds renowned for their role in the coloration of flowers and fruits, are found in large quantities in propolis and pollen. Scientific literature identifies several major types of flavonoids present in these substances, including flavones, flavonols, flavanones, flavanonols, chalcones, dihydrochalcones, isoflavones, isodihydroflavones, flavans, isoflavans, and neoflavonoids. Among the flavonoids in propolis, the main ones include chrysin, pinocembrin, apigenin, rutin, luteolin, galangin, kaempferol, myricetin,

catechin, naringenin, quercetin, tectochrysin, pinostrobin, and acacetin. These compounds are abundantly represented in pollen and propolis extracts, highlighting their importance in therapeutic and nutritional applications **(Huang et al., 2014; Sforcin, 2016; Przybyłek and Karpiński, 2019; Thakur and Nanda, 2020)**.

II.4. Biological activities

II.4.1. Antioxidant activity

Antioxidants are molecules capable of inhibiting the excessive production of free radicals and neutralizing their harmful effects. Free radicals, when overproduced, can cause oxidative stress, leading to damage to biological molecules and contributing to various diseases including cancer, cardiovascular diseases, renal failure, and neurodegenerative conditions such as Alzheimer's disease. Therefore, antioxidants play a crucial role in protecting cells from these detrimental effects **(Chaudhary et al., 2023).**

Pollen is recognized for its richness in natural antioxidants, predominantly polyphenolic compounds and flavonoids, which contribute significantly to its antioxidant activity. These compounds function by scavenging free radicals and chelating metal ions involved in oxidation reactions, owing to their unique chemical structures **(Tosi et al., 2006)** .

It is important to note that the antioxidant activity of pollen and propolis can vary based on several factors, including the plant species, harvest time, and post-harvest treatments. Consequently, study outcomes may differ depending on these variables **(Cardinault et al., 2012; Aylanc et al., 2021; Kinghorn et al., 2021).**

Propolis stands out among beekeeping products due to its robust antioxidant properties, which often surpass those of other products. Propolis extracts, enriched with vitamins E, C, and phenolic substances, exhibit notable antioxidant activity, largely attributed to their(Siheri et al., 2017) high polyphenol content, as documented in numerous studies**.** Research by **Kumazawa et al. (2007)** and **Siheri et al. (2017)** highlights specific antioxidant compounds in propolis, such as kaempferol and phenethyl caffeate, identified through assessments like the DPPH test**.**

II.4.2. Anti-inflammatory activity

Inflammation is a necessary response for tissue repair but can become problematic when chronic, associated with various diseases. Anti-inflammatory mechanisms are essential for controlling this response, involving various substances such as enzymes, cytokines, and eicosanoids **(Soliman and Barreda, 2022).**

Caffeic acid phenethyl ester (CAPE) and galangin, both present in propolis, have been observed for their anti-inflammatory properties, showing the ability to inhibit carrageenan-

induced pleurisy, carrageenan-induced edema, and arthritic inflammations in rats. On the other hand, pollen possesses anti-inflammatory properties due to its richness in phenolic compounds and fatty acids (phytosterols), which act by inhibiting the activity of cyclooxygenase and lipoxygenase enzymes. These enzymes are responsible for the conversion of arachidonic acid into compounds that amplify inflammation, such as prostaglandins and leukotrienes **(Rossi et al., 2002).**

II.4.3. Antimicrobial activity

II.4.3.1. Antibacterial activity

Faced with the increase in antibiotic-resistant bacteria, pollen is gaining attention for its remarkable antibacterial properties **(Sayari et al., 2016)**. Its *in vitro* efficacy against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) has motivated numerous studies.

The antibacterial efficacy of pollen lies in its richness in phenolic compounds such as quercetin and kaempferol. These compounds act by disrupting the bacterial cell wall, altering its integrity and function. The mechanism of action involves forming complexes with adhesins and polypeptides exposed on the bacterial cell surface, as well as with cell membrane enzymes. This interaction disrupts ion channels, impeding the vital processes of the bacteria **(Didaras et al., 2020).**

Propolis, with its components like galangin and pinocembrin, as well as benzoic and caffeic acids, demonstrates powerful antibacterial activity. It acts bacteriostatically and bactericidally against a variety of bacteria, including Gram-positive strains, with particular effectiveness. Studies suggest that its mechanism of action may disrupt cell division, disorganize the cytoplasm, inhibit protein synthesis, or hinder bacterial adherence **(Cardinault et al., 2012).**

II.4.3.2. Antifungal activity

The antifungal properties of bee pollen have been extensively studied against various pathogenic fungi, including Candida species *(C. albicans, C. glabrata, and C. krusei*), Aspergillus species, and others. This antifungal activity is primarily attributed to the phenolic compounds and flavonoids present in bee pollen, which possess strong antioxidant properties that disrupt fungal cellular processes. Phenolamides, also present in bee pollen, enhance its antifungal efficacy by targeting different aspects of fungal physiology **(Rodríguez-Pólit et al., 2023).**

Propolis also exhibits significant antifungal properties due to its complex mixture of bioactive substances, including flavonoids and phenolic acids. It has demonstrated antimycosic effects against various yeasts, such *as C. pelliculosa, C. parapsilosis, Pichia ohmeri*, and *C. famata*, by disrupting fungal cell membranes and inhibiting key metabolic pathways. These findings highlight the potential of bee products as natural antifungal agents, offering a promising alternative to synthetic drugs with fewer side effects and a reduced risk of resistance **(Zulhendri et al., 2021).**

Chapter III

Materials and Methods

III. Materials and Methods

III.1. Materials

Details regarding the chemicals, reagents, glassware, equipment, and other non-biological materials employed in the experiment are listed in **Appendix 1**.

III.1.1. Plant material

This study focuses on the biological properties of two hive products: bee pollen and propolis. These products were sourced from a beekeeper in the commune of Ouacif, located in the wilaya of Tizi Ouzou at an altitude of 800 meters. The samples are shown in Figure 5.

Figure 5: Plant material (propolis and pollen) used in this study.

III.1.2. Preparation of plant matrix

After collection, the hive products were cleaned to remove impurities such as bee wings and dust. They were then dried in an oven at 40°C. Once dried, the pollen and propolis were each ground using a coffee grinder to obtain a uniform powder. This powder was then passed through a 200 µm sieve to ensure consistency. The fine and homogeneous powder was stored in airtight glass containers, protected from light, until use.

Figure 6 : Clean, dried and sieved pollen and propolis powders.

III.2. Methods

III.2.1. Extraction of phenolic compounds from hive products

The extraction techniques used in this study include ultrasonic extraction and maceration. These solid-liquid extraction processes involve contacting the pollen and propolis powders with a solvent to isolate the active compounds they contain.

III.2.1.1. Ultrasonic-assisted extraction

Ultrasonic-assisted extraction was performed following the optimal extraction conditions described by **Oroian et al. (2020)**. For the extraction of phenolic compounds from propolis and pollen, 10 g of each powdered sample were combined with 100 mL of 70% ethanol. The extraction process was conducted in an ultrasonic bath for 30 minutes at 65°C. The resulting ethanolic extracts were filtered using Wattman paper (No. 02). After filtration, the propolis and pollen extracts were evaporated using a rotary evaporator, then lyophilized, and stored at -20°C for further analysis.

III.2.1.2. Maceration extraction

Propolis and pollen samples, each weighing 10 g, were individually combined with 100 mL of 70% ethanol at a ratio of 1:10 (w/v). After 24 hours of maceration at room temperature with gentle and constant agitation, the mixtures were filtered using filter paper to separate the solid residues from the liquid extract. After filtration, the extracts were evaporated using a rotary evaporator, then lyophilized, and stored at -20°C for further analysis **(Falleh et al., 2008)**.

III.2.2. Quantification of phenolic compounds

III.2.2.1. Total polyphenol quantification

Total polyphenols were determined using the Folin-Ciocalteu method, which is based on a redox reaction where phenolic compounds reduce the Folin-Ciocalteu reagent, a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMO_{12}O_{40}$). This reduction leads to the formation of a blue complex composed of tungsten and molybdenum oxides. The intensity of this coloration is correlated with the concentration of oxidized phenolic compounds in the sample **(Boizot and Charpentier, 2006)**.

The protocol described by **Singleton and Rossi (1965)** was followed to determine the total polyphenol content. Briefly, 1000 µL of Folin-Ciocalteu reagent (diluted 1:10) was combined with 200 µL of extracts prepared at an appropriate concentration. After a 4-minute incubation in the dark, 800 µL of sodium carbonate (7.5% w/v) was added and the absorbance

was measured at 765 nm using a UV-VIS spectrophotometer after a 30-minute incubation in the dark.

Results were expressed in µg of gallic acid equivalents per mg of sample (µg GAE/mg extract) using the gallic acid calibration curve **(Appendix 2)**.

III.2.2.2. Flavonoid quantification

The method used to quantify flavonoids is based on the chelation reaction between aluminum chloride ($AlCl_3$) and oxygen atoms in flavonoids, resulting in a distinct complex formation. This approach establishes a relationship between the flavonoid concentration in the extract and the intensity of the resulting yellow coloration. Thus, the observed color intensity is directly correlated with the flavonoid concentration in the extract **(Bahorun et al., 1996; Ali-Rachedi et al., 2018).**

The determination of flavonoid content followed the procedure described by **Haddouchi et al. (2016) .** Specifically, 500 µL of a 2% (w/v) ethanolic solution of $AlCl_3$ was added to 500 µL of extracts prepared at an appropriate concentration. After a 1-hour incubation at room temperature in the dark, the absorbance was measured at 450 nm.

A quercetin calibration curve **(Appendix 3)** was used to determine the flavonoid content of the pollen and propolis extracts. Results were presented in µg of quercetin equivalents per mg of sample (µg QE/mg extract).

III.2.3. *In Vitro* antioxidant activity evaluation

To evaluate the antioxidant potential of the hive products, several tests were conducted, including DPPH and $ABTS^{\bullet+}$ radical scavenging assays, as well as the ferric reducing power assay.

III.2.3.1. DPPH radical scavenging activity

The chemical compound 2,2-diphenyl-1-picrylhydrazyl, commonly known as DPPH, is a stable free radical with a violet color. In the presence of antioxidants, this radical undergoes a reduction via hydrogen transfer, resulting in a yellowish color change **(Alara et al., 2018)**.

The DPPH radical scavenging capacity of pollen and propolis extracts was evaluated using the protocol described by **Alara, Abdurahman et al. (2018).** Briefly, 1 mL of a freshly prepared 0.1 mM DPPH solution was mixed with 100 µL of samples prepared at various concentrations. The mixtures were incubated in the dark for 30 minutes, after which the absorbance was measured at 517 nm using a UV-vis spectrophotometer. Ascorbic acid, a standard antioxidant, served as a positive control and was measured under the same conditions **(Appendix 04)**.

The percentage of DPPH radical inhibition (PI%) was determined using the following equation:

$$I(\%) = \frac{\text{Abs}orbance\ \text{control} - \text{Abs}orbance\ sample}{\text{Abs}orbance\ \text{control}} \times 100$$

III.2.3.2. $ABTS^{\bullet+}$ radical scavenging activity

The ABTS assay evaluates the relative effectiveness of antioxidants to neutralize the $ABTS^{\bullet+}$ radical, formed by a reaction between ABTS salt and potassium persulfate. The reduction of the blue-green $ABTS^{\bullet+}$ radical by hydrogen-donating antioxidants is indicated by a color change in the solution **(Djeridane et al., 2006)**.

To evaluate the capacity of pollen and propolis extracts to scavenge the $ABTS^{\bullet+}$ radical, a method described by **Re et al. (1999)** was used with slight adjustments. The $ABTS^{\bullet+}$ stock solution was prepared by mixing a 7 mM ABTS solution with a 2.45 mM potassium persulfate solution in distilled water. This mixture was allowed to react for 16 hours in the dark at room temperature.

