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DEPARTMENT OF BIOLOGICAL AND MEDICAL SCIENCES

Diploma thesis

Propolis and its effects as a supplement

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HRADEC KRÁLOVÉ

2024

Acknowledgements

I would want to express my profound gratitude to my supervisor for their consistent support, intelligent criticism, and constant encouragement while I completed my thesis. I am appreciative of my family's support and affection, which motivated me to undertake this academic quest. Additionally, I would want to thank my friends for their support and encouragement along this academic journey. Their assistance has been crucial to finishing this thesis successfully.

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HRADEC KRALOVE, 2024

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Contents

Abstrakt in Czech

Více jak 80 % pacientů na celém světě jsou osoby, které spoléhají na doplňkové a alternativní léčivé přípravky alespoň pro část své primární péče, vzhledem k rychlému nárůstu jejich užívání v posledních desetiletích. Propolis, včelí produkt bohatý na bioaktivní sloučeniny, je jedním z nich, jehož terapeutické využití sahá až do starověku a stále je jedním z nejpoužívanějších přírodních produktů. Výzkum propolisu jako alternativního doplňkového léčiva pro možnou léčbu různých akutních a chronických poruch se zvýšil v důsledku technických průlomů ve farmaceutickém a lékařském oboru s hlášenými širokými terapeutickými přínosy.

Kromě zaměření na hlavní bioaktivní látky, včetně flavonoidů a fenolových kyselin, se tato práce dále zabývá biologickou aktivitou a terapeutickým potenciálem propolisu. Propolis vykazuje značné antibakteriální, protizánětlivé, antioxidační a protirakovinné účinky, jak prokázaly experimentální testy. Jeho účinnost v boji proti infekcím, regulaci zánětlivých reakcí a prevenci oxidačního stresu je prokázána klinickými výzkumy a testováním in vitro a in vivo. Heterogenita ve složení způsobená vnějšími podmínkami však představuje problém pro standardizaci. Tato práce zdůrazňuje význam standardizovaných extrakčních technik pro zajištění konzistentní účinnosti a bezpečnosti v klinických podmínkách. Budoucí výzkum by se měl zaměřit na synergické výhody s jinými přírodními sloučeninami a také na vývoj inovativních metod podávání ke zvýšení biologické dostupnosti propolisu.

Abstract in English

Over 80% of patients worldwide are identified as relying on complementary and alternative medicinal products for at least some of their primary care, given the rapid increase in their use in recent decades. Propolis, a honeybee product rich in bioactive compounds, is one of them, as its therapeutic use dates back to ancient times and is still one of the most widely utilized natural products. Research on propolis as an alternative supplemental medicine for the possible treatment of a variety of acute and chronic disorders has increased as a result of technical breakthroughs in the pharmaceutical and medical fields, with a reported wide range of therapeutic benefits.

In addition to focusing on the main bioactive substances, including flavonoids and phenolic acids, this thesis delves further into the biological activity and therapeutic potential of propolis. Propolis exhibits considerable antibacterial, anti-inflammatory, antioxidant, and anticancer activities, as demonstrated by experimental tests. Its efficiency in combating infections, regulating inflammatory reactions, and preventing oxidative stress is demonstrated by clinical investigations and in vitro and in vivo testing. However, heterogeneity in composition due to external conditions poses a difficulty for standardization. This thesis underlines the importance of standardized extraction and formulation techniques for ensuring consistent efficacy and safety in clinical settings. Future research should look into synergistic benefits with other natural compounds as well as developing innovative delivery methods to increase propolis bioavailability

1. Introduction

A promising source for the development of novel medications is natural ingredients. Apiculture is the art and science of extending, supporting, and preserving health via the use of honeybee-derived products. Numerous studies on the biological characteristics and content of propolis have been published in recent decades, indicating the interest that scientists have in this bee product and its potential for the creation of novel medications. Propolis is one of the significant medicinal compounds that honeybees make and it's considered an animal-derived substance since plant material comes into contact with the honeybee's digestive tract, particularly saliva, before being deposited into the beehive compartments. Bees gather buds or resinous materials