A freshly prepared diluted $ABTS^{\bullet+}$ solution with an absorbance of 0.700 at 734 nm was used for the experiment. Then, 50 µL of propolis and pollen extracts, prepared at various concentrations, were added to 1000 µL of the $ABTS^{\bullet+}$ solution. After a 10 min incubation in the dark, the absorbance of the mixture was measured at 734 nm.

The inhibition percentage was calculated using the previously described method, and the IC_{50} value of each extract was established. Ascorbic acid was used as a positive control **(Appendix 05)**.

III.2.3.3. Ferric reducing power

The ferric reducing power assay is designed to evaluate the ability of extracts to convert ferric ion (Fe^{3+}) in the $K_3Fe(CN)_6$ complex to ferrous ion (Fe^{2+}). Initially yellow, ferric ion becomes blue or green when reduced by an electron donor. The color change to blue or green is directly linked to the antioxidant activity **(Dudonne et al., 2009).**

The reducing power of the extracts was evaluated according to the method described by **Le et al. (2007)**. A volume of 250 µL of propolis and pollen samples prepared at various concentrations was mixed with 250 µL of phosphate buffer (0.2 M, pH 6.6) and 250 µL of 1% (w/v) potassium ferricyanide. After a 20-minute incubation at 50°C, 250 µL of 10% trichloroacetic acid (TCA) was added to stop the reaction. The tubes were then centrifuged at

7000 × g for 10 min. Then, 500 µL of each supernatant was combined with 100 µL of 0.1% (w/v) $FeCl_3$ solution and kept in the dark for 10 minutes before measuring the absorbance at 700 nm.

III.2.4. Protein extraction

Initially, the lipid fraction was extracted from the pollen and propolis powders through delipidation using a Soxhlet extractor with n-hexane as the solvent, maintaining a solvent/sample ratio of 1:10 (w/v). This procedure was conducted for 6 hours at 40°C **(Cheikh-Rouhou et al., 2006).**

Following delipidation, the powders were left under a fume hood to ensure complete solvent evaporation, then sieved through a 200 µm sieve and stored in airtight containers at - 20°C.

Proteins from propolis and pollen were extracted using a modified version of **Joshi et al. (2011)**. A quantity of 10 g of delipidated pollen and propolis powders was combined with 100 mL of Tris-HCl buffer (pH 8). This mixture was agitated for one hour at a constant speed of 300 rpm. Subsequently, the mixture was centrifuged at 8000 × g for 15 minutes to collect the supernatant, which contains the proteins. The supernatant was then mixed with two volumes of cold acetone to precipitate the proteins and stored at 4°C for 24 hours. Finally, the precipitated proteins were recovered by centrifugation at 8000 × g for 15 min and lyophilized.

III.2.5. Total protein quantification

The protein content was measured using the **Bradford (1976)** method This colorimetric technique is based on the absorbance shift caused by the interaction between Coomassie blue dye and proteins. The dye binds to basic and hydrophobic amino acid residues, resulting in a color change.

A volume of 20 µL of each sample (propolis and pollen protein isolates) was mixed with 1 mL of prepared Coomassie brilliant blue G-250 reagent and kept in the dark for 5 min. The absorbance was then recorded at 595 nm using a UV-vis spectrophotometer.

The protein concentration was determined using a bovine serum albumin (BSA) standard curve **(Appendix 6)**, and the results are expressed as a percentage.

III.2.6. Functional properties of proteins

III.2.6.1. Solubility

The solubility of protein isolates from pollen and propolis was assessed using the method described by **Stone et al. (2015)** . Briefly, 100 mg of the protein isolate was mixed with 10 mL of a 1M NaCl solution (pH 7). This mixture was stirred for 1 hour at room

temperature and then centrifuged at 8000 × g for 10 min. The protein content in the supernatant was determined using the **Bradford (1976)** assay, with BSA as the standard. Solubility was quantified by dividing the protein content in the supernatant by the total protein content of the isolate, and the results were expressed as a percentage.

III.2.6.2. Water and oil holding capacity

The water and oil holding capacities of the protein isolates from pollen and propolis were evaluated following the procedure described by **Stone et al. (2015).** In this method, 100 mg of the protein isolates were placed in pre-weighed centrifuge tubes, to which 1 mL of water or sunflower oil was added. The mixtures were vortexed for one minute and then allowed to stand at room temperature for 30 min. After incubation, the samples were centrifuged at 8000 × g for 10 min. The holding capacities were then determined and expressed as grams of water or oil retained per gram of protein sample.

III.2.6.3. Emulsifying properties

The emulsifying activity of protein isolates from pollen and propolis was evaluated using the method detailed by **Wang et al. (2018)**. In this approach, 3 mL of a 0.2% (w/v) protein isolate solution was mixed with 1 mL of sunflower oil. The mixture was vigorously vortexed for 5 min to thoroughly blend the two phases, then centrifuged at 8000 × g for 10 min. The emulsion stability was measured at 10, 30, 60, and 90 min after homogenization. The emulsifying capacity and stability were calculated using the following formulas:

$$Emulsifying\ capacity\ (\%) = \frac{Emulsion\ volume}{\text{Total } volume} \times 100$$

$$Emulsion\ stability\ (\%) = \frac{Final\ emulsion\ volume}{Total\ volume} \times 100$$

III.2.6.4. Foaming properties

The foaming capacity and stability of protein isolates from pollen and propolis were assessed following the method of **Wang et al. (2018)**, with slight modifications. A 1% (w/v) protein suspension was homogenized using an electric mixer for 5 minutes. The foam volume was measured immediately (0 min) to determine the foaming capacity, and then at 30, 60, and 90 min to evaluate foam stability at room temperature. Foaming capacity and stability were calculated using the following formulas:

$$\text{Foaming capacity (\%)} = \frac{\text{Initial foam volume}}{\text{Total suspension volume}} \times 100$$

III.2.7. Evaluation of antimicrobial activity

The method used to evaluate the antibacterial activity of various concentrations of propolis and pollen extracts obtained through maceration and ultrasound is the paper disc diffusion method. This technique is based on the appearance of an inhibition zone in the culture medium around the disc containing the tested extract.

III.2.7.1. Bacterial strains

To evaluate the antimicrobial activity, four bacterial strains were used as microbial supports (**Table IV**). These strains were provided by Dr. Djenadi K., a teacher in the Department of Biology at the Faculty of SNV-ST, University of Bouira.

The selected strains cover a wide spectrum of bacteria, including both Gram-positive and Gram-negative species, to ensure a comprehensive evaluation of the antimicrobial activity of the tested extracts.

Table IV : General Characteristics of the Tested Bacterial Strains.

Strain Name	Reference	Gram
Escherichia coli	ATCC25922	Negative
Staphylococcus aureus	ATCC23235	Positive
Pseudomonas aeruginosa	ATCC 6633	Negative
Klebsiella pneumoniae		Negative

III.2.7.2. Preparation of bacterial strains (subculturing)

Antibacterial tests must be conducted using young cultures aged 18 to 24 hours in the exponential growth phase. The subculturing of strains (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*) was performed by inoculating each bacterial species into its specific medium (Chapman for *Staphylococcus aureus*, EMB for E. coli and *Klebsiella pneumoniae*, and King's medium for *Pseudomonas aeruginosa),* followed by incubation at 37°C for 18 to 24 hours **(Moroh et al., 2008).**

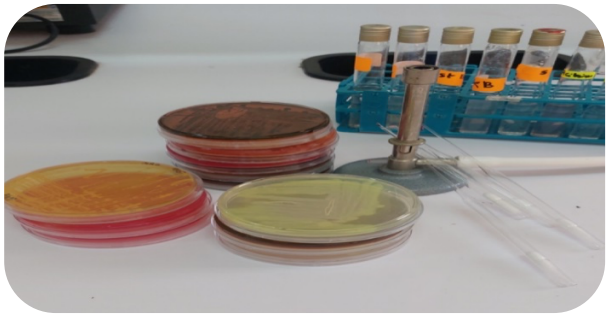

Figure 7: Bacterial strains replated on isolation media (original photograph).

III.2.7.3. Preparation of the inoculum

After 24 hours of incubation, 1 to 2 well-isolated and identical colonies in the exponential growth phase were taken from each tested strain using a platinum loop. These colonies were then placed in a tube containing sterile physiological water with 0.9% NaCl. The tubes were homogenized using a vortex to achieve an optical density between 0.08 and 0.1 at a wavelength of 620 nm, corresponding approximately to 10^8 CFU/ml.

III.2.7.4. Preparation of extract dilutions

The various pollen and propolis extracts obtained through maceration and ultrasound were dissolved in dimethyl sulfoxide (DMSO) at an initial concentration of 100 mg of each extract per 1 ml of DMSO solution. Subsequently, the stock solution was diluted to 1/2 and 1/4 to achieve concentrations of 50 mg/ml and 25 mg/ml, respectively.

III.2.7.5. Aromatogram

A bacterial suspension was swabbed from top to bottom with closely spaced streaks on the surface of Muller-Hinton agar. Discs impregnated with 20 µl of each extract, at concentrations of 100 and 50 mg/ml prepared in advance, were placed on the surface of the inoculated agar using sterile forceps. The Petri dishes were then left for 2 hours at room temperature to allow proper diffusion of the tested extracts before being incubated at 37°C for 24 hours. Antimicrobial activity was determined by measuring the inhibition zones that appeared around the discs **(Mothana and Lindequist, 2005; Bolou et al., 2011).**

III.2.7.5.1. Reading of aromatogram

The reading of the aromatograms was done by measuring the diameters of the inhibition zones around the discs.

The results are expressed by the diameter of the inhibition zone**:** Inhibition zones ≥ 15 mm were classified as strong, 8 to 15 mm as moderate, and ≤ 8 mm as resistant **(Bansemir et al., 2006)**.

II.2.7. Statistical analysis

Experiments were conducted in triplicate, with data presented as mean ± standard deviation. One-way ANOVA was used to analyze differences between groups, while Two- way ANOVA evaluated the impact of two variables. Significance was determined at $p < 0.05$.

Chapter IV

Results and Discussion

IV. Results and Discussion

This study focuses on propolis and pollen extracts obtained by two extraction methods: maceration and ultrasound. For each method, the extraction yield was calculated. The polyphenol and flavonoid contents were also determined. The antioxidant activity of the extracts was analyzed using various tests, including DPPH, ABTS and FRAP. In addition, the antimicrobial activity of the extracts was assessed.

IV.1. Extraction yields of polyphenolic extracts from propolis and pollen

Extraction yield results for both extraction techniques are presented in **Table V**. They show that ultrasound extraction rates are higher than those obtained by maceration, for both pollen and propolis, although the extraction conditions applied to both methods are identical in terms of quantity of plant material and type of extraction solvent (10 g powder and 100 ml 70% ethanol).

Table V : yields of polyphenolic extracts from pollen and propolis using different methods

Extract	**Propolis**		**Pollen**	
Method used	**Maceration**	**Ultrasound**	**Maceration**	**Ultrasound**
Yield (%)	32 ± 0.254^{b}	49 ± 0.7^{a}	23 ± 0.12^{c}	35 ± 0.97^{b}

Letters indicate significant differences between samples ($p < 0.05$). Samples with the same letters are not significantly different.

It should also be noted that propolis showed the highest extraction rates, reaching 49% with ultrasonic extraction and 32% with maceration extraction. In comparison, pollen extraction rates were 35% with ultrasonic extraction and 23% with maceration extraction. Ethanol was chosen as the extraction solvent in this study due to its low toxicity and frequent use in the composition of many therapeutic preparations **(Brehon et al., 2000)**. It evaporates easily and solubilizes the active components of pollen and propolis **(Krell, 1996)** .

A study by **Ouahab et al. (2023)** on propolis extracts of prepared by maceration with 70% ethanol gave an extraction rate of 31.2%, in line with those obtained in our study.

Several comparative studies have demonstrated the superiority of ultrasound extraction for propolis and pollen over maceration. Ultrasound, by producing cavitation bubbles and intense shear, facilitates more complete and rapid extraction. They improve contact between matrix and solvent, fragment the matrix, increase cell pore size and accelerate mass exchange, resulting in higher extraction yields **(Oroian et al., 2020).**

According to the literature, variations in the extraction rates of phenolic compounds can be influenced by several factors such as the solubility of phenolic compounds in extraction solvents, the types of solvents used and their purity levels, the extraction method, extraction time, particle size, and temperature **(Hayat et al., 2009).**

IV.2. Total polyphenol content

The polyphenol content in pollen and propolis was measured using the Folin-Ciocalteu method. The results of the total polyphenol content in the ethanolic extracts of pollen and propolis are presented in **Figure 8**.

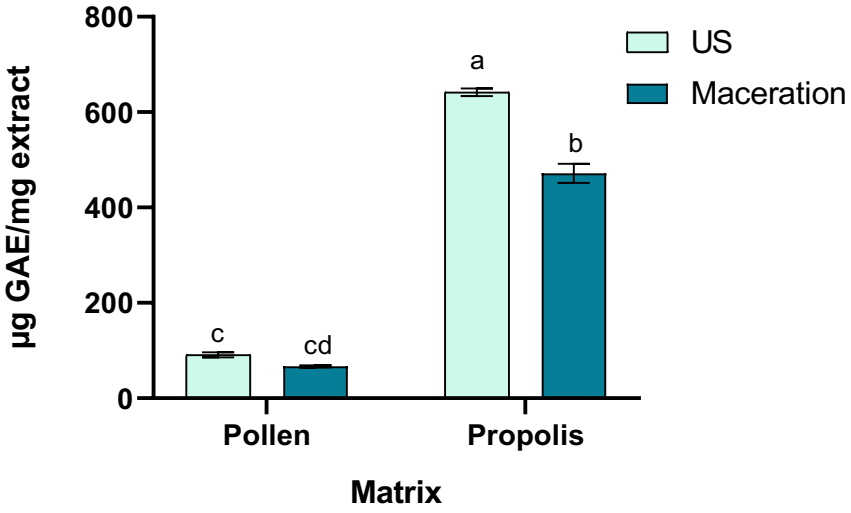

Figure 8 : Polyphenolic compound content of various extracts.

The findings reveal that all hydroethanolic extracts of the bee products studied are rich in polyphenols, though their quantities vary. As shown in **Figure 8**, the hydroethanolic extract of propolis consistently exhibits the highest phenolic compound content, regardless of the extraction method used, with a concentration of 641.616 ± 7.95 µg GAE/mg for the ultrasound extract and 471.515 ± 19.841 µg GAE/mg for the maceration extract. Conversely, pollen shows the lowest content, with 66.936 ± 2.386 µg GAE/mg and 91.043 ± 5.41 µg GAE/mg for the maceration and ultrasound extracts, respectively.

Statistical analysis indicates no significant difference between the two extraction methods for pollen. However, significant differences are observed between the two methods for propolis, suggesting that ultrasound is significantly more effective than maceration.

The results obtained in our study surpass those reported by **Mouhoubi-Tafinine et al. (2016),** who found a polyphenol content in propolis of 53.512 µg GAE/mg. Similarly, **Blanc (2010)** reported a polyphenol content in pollen of 29.38 g GAE/100g, which are lower than those we obtained. Furthermore, the results obtained in our study are quite higher compared to the result **of Pascoal et al. (2014)** (32.15 ± 2.12 mg GAE/g pollen).

These variations in content can be attributed to the botanical and geographical origin of the bee products, as well as the diversity of pollen profiles **(Ouchemoukh et al., 2007)**. These findings highlight the importance of bee products as a promising source of polyphenols, paving the way for future research into their applications in various fields.

IV.3. Total flavonoid content

The aluminum chloride method was used to quantify flavonoids in the ethanolic extracts of pollen and propolis. The total flavonoid concentrations are presented in **Figure 9**.

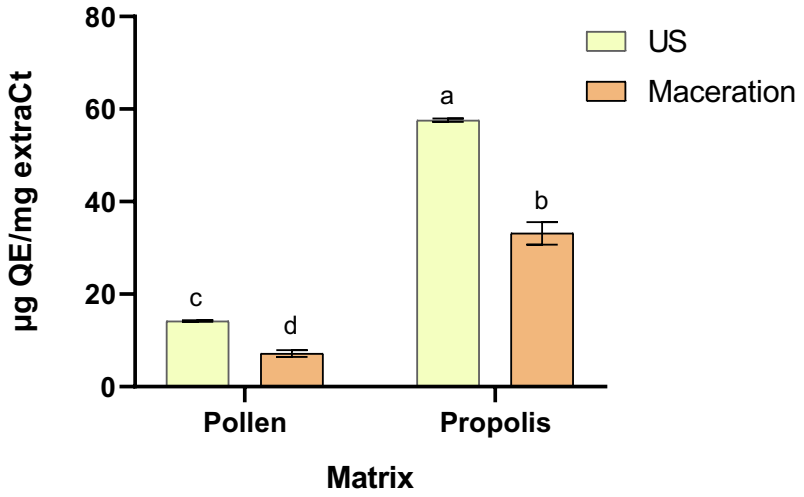

Figure 9: Flavonoid content of various extracts.

Similar to the polyphenol content, this test reveals a significant variation in the total flavonoid content among the different extracts. Extracts obtained by ultrasonication show a significantly higher flavonoid concentration than those obtained by maceration. Notably, the hydroethanolic extract of propolis shows higher flavonoid content for both extraction methods, with 57.598 ± 0.352 µg QE/mg for ultrasonication and 33.158 ± 2.437 µg QE/mg for maceration. Conversely, pollen displays the lowest content, with 7.179 ± 0.716 µg QE/mg and 14.188 ± 0.154 µg QE/mg for maceration and ultrasound extraction, respectively.

The amount of flavonoids present in propolis is also higher than that reported by **Lanez et al. (2014)** for Khenchla propolis, which was 3.46 mg QE/g.

The flavonoid content of the pollen obtained in this study is higher than the results obtained by **Mărghitaş et al. (2009)** for Romanian pollen (8.8 ± 0.1 to 12.7 ± 0.1 mg QE/g). This difference could be explained by the floral origin (each plant has its own flavonoids) and climatic conditions.

IV.4. Antioxidant activity

IV.4.1. Ferric reducing antioxidant power (FRAP)

The FRAP test involves an electron transfer reaction, using a ferric salt as the oxidizing agent. During this test, the yellow color of the solution changes to various shades of green and blue, depending on the reducing potential of the antioxidant samples **(Gülçin et al., 2010)**. An increase in absorbance indicates an increase in reducing power. The following figure presents the results of the reducing power assessment.

The results indicate that all extracts exhibit varying degrees of reducing power, with statistically significant differences ($p < 0.05$). Among them, the extracts obtained by maceration show the highest reducing power, with $A_{700nm} = 3 \pm 0.00$ for propolis extract and $A_{700nm} = 0.819 \pm 0.007$ for pollen extract tested at a concentration of 1500 µg/mL. Similarly, the extracts obtained by ultrasonication show that propolis retains the highest reducing power ($A_{700nm} = 2.975 \pm 1.03$), followed by pollen ($A_{700nm} = 0.64 \pm 0.01$) at the same concentration. Thus, the propolis extract exhibits the highest reducing power regardless of the extraction method used: maceration or ultrasonication.

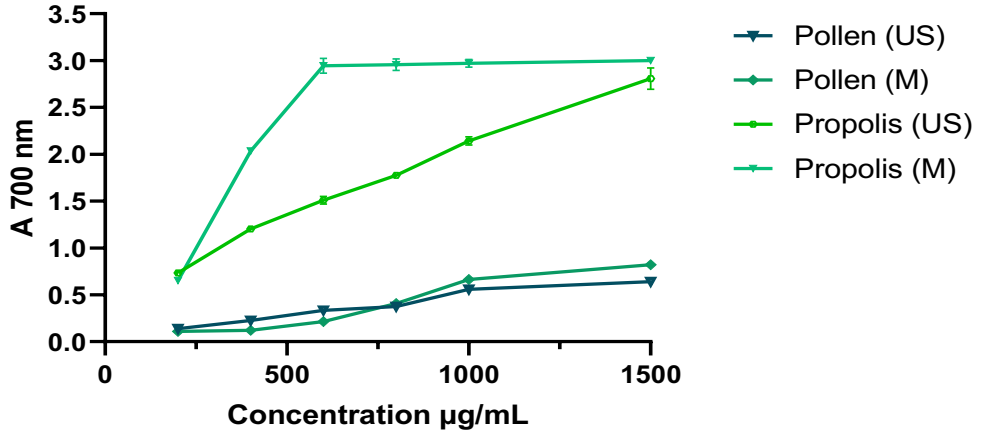

Figure 10: Reducing power of propolis and pollen extracts.

IV.4.2. DPPH radical scavenging activity

The results concerning the antiradical activity of hydro-ethanol extracts of pollen and propolis are presented in the figure. The results obtained show that pollen and propolis extracts obtained by maceration and ultrasound have a DPPH˙ radical scavenging capacity, with percentages ranging from 61.31 ± 0.181% to 100 ± 0.00% for a concentration of 1500 µg /mL extract.

To assess the antioxidant activity towards the DPPH˙ radical of pollen and propolis extracts, IC50 values were calculated and are presented in **Figure 11.a**. IC_{50} is a widely used parameter for measuring the antioxidant activity of samples, defined as the concentration required to reduce the initial DPPH- concentration by 50%. The lower the IC_{50} value, the higher the antioxidant activity. It should be noted that IC_{50} is inversely related to the antioxidant capacity of the compound, with a lower IC_{50} value indicating better antioxidant activity.

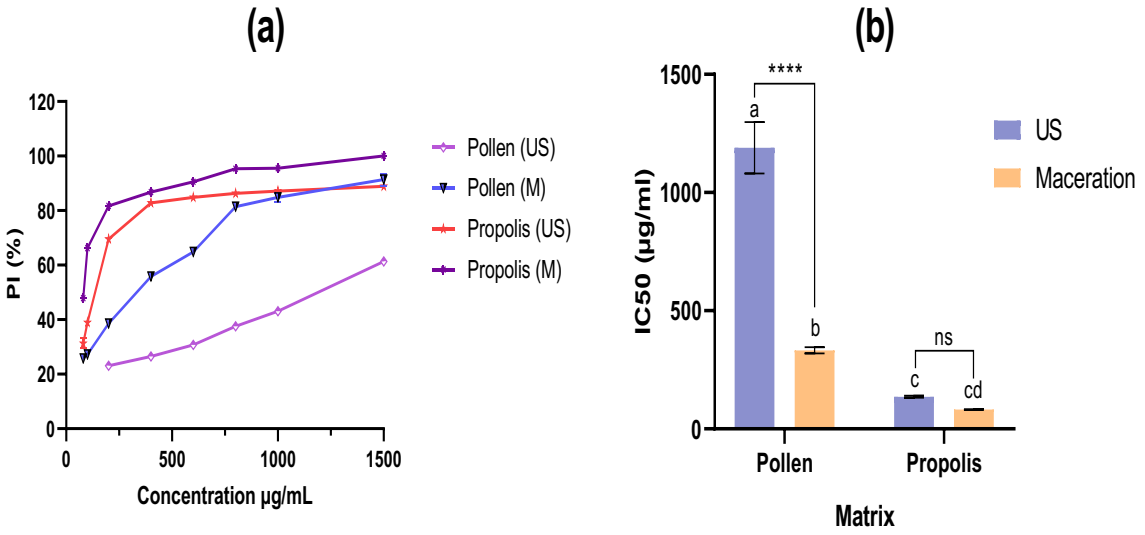

Figure 11: DPPH radical scavenging activity of various extracts, expressed as inhibition percentage (%) (a) and IC_{50} (b).

The results indicate that the propolis extract has a better antioxidant capacity against the DPPH˙ radical, with an IC_{50} of 82.341 ± 1.095 µg/mL for maceration and 136.227 ± 4.655 µg/mL for ultrasonication, followed by pollen with IC_{50} values of 1189.36 ± 108.95 µg/mL and 332.417 ± 12.77 µg/mL for the ultrasound and maceration extracts, respectively. This capacity can be attributed to the chemical structure of phenolic acids and flavonoids present in the pollen

and propolis extracts, which have hydrogen-donating ability and form a stable phenoxyl radical. However, when compared to the IC_{50} value of 39 ± 0.024 µg/mL for ascorbic acid, a synthetic antioxidant, its antioxidant activity is notably stronger.

Statistical analysis reveals no significant difference between the two extraction methods for propolis, whereas for pollen, a significant difference is observed, showing that maceration is significantly more effective than ultrasonication.

According to **Ferreira et al. (2024)**, the propolis extract exhibits DPPH$^{\bullet}$ radical scavenging activity with an IC_{50} of 18.9 ± 0.01 µg/mL, a better activity than that obtained in this study.

IV.4.3. ABTS$^{\bullet+}$ radical scavenging activity

The ABTS$^{\bullet+}$ radical scavenging activity of pollen and propolis extracts obtained by two distinct extraction methods was evaluated, and their inhibition percentages as well as IC_{50} values are presented in **Figure 12**. Both extracts show a dose-dependent relationship, with a notable increase in ABTS$^{\bullet+}$ radical inhibition percentage as extract concentrations increase.

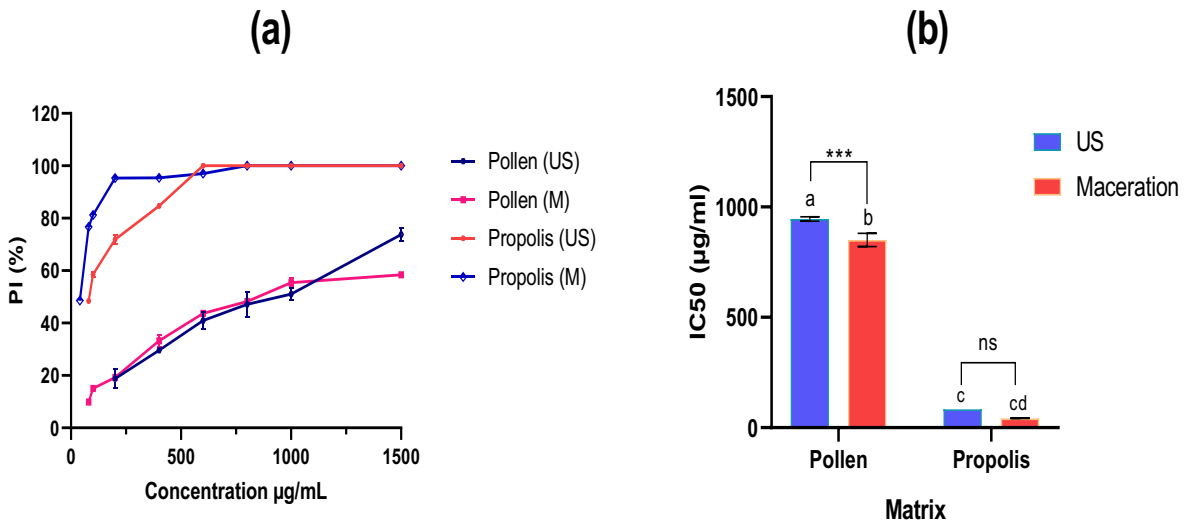

Figure 12: ABTS$^{\bullet+}$ radical scavenging activity of various extracts, expressed as inhibition percentage (%) (a) and IC_{50} (b).

Among the propolis extracts, the extract obtained by maceration showed the highest activity, with an IC_{50} value of 42.044 ± 0.73 µg/mL, higher than that of ascorbic acid (51 ±

0.003 µg/mL). In contrast, the propolis extract obtained by ultrasound extraction did not show a significant difference ($p > 0.05$), recording an IC_{50} value of 83.14 ± 0.401 µg/mL. These results are significantly better than those of the pollen extracts, which exhibit IC_{50} values of 850.30 ± 29.91 µg/mL and 945.45 µg/mL for the maceration and ultrasound extraction methods, respectively.

According to **Miguel et al. (2014)**. Immouzzer propolis has an $ABTS^{\bullet+}$ radical scavenging capacity with an IC_{50} of 9 µg/mL, an activity superior to that found in this study. This variation could be explained by differences in geographical origin.

IV.5. Protein extraction yield

The protein extraction yield from propolis is 2.8%, reflecting its relatively low protein content, consistent with its known chemical composition. Propolis is predominantly composed of resins, balms, essential oils, and flavonoids, with a scarcity of proteins. The intricate and resinous nature of propolis poses challenges for protein extraction, as noted in several studies.

Table VI: Extraction yield of protein from pollen and propolis.

Extract	Pollen	Propolis
Yield (%)	11 ± 1.2	2.8 ± 0.6

Kujumgiev et al. (1999) demonstrated that propolis contains various bioactive compounds but has a low protein content, which accounts for the low extraction yield. Similarly, **Bankova et al. (2000)** confirmed that phenolics and flavonoids are the primary constituents of propolis rather than proteins.

In contrast, the protein extraction yield from pollen is notably higher at 11%, underscoring its richness in proteins. Pollen serves as a crucial nutritional source for bees, containing substantial amounts of proteins and essential amino acids.

Evans et al. (1991) reported a protein extraction yields of 26%, surpassing the yield of our study, which was only 11%.

This revised version maintains clarity while incorporating the findings from cited studies and providing a cohesive flow between the information on propolis and pollen.

IV.6. Functional properties of pollen and propolis protein extracts

IV.6.1. Solubility

Protein solubility is a critical indicator affecting their functional properties. The solubility of each protein extract was determined, with the results shown in **Table VII**. Significant differences ($p < 0.0001$) were observed between the two protein samples. The propolis protein extract exhibited higher solubility (10.627 ± 0.2388%) compared to pollen (2.176 ± 0.082%), even though pollen had a higher protein content (81.927 ± 2.81%).

Studies have shown that higher protein concentrations can lead to protein-protein interactions, aggregation, and precipitation, thus reducing solubility. Conversely, lower protein concentrations minimize these interactions, resulting in better solubility. Additionally, the lower protein concentration in the propolis extract suggests the presence of other highly soluble components in aqueous solutions **(Chandran et al., 2023)**.

Table VII: Solubility and protein content of pollen and propolis protein extracts.

Protein Extract	Protein Content (%)	Solubility (%)
Pollen	81.927 ± 2.812 [a]	2.176 ± 0.082 [b]
Propolis	5.123 ± 0.158 [b]	10.627 ± 0.2388 [a]

Letters indicate significant differences between samples ($p < 0.05$) in the same column. Samples with the same letters are not significantly different.

Kostić et al. (2015) reported solubility for 25 Serbian pollen extracts ranging from 2.79% to 25.9%. In the food industry, common plant protein sources include legumes, cereals, and oilseeds. Comparing our results with commercial protein sources, the solubility of propolis and pollen is lower than that of soy (14.9%) **(Stone et al., 2015)** and peanut (19.18%) **(Wu et al., 2009)**, but higher than wheat protein isolate (0.7%) **(Stone et al., 2015).** Pollen solubility is lower, while propolis solubility is higher than pea protein isolate (5%) **(Stone et al., 2015)**.

IV.6.2. Emulsifying activity and emulsion stability

The emulsifying activity and emulsion stability of pollen and propolis protein extracts over time are detailed in **Table VIII**. Pollen protein extract exhibited an emulsifying activity of 24 ± 1.05%, which was not significantly higher ($p > 0.05$) than the 20 ± 1.11% shown by propolis protein extract. Emulsifying activity refers to the ability of proteins to interact with

both oil and water to form stable emulsions, depending on factors like ionic charge and surface hydrophobicity **(Zhang et al., 2020)**. Key factors influencing emulsifying activity are protein solubility and surface hydrophobicity **(Liu et al., 2011).** Therefore, the low emulsifying activity of both pollen and propolis protein extracts can be attributed to their low solubility.

Table VIII: Emulsifying activity and emulsion stability of protein extracts.

Protein Extract	Emulsifying Activity (%)	Emulsion Stability (%)		
		30 min	60 min	90 min
Pollen	24 ± 1.05^{a}	20 ± 0.00^{a}	20 ± 0.00^{a}	20 ± 0.00^{a}
propolis	20 ± 1.11^{a}	18 ± 0.1^{a}	14 ± 0.00^{b}	14 ± 0.00^{b}

Letters indicate significant differences between samples ($p < 0.05$) in the same column. Samples with the same letters are not significantly different.

Emulsion stability refers to the ability of proteins to prevent destabilization phenomena such as creaming, flocculation, and coalescence over time **(Afizah and Rizvi, 2014; Gong et al., 2016).** Results revealed that emulsion stability decreased after 30 min at room temperature, reaching 20 ± 0.00% for pollen protein extract and 18 ± 0.1% for propolis protein extract. After 60 and 90 minu, pollen protein extract maintained its stability, indicating its capacity to sustain emulsions over a longer period. In contrast, propolis protein extract's stability decreased to 14 ± 0.00% after 30 min and remained unchanged after 90 min.

Emulsion stability is maintained by electrostatic repulsions between proteins adsorbed at the interfacial film, influenced by the protein's surface charge. However, the observed stability decrease over time suggests a weakening of the interfacial film due to reduced protein-protein interactions **(Mundi and Aluko, 2012; Wani et al., 2015)**.

IV.6.3. Water and oil holding capacity

Water holding capacity (WHC) and oil holding capacity (OHC) are essential protein properties determining their ability to absorb water and oil, respectively. These characteristics make proteins valuable as additives in food products, enhancing quality, including shelf life, texture, and flavor **(Mohan and Mellem, 2020; Zhang et al., 2020).**

The WHC and OHC of pollen and propolis protein extracts are presented in **Table IX**. Pollen protein extract demonstrated a WHC of 1.06 ± 0.12 g/g, comparable to Serbian pollen

extracts with values ranging from 0.92 to 2.25 g/g. Protein WHC largely depends on their amino acid composition **(Aryee et al., 2018)**. The relatively low WHC of pollen protein extract suggests a deficiency in hydrophilic amino acids essential for water binding **(Sathe et al., 1982; Afizah and Rizvi, 2014)**

Table IX: Water and oil holding capacity of pollen and propolis protein extracts.

Protein Extract	WHC (g/g)	OHC (g/g)
Pollen	1.06 ± 0.12^{b}	5.868 ± 0.384^{a}
Propolis	4.938 ± 0.26^{a}	4.88 ± 0.345^{a}

Letters indicate significant differences between samples ($p < 0.05$) in the same column. Samples with the same letters are not significantly different.

Conversely, the propolis protein extract showed significantly higher WHC ($p < 0.001$) than pollen protein extract, with a value of 4.93 ± 0.26 g/g, indicating a rich presence of hydrophilic amino acids capable of binding water molecules.

Regarding OHC, pollen protein extract exhibited a substantial capacity of 5.86 ± 0.384 g/g, surpassing reported values for Serbian bee pollen extracts, ranging from 1.00 to 3.53 g/g. High OHC is likely due to numerous non-polar side chains retaining oil through associative binding **(Aryee et al., 2018)**. This suggests that pollen protein extract has a higher content of hydrophobic amino acids.

Propolis protein extract had an OHC of 4.88 ± 0.345 g/g, not significantly different ($p > 0.05$) from that of pollen protein extract. The similar OHC and WHC values in propolis protein extract may indicate a balanced ratio of hydrophilic and hydrophobic amino acids.

IV.6.4. Foaming property

The foaming capacity of proteins is determined by their ability to unfold under certain conditions and form a cohesive layer around air bubbles, creating foam **(Jiang et al., 2021)**. The foaming capacities of pollen and propolis protein extracts are shown in **Table X**. Pollen protein extract exhibited a foaming capacity of 3.8% ± 0.16%, higher than the 0.2% ± 0.00% displayed by propolis protein extract. The low foaming capacity observed in both samples can be attributed to their low solubility. Good solubility is crucial for proteins to form foam effectively, as it allows them to rapidly migrate to the air-water interface, reduce interfacial

tension, and undergo conformational changes such as unfolding and interaction to form a protective film trapping air particles **(Wani et al., 2015; Li et al., 2018).**

Table X: Foaming capacity and stability of protein extracts.

Extract	Foaming capacity (%)	Foaming stability (%)		
		30 min	60 min	90 min
Pollen	$3.8 \pm 0,16^{a}$	$0.8 \pm 0,00^{a}$	$0.8 \pm 0,00^{a}$	$0.8 \pm 0,00^{a}$
propolis	$0.2 \pm 0,00^{a}$	$0.2 \pm 0,00^{a}$	$0.2 \pm 0,00^{b}$	$0.2 \pm 0,00^{b}$

Letters indicate significant differences between samples ($p < 0.05$) in the same column. Samples with the same letters are not significantly different.

Examining foam stability over time, it was found that foam stability decreased to 0.8% ± 0.00% for pollen protein extract and to 0.2% ± 0.00% for propolis protein extract after 10 minutes at room temperature, with these values remaining constant for the rest of the testing period. This reduction in foam stability suggests that the interfacial film was not cohesive and viscous enough to maintain stable air bubbles over an extended period **(Fidantsi and Doxastakis, 2001).** For good foam stability, forming a strong interfacial film of adsorbed proteins is crucial, resulting from significant protein-protein interactions at the interface. This interaction increases viscosity by enhancing interactions between water and protein molecules, facilitating the formation of a cohesive multilayer film **(Zhang et al., 2020).**

IV.7. Antimicrobial activity

IV.7.1. Aromatogram

The results concerning the antibacterial activity of ethanolic extracts of pollen and propolis at different concentrations on bacterial strains are presented in **Table XI**.

The results of the inhibition zone diameters show a proportional correlation with the concentrations of the extracts used. The propolis extract obtained by ultrasound exhibited the largest diameters for both *S. aureus* and *E. coli.*

According to the table, the most significant inhibition zones were observed for *S. aureus ATCC 29213,* with a notable zone of 18.0 ± 1.41 mm, and for *E. coli*, with a zone of 10 mm, at a concentration of 100 mg/ml. These findings are consistent with those of **Bonvehí and**

Gutiérrez (2012), who reported inhibition zones ranging from 10 to 16 mm for *S. aureus*, confirming strong antibacterial activity of their propolis.

Table XI : Aromatogram results of ethanolic extracts of pollen and propolis.

Concentration mg/mL	***E.coli***				***S. aureus* ATCC 29213**				***P. aeruginosa***				***Klebsiella pneumonie***			
Extract	**Pollen**		**Propolis**		**Pollen**		**Propolis**		**Pollen**		**Propolis**		**Pollen**		**Propolis**	
Extraction technique	**M**	**US**	**M**	**US**	**M**	**US**	**M**	**US**	**M**	**US**	**M**	**US**	**M**	**US**	**M**	**US**
100	-	-	9±0.16	10±0.54	13± 0	15± 0 .0	16±1.12	18 ± 1.41	-	-		7 ± 0.2	-	-	-	-
50	-	-	8±0.0	8±0.0	11 ± 1.3	14± 1.23	15±0.03	15± 1.1	-	-	-	-	-	-	-	-
25	-	-	7±0.0	7±0.0	10 ± 0.9	12± 1.00	12±0.15	13±0.45	-	-	-		-	-	-	-

Our observations regarding *E. coli* are supported by the studies of **Segueni et al. (2011)** and **Boufadi et al. (2016)**, which explored the antibacterial activity of Algerian propolis from Tizi Ouzou, Mostaganem, Mila, and Jijel, demonstrating inhibition zones of 11 mm, 11.5 mm, 10 mm, and 14 mm respectively.

Regarding pollen, the ethanolic extract obtained by ultrasound showed the strongest inhibitory effect against *S. aureus ATCC 29213* at a concentration of 100 mg/ml, producing an inhibition zone of 15.0 ± 0 mm. However, no inhibition was observed for *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Our results confirmed that the propolis polyphenolic extract has good activity against both Gram-positive and Gram-negative bacteria. However, Gram-positive bacteria were more sensitive than Gram-negative bacteria. **Silici and Kutluca (2005)** studied the antimicrobial activity of three types of propolis collected by three different bee races against *S. aureus, E. coli*, Pseudomonas aeruginosa, and Candida albicans, and found that ethanolic extracts of propolis (EEP) showed strong activity against S. aureus but weak activity against Gram-negative bacteria (*E. coli, P. aeruginosa, and C. albicans*).

The antibacterial activity of propolis may due to polyphenols, aromatic acids and esters. The mechanism of this activity is attributed to a synergy between phenolic compounds and other compounds in the resin **(Boufadi et al., 2016).**

The low susceptibility identified in Gram-negative bacteria is consistent with numerous previous studies, suggesting these bacteria have very limited sensitivity to the bactericidal action of propolis and pollen **(Kujumgiev et al., 1999; Moreno et al., 1999)**. The most plausible explanation for the low sensitivity demonstrated by Gram-negative bacteria could be their outer membrane, which inhibits and/or delays the penetration of propolis and pollen.

Literature indicates that the susceptibility of microorganisms and the differences in active components of propolis with antibacterial and antifungal activities are strongly influenced by geographical variations in propolis sources **(Bankova et al., 2000)**. Ultrasound-assisted extraction proves more effective due to the use of high-frequency sound waves creating cavitations in the solvent, thereby enhancing the extraction of bioactive compounds from propolis, particularly phenolic compounds and flavonoids known for their antimicrobial properties.

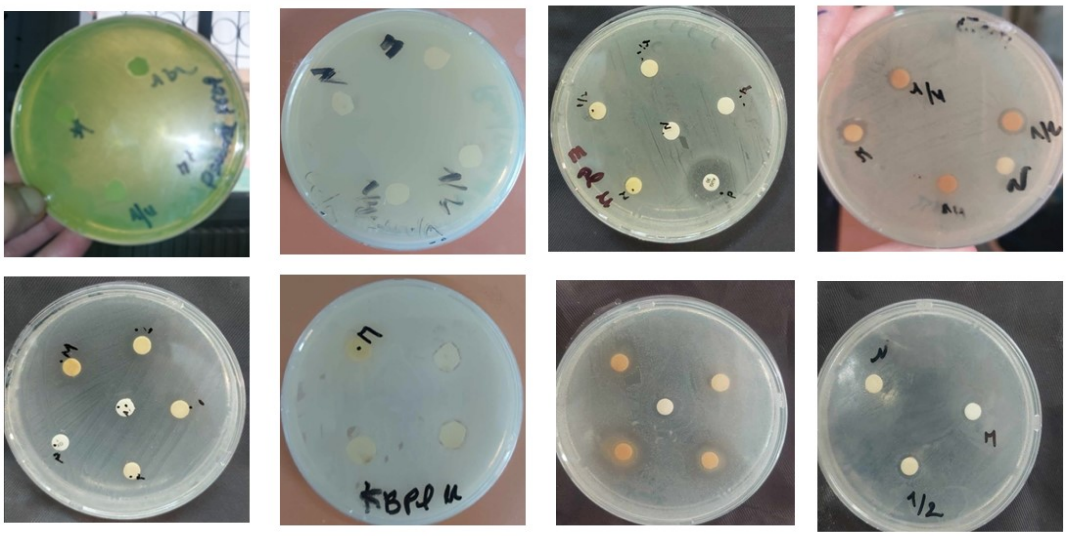

Figure 13 : Antimicrobial activity of propolis and pollen extracts after 24 hours of incubation.

General

Conclusion and perspectives

General conclusion and perspectives

Natural substances play a crucial role in our daily lives, particularly by offering a wide range of therapeutic and nutritional options. In particular, propolis and bee pollen represent a rich source of chemical compounds beneficial to our well-being.

This study aims to quantitatively evaluate the phenolic compounds and total proteins in two bee products, propolis and pollen, collected from the Wilaya of Tizi Ouzou. Additionally, we investigated their antioxidant and antimicrobial activities, as well as their functional properties. To achieve these objectives, we utilized maceration extraction and ultrasound-assisted extraction methods with ethanol as the solvent for polyphenol extraction, and the acetone precipitation method for protein extraction. Our findings indicate that propolis extract exhibited the highest extraction efficiency, regardless of the extraction method employed.

The phenolic compound and flavonoid contents vary between the two samples. The propolis extract demonstrates notably high levels of total phenolic compounds and flavonoids, measuring 641.616 ± 7.95 µg GAE/mg and 57.598 ± 0.352 µg QE/mg, respectively.

The investigation of antioxidant activity using various methods has revealed that propolis extract exhibits the highest antioxidant potential. This is evidenced by its superior performance in the FRAP test ($A_{700\ nm}$ = 3 ± 0.00), its potent DPPH radical scavenging activity (IC_{50} = 83.341 ± 1.095 µg/mL), and its effective $ABTS^{\bullet+}$ radical scavenging activity (IC_{50} = 42.044 ± 0.73 µg/mL).

Evaluation of the protein content in the extracts obtained from pollen and propolis revealed that the pollen protein extract has the highest protein content (81.92 ± 2.81%). Further investigation into functional properties demonstrated that the pollen protein extract exhibited superior emulsifying and foaming properties, as well as a higher oil-holding capacity, compared to the propolis protein extract. Conversely, the propolis extract displayed greater solubility and water-holding capacity.

The antibacterial activity of the ethanolic extracts was assessed using the disk diffusion method on both wild and resistant bacterial strains. Propolis and pollen extracts exhibited the highest activity against S. aureus strains.

Based on the results obtained, we can conclude that propolis and bee pollen are invaluable natural products with a remarkable diversity of applications and benefits. Their unique properties underline their importance in a variety of fields.

In future research, there are several key areas worth exploring to better understand and utilize the potential of bee products:

- Increase sample diversity and compare propolis and bee pollen with other bee products, such as royal jelly and venom.
- Conduct further research into the optimization of extraction, fractionation and identification methods.
- Evaluate other biological activities, such as antifungal, antidiabetic and wound-healing properties.
- Enhance the value of hive products in the fields of nutrition, pharmacology and cosmetology.

References

References

Afizah, M.N., Rizvi, S.S., 2014. Functional properties of whey protein concentrate texturized at acidic pH: effect of extrusion temperature. LWT-Food Science and Technology 57, 290-298.

Alara, O.R., Abdurahman, N.H., Ukaegbu, C.I., Azhari, N.H., 2018. Vernonia cinerea leaves as the source of phenolic compounds, antioxidants, and anti-diabetic activity using microwave-assisted extraction technique. Industrial Crops and Products 122, 533-544.

Ali-Rachedi, F., Meraghni, S., Touaibia, N., Mesbah, S., 2018. Analyse quantitative des composés phénoliques d'une endémique algérienne Scabiosa Atropurpurea sub. Maritima L. Bulletin de la société royale des sciences de liège.

Aryee, A., Agyei, D., Udenigwe, C., 2018. Impact of processing on the chemistry and functionality of food proteins, Proteins in food processing. Elsevier, pp. 27-45.

Aylanc, V., Tomás, A., Russo-Almeida, P., Falcão, S.I., Vilas-Boas, M., 2021. Assessment of bioactive compounds under simulated gastrointestinal digestion of bee pollen and bee bread: Bioaccessibility and antioxidant activity. Antioxidants 10, 651.

Bahorun, T., Gressier, B., Trotin, F., Brunet, C., Dine, T., Luyckx, M., Vasseur, J., Cazin, M., Cazin, J., Pinkas, M., 1996. Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. Arzneimittel-forschung 46, 1086-1089.

Bankova, V., Dyulgerov, A., Popov, S., Evstatieva, L., Kuleva, L., Pureb, O., Zamjansan, Z., 1992. Propolis produced in Bulgaria and Mongolia: phenolic compounds and plant origin. Apidologie 23, 79-85.

Bankova, V.S., de Castro, S.L., Marcucci, M.C., 2000. Propolis: recent advances in chemistry and plant origin. Apidologie 31, 3-15.

Bansemir, A., Blume, M., Schröder, S., Lindequist, U., 2006. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture 252, 79-84.

Blanc, M., 2010. Propriétés et usage médical des produits de la ruche. SCD de l'Université de Limoges.

Boizot, N., Charpentier, J.-P., 2006. Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier. Le Cahier des Techniques de l'INRA, In: Numéro spécial, 79-82.

Bolou, G., Attioua, B., N'guessan, A., Coulibaly, A., N'guessan, J., Djaman, A., 2011. Évaluation in vitro de l'activité antibactérienne des extraits de Terminalia glaucescens

planch. sur Salmonella typhi et Salmonella typhimurium. Bulletin de la société royale des sciences de liège.

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 and Biotechnology 28, 1351-1358.

Boufadi, Y.M., Soubhye, J., Nève, J., Van Antwerpen, P., Riazi, A., 2016. Antimicrobial effects of six Algerian propolis extracts. International Journal of Food Science & Technology 51, 2613-2620.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry 7.254-248 ,2

Brehon, S., Giraud, C., Certain, A., 2000. L'alcool dans les médicaments: analyse des risques et de l'information des spécialités administrées par voie orale ou injectable. Journal de pharmacie clinique (Paris) 19, 32-48.

Campos, M.G., Bogdanov, S., de Almeida-Muradian, L.B., Szczesna, T., Mancebo, Y., Frigerio, C., Ferreira, F., 2008. Pollen composition and standardisation of analytical methods. Journal of Apicultural Research 47, 154-161.

Cardinault, N., Cayeux, M.-O., Percie du Sert, P., 20 .12Propolis: origin, composition and properties. Phytothérapie 10, 298-304.

Chandran, A.S., Suri, S., Choudhary, P., 2023. Sustainable plant protein: A recent overview of sources, extraction techniques and utilization ways. Sustainable Food Technology.

Chaudhary, P., Janmeda, P., Docea, A.O., Yeskaliyeva, B., Abdull Razis, A.F., Modu, B., Calina, D., Sharifi-Rad, J., 2023. Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Frontiers in chemistry 11, 1158198.

Chavda, V.P., Chaudhari, A.Z., Teli, D., Balar, P., Vora, L., 2023. Propolis and their active constituents for chronic diseases. Biomedicines 11, 259.

Cheikh-Rouhou, S., Hentati, B., Besbes, S., Blecker, C., Deroanne, C., Attia, H., 2006. Chemical composition and lipid fraction characteristics of Aleppo pine (Pinus halepensis Mill.) seeds cultivated in Tunisia. Food science and technology international 12, 407-415.

Choudhari, M.K., Punekar, S.A., Ranade, R.V., Paknikar, K.M., 2012. Antimicrobial activity of stingless bee (Trigona sp.) propolis used in the folk medicine of Western Maharashtra, India. Journal of ethnopharmacology 141, 363-367.

Denisow, B., Denisow-Pietrzyk, M., 2016. Biological and therapeutic properties of bee pollen: a review. Journal of the Science of Food and Agriculture 96, 4303-4309.

Didaras, N.A., Karatasou, K., Dimitriou, T.G., Amoutzias, G.D., Mossialos, D., 2020. Antimicrobial activity of bee-collected pollen and beebread: State of the art and future perspectives. Antibiotics 9, 811.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal, N., 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry 97, 654-660.

Donadieu, Y., 2008. La Propolis Editions Dangles. Paris. P 90.

Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., Mérillon, J.-M., 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD ,and ORAC assays. Journal of agricultural and food chemistry 57, 1768-1774.

El Ghouizi, A., Bakour, M., Laaroussi, H., Ousaaid, D., El Menyiy, N., Hano, C., Lyoussi, B., 2023. Bee Pollen as Functional Food: Insights into Its Composition and Therapeutic Properties. Antioxidants 12, 557.

Evans, D., Taylor, P., Singh, M., Knox, R., 1991. Quantitative analysis of lipids and protein from the pollen of Brassica napus L. Plant science 73, 117-126.

Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi ,N., Boulaaba, M., Abdelly, C., 2008. Phenolic composition of Cynara cardunculus L. organs, and their biological activities. Comptes Rendus. Biologies 331, 372-379.

Ferhoum, F., 2010. Analyses physico chimiques de la propolis locale selon les étages bioclimatiques et les deux races d'abeille locales (Apis mellifica intermissa et Apis mellifica sahariensis). Boumerdés, Université M'hamed Bougara. Faculté des Sciences de L'ingénieur.

Ferreira, L.M.d.M.C., Souza, P.D.Q.d., Pereira, R.R., da Silva, E.O., Barbosa, W.L.R., Silva-Júnior, J.O.C., Converti, A., Ribeiro-Costa, R.M., 2024. Preliminary study on the chemical and biological properties of propolis extract from stingless bees from the Northern region of Brazil. Processes 12, 700.

Fidantsi, A., Doxastakis, G., 2001. Emulsifying and foaming properties of amaranth seed protein isolates. Colloids and surfaces B: Biointerfaces 21, 119-124.

Fontaine, É., 2007. Radicaux libres et vieillissement. Cahiers de Nutrition et de Diététique 42, 110-115.

Franko, Y., 20 .20Nutritional Advantages and the Impact of Bee Pollen in the Feed of Exotic Animals.

Gardana, C., Simonetti, P., 2011. Evaluation of allergens in propolis by ultra-performance liquid chromatography/tandem mass spectrometry. Rapid communications in mass spectrometry 25, 1675-1682.

Gong, K.-J., Shi, A.-M., Liu, H.-Z., Liu, L., Hu, H., Adhikari, B., Wang, Q., 2016. Emulsifying properties and structure changes of spray and freeze-dried peanut protein isolate. Journal of Food Engineering 170, 33-40.

Gülçin ,I., Bursal, E., Şehitoğlu, M.H., Bilsel, M., Gören, A.C., 2010. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food and Chemical Toxicology 48, 2227-2238.

Haddouchi, F., Chaouche, T., Halla, N .2016 ,.Screening phytochimique, activités antioxydantes et pouvoir hémolytique de quatre plantes sahariennes d'Algérie. Phytothérapie 16, S254-S262.

Hayat, K., Hussain, S., Abbas, S., Farooq, U., Ding, B., Xia, S., Jia, C., Zhang, X., Xia, W., 2009. Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity in vitro. Separation and Purification Technology 70, 63-70.

Hossain, R., Quispe, C., Khan, R.A., Saikat, A.S.M., Ray, P., Ongalbek, D ,.Yeskaliyeva, B., Jain, D., Smeriglio, A., Trombetta, D., 2022. Propolis: An update on its chemistry and pharmacological applications. Chinese medicine 17, 100.

Huang, S., Zhang, C.-P., Wang, K., Li, G.Q., Hu, F.-L., 2014. Recent advances in the chemical composition of propolis. Molecules 19, 19610-19632.

Irigoiti, Y., Navarro, A., Yamul, D., Libonatti, C., Tabera, A., Basualdo, M., 2021. The use of propolis as a functional food ingredient: A review. Trends in Food Science & Technology 115, 297-306.

Jiang, Y., Zhu, Y., Zheng, Y., Liu, Z., Zhong, Y., Deng, Y., Zhao, Y., 2021. Effects of salting-in/out-assisted extractions on structural, physicochemical and functional properties of Tenebrio molitor larvae protein isolates. Food Chemistry 338, 128158.

Joshi ,M., Adhikari, B., Aldred, P., Panozzo, J., Kasapis, S., 2011. Physicochemical and functional properties of lentil protein isolates prepared by different drying methods. Food Chemistry 129, 1513-1522.

Kebede, I., Gebremeskel, H., Ahmed, A., 2024. Bee products and their processing: a review. Pharm Pharmacol Int J 12, 5-12.

Khalifa, S.A., Elashal, M.H., Yosri, N., Du, M., Musharraf, S.G., Nahar, L., Sarker, S.D., Guo, Z., Cao, W., Zou, X., 2021. Bee pollen: Current status and therapeutic potential. Nutrients 13, 1876.

Kinghorn, A.D., Falk, H., Gibbons, S., Asakawa, Y., Liu, J.-K., Dirsch, V.M., 2021. Progress in the chemistry of organic natural products. Springer.

Komosinska-Vassev, K., Olczyk, P., Kaźmierczak, J., Mencner, L., Olczyk, K., 2015. Bee pollen :chemical composition and therapeutic application. Evidence-Based Complementary and Alternative Medicine 2015, 297425.

Kostić, A.Ž., Barać, M.B., Stanojević, S.P., Milojković-Opsenica, D.M., Tešić, Ž.L., Šikoparija, B., Radišić, P., Prentović, M., Pešić ,M.B., 2015. Physicochemical composition and techno-functional properties of bee pollen collected in Serbia. LWT-Food Science and Technology 62, 301-309.

Krell, R., 1996. Value-added products from beekeeping. Food & Agriculture Org.

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, 235-240.

Kumazawa, S., Ueda, R., Hamasaka, T., Fukumoto, S ,.Fujimoto, T., Nakayama, T., 2007. Antioxidant prenylated flavonoids from propolis collected in Okinawa, Japan. J. of Agricultural and Food Chemistry 55, 7722-7725.

Lanez, T., Rebiai, A., Belfar, M.L., 2014. Total polyphenol contents, radical scavenging and cyclic voltammetry of Algerian propolis. Int J Pharm Pharm Sci 6, 395-400.

Le, K., Chiu, F., Ng, K., 2007. Identification and quantification of antioxidants in Fructus lycii. Food Chemistry 105, 353-363.

Li, M., Wen, X., Peng, Y., Wang, Y., Wang, K., Ni, Y., 2018. Functional properties of protein isolates from bell pepper (Capsicum annuum L. var. annuum) seeds. LWT 97, 802-810.

Liolios, V., Tananaki, C., Dimou, M., Kanelis, D., Rodopoulou, M.-A., Thrasyvoulou, A., 2018. Exploring the sugar profile of unifloral bee pollen using high performance liquid chromatography. Journal of Food & Nutrition Research 57.

Liu, Y., Zhao, G., Ren, J., Zhao, M., Yang, B., 2011. Effect of denaturation during extraction on the conformational and functional properties of peanut protein isolate. Innovative Food Science & Emerging Technologies 12, 375-380.

Lu, X., Ye, X., Liu, J., 2022. Morphological differences between anemophilous and entomophilous pollen. Microscopy Research and Technique 85, 1056-1064.

Mărgăoan, R., Mărghitas, L., Dezmirean, D.S., Bobis, O., Mihai, C.M., 2012. Physical-Chemical composition of fresh bee pollen from Transylvania. Bull. UASVM Anim. Sci. Biotechnol 69, 351-355.

Mărghitaş, L.A., Stanciu, O.G., Dezmirean, D.S., Bobiş, O., Popescu, O., Bogdanov ,S., Campos, M.G., 2009. In vitro antioxidant capacity of honeybee-collected pollen of selected floral origin harvested from Romania. Food Chemistry 115, 878-883.

Miguel, M.d.G., Doughmi, O., Aazza, S., Antunes, D., Lyoussi, B., 2014. Antioxidant, anti-inflammatory and acetylcholinesterase inhibitory activities of propolis from different regions of Morocco. Food Science and Biotechnology 23, 313-322.

Mohan, N., Mellem, J.J., 2020. Functional properties of the protein isolates of hyacinth bean [Lablab purpureus (L.) Sweet]: An effect of the used procedures. Lwt 129, 109572.

Moreno, M.N., Isla, M., Cudmani, N., Vattuone, M., Sampietro, A., 1999. Screening of antibacterial activity of Amaicha del Valle (Tucumán, Argentina) propolis. Journal of ethnopharmacology 68, 97-102.

Mothana, R.A., Lindequist, U., 2005. Antimicrobial activity of some medicinal plants of the island Soqotra. Journal of ethnopharmacology 96, 177-181.

Mouhoubi-Tafinine, Z., Ouchemoukh, S., Tamendjari, A., 2016. Antioxydant activity of some algerian honey and propolis. Industrial Crops and Products 88, 85-90.

Mundi, S., Aluko, R.E., 2012. Physicochemical and functional properties of kidney bean albumin and globulin protein fractions. Food research international 48, 299-306.

Oroian, M., Ursachi, F., Dranca, F., 2020. Ultrasound-assisted extraction of polyphenols from crude pollen. Antioxidants 9, 322.

Ouahab, A., Grara, N., Menaiaia, K., Khaldi, K., Bensouici, C., 2023. Phytochemical analysis, antioxidant, and acetylcholinesterase inhibitory activity of propolis from northeastern Algeria. Phytothérapie 21, 119.

Ouchemoukh, S., Louaileche, H., Schweitzer, P., 2007. Physicochemical characteristics and pollen spectrum of some Algerian honeys. Food control 18, 52-58.

Pascoal, A., Rodrigues, S ,.Teixeira, A., Feás, X., Estevinho, L.M., 2014. Biological activities of commercial bee pollens: Antimicrobial, antimutagenic, antioxidant and anti-inflammatory. Food and Chemical Toxicology 63, 233-239.

Pietta, P., Gardana, C., Pietta, A., 2002. Analytical methods for quality control of propolis. Fitoterapia 73, S7-S20.

Przybyłek, I., Karpiński, T.M., 2019. Antibacterial properties of propolis. Molecules 24, 2047.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine 26, 1231-1237.

Rodríguez-Pólit, C., Gonzalez-Pastor, R., Heredia-Moya, J., Carrera-Pacheco, S.E., Castillo-Solis, F., Vallejo-Imbaquingo, R., Barba-Ostria, C., Guamán, L.P., 2023. Chemical Properties and Biological Activity of Bee Pollen. Molecules 28, 7768.

Rossi, A., Ligresti, A., Longo, R., Russo, A., Borrelli, F., Sautebin, L., 2002. The inhibitory effect of propolis and caffeic acid phenethyl ester on cyclooxygenase activity in J774 macrophages. Phytomedicine 9, 530-535.

Salami, F., Mohebbati, R., Hosseinian, S., Shahraki, S., Hossienzadeh, H., Rad, A.K., 2024. Propolis and its therapeutic effects on renal diseases: A review. Iranian Journal of Basic Medical Sciences 27, 383.

Sathe, S., Deshpande, S., Salunkhe, D., 1982. Functional properties of lupin seed (Lupinus mutabilis) proteins and protein concentrates. Journal of food science 47, 491-497.

Sayari, N., Saidi, M.N., Sila, A., Ellouz-Chaabouni, S., Bougatef, A., 2016. Chemical composition, angiotensin I-converting enzyme (ACE) inhibitory, antioxydant and antimicrobial activities of Ononis natrix leaves extracts. Free Radicals and Antioxidants 6, 23-33.

Segueni, N., Magid, A.A., Decarme ,M., Rhouati, S., Lahouel, M., Antonicelli, F., Lavaud, C., Hornebeck, W., 2011. Inhibition of stromelysin-1 by caffeic acid derivatives from a propolis sample from Algeria. Planta medica 77, 999-1004.

Sforcin, J.M., 2016. Biological properties and therapeutic applications of propolis. Phytotherapy research 30, 894-905.

Siheri, W., Alenezi, S., Tusiimire, J., Watson, D.G., 2017. The chemical and biological properties of propolis. Bee products-chemical and biological properties, 137-178.

Silici, S., Kutluca, S., 2005. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. Journal of ethnopharmacology 99, 69-73.

Silori, G.K., Kushwaha, N., Kumar, V., 2019. Essential oils from pines: Chemistry and applications. Essential oil research: Trends in biosynthesis, analytics, industrial applications and biotechnological production, 275-297.

Simone-Finstrom, M., Spivak, M., 2010. Propolis and bee health: the natural history and significance of resin use by honey bees. Apidologie 41, 295-311.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture 16, 144-158.

Siuda, M., Wilde, J., Bąk, T .2012 ,.The effect of various storage methods on organoleptic quality of bee pollen loads. Journal of Apicultural Science 56, 71-79.

Soliman, A.M., Barreda, D.R., 2022. Acute inflammation in tissue healing. International Journal of Molecular Sciences 24, 6.41

Soto, M.L., Moure, A., Domínguez, H., Parajó, J.C., 2011. Recovery, concentration and purification of phenolic compounds by adsorption: A review. Journal of Food Engineering 105, 1-27.

Stojanović, S.T., Najman, S.J., Popov, B.B., Najman, S.S., 2020. Propolis: chemical composition, biological and pharmacological activity–a review. Acta Medica Medianae 59.

Stone, A.K., Avarmenko, N.A., Warkentin, T.D., Nickerson, M.T., 2015. Functional properties of protein isolates from different pea cultivars. Food Science and Biotechnology 24, 827-833.

Thakur, M., Nanda, V., 2020. Composition and functionality of bee pollen: A review. Trends in Food Science & Technology 98, 82-106.

Tosi, E.A., Ciappini, M.C., Cazzolli, A.F., Tapiz, L.M., 2006. Physico chemical characteristics of propolis collected in Santa Fe (Argentine). Apiacta 41, 110-120.

Velikova, M., Bankova, V., Sorkun, K., Houcine, S., Tsvetkova, I., Kujumgiev, A., 2000. Propolis from the Mediterranean region: chemical composition and antimicrobial activity .Zeitschrift für Naturforschung c 55, 790-793.

Wang, L., Zhang, B., Xiao, J., Huang, Q., Li, C., Fu, X., 2018. Physicochemical, functional, and biological properties of water-soluble polysaccharides from Rosa roxburghii Tratt fruit. Food Chemistry 249, 127.135-

Wani, I.A., Sogi, D.S., Shivhare, U.S., Gill, B.S., 2015. Physico-chemical and functional properties of native and hydrolyzed kidney bean (Phaseolus vulgaris L.) protein isolates. Food Research International 76, 11-18.

Wu, H., Wang, Q., Ma, T., Ren ,J., 2009. Comparative studies on the functional properties of various protein concentrate preparations of peanut protein. Food Research International 42, 343-348.

Zhang, Z., He, S., Liu, H., Sun, X., Ye, Y., Cao, X., Wu, Z., Sun, H., 2020. Effect of pH regulation on the components and functional properties of proteins isolated from cold-

pressed rapeseed meal through alkaline extraction and acid precipitation. Food Chemistry 327, 126998.

Zulhendri, F., Chandrasekaran, K., Kowacz, M., Ravalia, M., Kripal ,K., Fearnley, J., Perera, C.O., 2021. Antiviral, antibacterial, antifungal, and antiparasitic properties of propolis: A review. Foods 10, 1360.

Appendices

Appendix 1: Chemicals, reagents, glassware, equipment, and other non-biological materials employed in this study.

Table XII : Non-biological materials used during the experiment.

Reagents and Chemicals	Equipment	Glassware and Small Equipment
Hydrochloric acid (HCl)	Magnetic stirrer (LMS-1003)	Test Tubes/Conical tubes
Gallic acid ($C_7H_6O_5$)	Ultarosonic bath (SELECTA)	Graduated cylinders
Sulfuric acid (H_2SO_4)	Analytical balance (OHAUS)	Beakers
Ammonia (NH_4OH)	Centrifuge (SIGMA 3-16L)	Erlenneyer flasks
Sodium carbonate (Na_2CO_3)	Oven (MEMMERT)	Flasks
Ferric chloride ($FeCl_3$)	Soxhlet extractor	Micropipettes (10-100µL, 100-1000µL)
DPPH ($C_{18}H_{12}N_5O_6$)	Fume hood (Sarl MBPL mobilier)	Filter paper Wattman
Anthron reagent	Water bath (MEMMERT)	Petri dishes
Ethanol	Spectrophotometer UV-VIS	Platinum loop
Sodium hydroxide (NaOH)	(OPTIZEN POP)	Eppendrof tubes
Folin-Ciocalteu reagent	Aluminium chloride ($AlCI_3$)	/

Appendix 02: Gallic acid calibration curve.

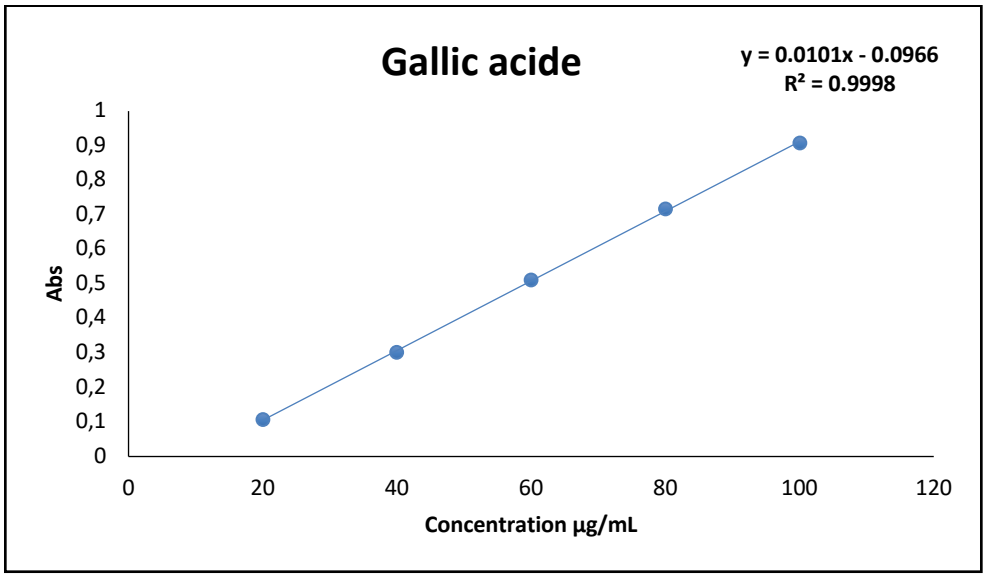

Figure 14: Gallic acid calibration curve used to determine polyphenols.

Appendix 03: Quercetin calibration curve.

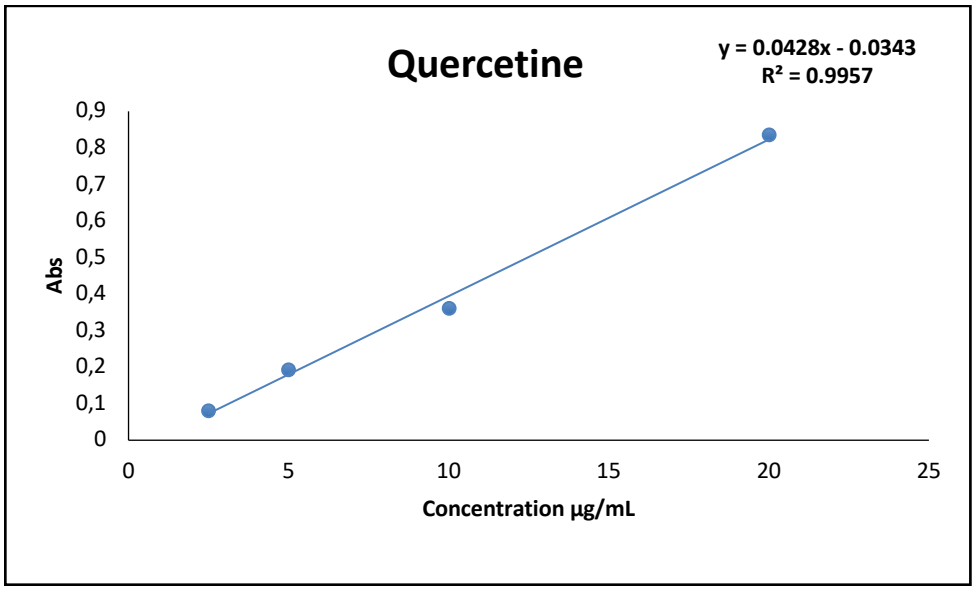

Figure 15 : Quercetin calibration curve used to determine total flavonoid content.

Appendix 04: DPPH radical scavenging activity of ascorbic acid.

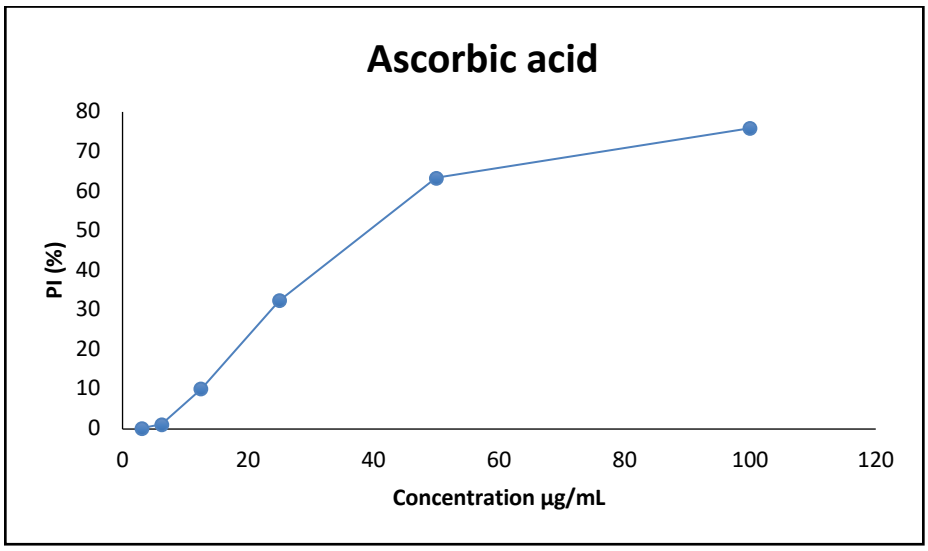

Figure 16: DPPH radical scavenging activity of ascorbic acid.

Appendix 05: $ABTS^{\bullet+}$ radical scavenging activity of ascorbic acid.

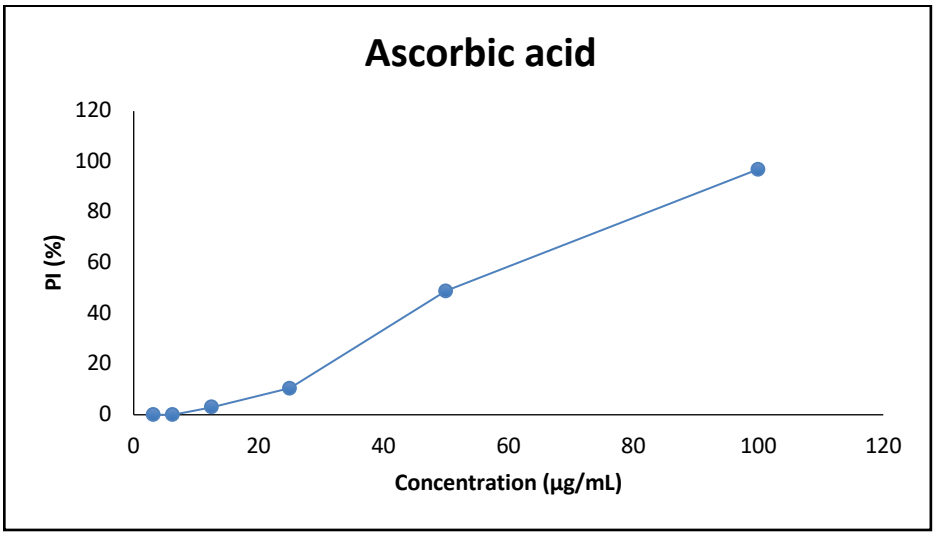

Figure 17 : $ABTS^{\bullet+}$ radical scavenging activity of ascorbic acid.

Appendix 06: BSA calibration curve.

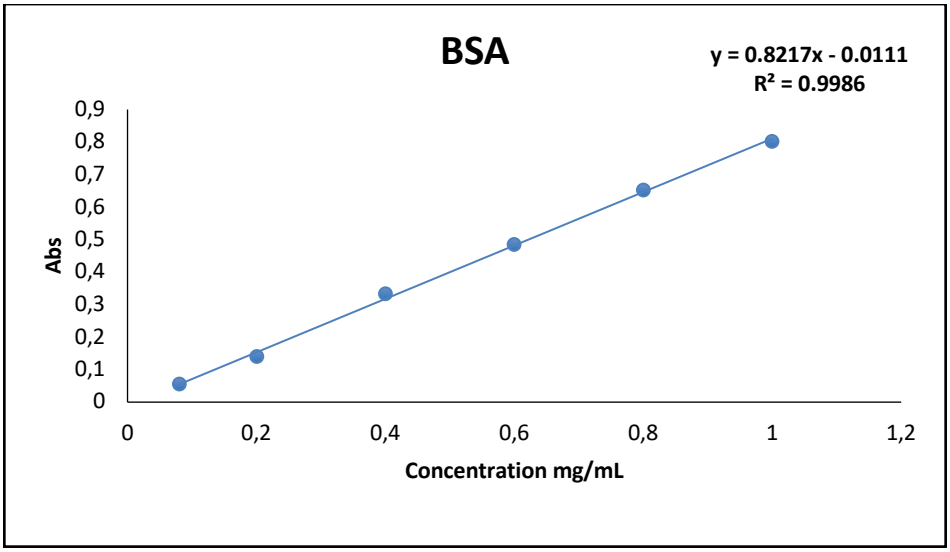

Figure 18 : BSA calibration curve used to determine total protein content.

Abstract

Natural substances significantly contribute to our daily lives by providing numerous therapeutic and nutritional benefits. Propolis and bee pollen, in particular, are rich in beneficial chemical compounds. This study aims to address the need for a quantitative evaluation of phenolic compounds and total proteins in these bee products, sourced from the Wilaya of Tizi Ouzou. It also examines their antioxidant and antimicrobial activities, as well as their functional properties. The findings reveal that propolis contains high levels of phenolic compounds and flavonoids (641.616 ± 7.95 µg GAE/mg and 57.598 ± 0.352 µg QE/mg), demonstrating superior antioxidant potential in FRAP, DPPH, and ABTS assays (IC50 values of 83.341 ± 1.095 µg/mL and 42.044 ± 0.73 µg/mL, respectively). Bee pollen shows the highest protein content (81.92 ± 2.81%) and excels in emulsification and foaming properties, while propolis has better solubility and water-holding capacity. Furthermore, propolis and bee pollen exhibits significant antibacterial activity against S. aureus strains, These results highlight the diverse therapeutic and nutritional applications of propolis and bee pollen.

Keywords: Propolis, bee pollen, phenolic compounds, proteins, antioxidant activity, antimicrobial activity, functional properties.

Résumé

Les substances naturelles contribuent de manière significative à notre vie quotidienne en apportant de nombreux avantages thérapeutiques et nutritionnels. La propolis et le pollen d'abeille, en particulier, sont riches en composés chimiques bénéfiques. Cette étude vise à répondre au besoin d'une évaluation quantitative des composés phénoliques et des protéines totales dans ces produits apicoles, provenant de la Wilaya de Tizi Ouzou. Elle examine également leurs activités antioxydantes et antimicrobiennes, ainsi que leurs propriétés fonctionnelles. Les résultats révèlent que la propolis contient des niveaux élevés de composés phénoliques et de flavonoïdes (641,616 ± 7,95 µg EAG/mg et 57,598 ± 0,352 µg EQ/mg), démontrant un potentiel antioxydant supérieur dans les essais FRAP, DPPH et ABTS (valeurs IC50 de 83,341 ± 1,095 µg/mL et 42,044 ± 0,73 µg/mL, respectivement). Le pollen d'abeille présente la teneur en protéines la plus élevée (81,92 ± 2,81 %) et excelle dans les propriétés d'émulsification et de moussage, tandis que la propolis présente une meilleure solubilité et une meilleure capacité de rétention de l'eau. En outre, la propolis et le pollen présentent une activité antibactérienne significative contre les souches de S. aureus. Ces résultats mettent en évidence les diverses applications thérapeutiques et nutritionnelles de la propolis et du pollen d'abeille.

Mots-clés : Propolis, pollen d'abeille, composés phénoliques, protéines, activité antioxydante, activité antimicrobienne, propriétés fonctionnelles.

ملخص

تساهم المواد الطبيعية بشكل كبير في حياتنا اليومية من خلال توفير العديد من الفوائد العلاجية والغذائية. والعكبر وحبوب لقاح النحل، على وجه الخصوص، غنية بالمركبات الكيميائية المفيدة. تهدف هذه الدراسة إلى تلبية الحاجة إلى إجراء تقييم كمي للمركبات الفينولية والبروتينات الكلية في منتجات النحل هذه، التي يتم الحصول عليها من ولاية تيزي وزو. كما تفحص الدراسة أيضاً أنشطتها المضادة للأكسدة والمضادة للميكروبات، فضلاً عن خصائصها الوظيفية. وتكشف النتائج أن البروبوليس يحتوي على مستويات عالية من المركبات الفينولية والفلافونويدات (641.616 ± 7.95 ميكروغرام من مكافئ حمض الغاليك/مغ و57.598 ± 0.352 ميكروغرام من مكافئ الكرستين/مغ)، مما يدل على قدرة فائقة على مقاومة الأكسدة في فحوصات FRAP و DPPH وABTS حيث تبلغ قيم IC50 83.341 ± 1.095 ميكروغرام/مل و42.044 ± 0.73 ميكروغرام/مل على التوالي .تُظهر حبوب لقاح النحل أعلى محتوى بروتيني (81.92 ± 2.81%) وتتفوق في خصائص الاستحلاب والرغوة، بينما يتميز العكبر بقدرة أفضل على الذوبان والاحتفاظ بالماء. وعلاوة على ذلك، يُظهر العكبرو حبوب لقاح النحل نشاطًا كبيرًا مضادًا للبكتيريا ضد سلالات بكتيريا *S. aureus* . تسلط هذه النتائج الضوء على التطبيقات العلاجية والغذائية المتنوعة للعكبر ولقاح النحل.

الكلمات المفتاحية: العكبر، وحبوب لقاح النحل، والمركبات الفينولية، والبروتينات، والنشاط المضاد للأكسدة، والنشاط المضاد للميكروبات، والخصائص الوظيفية.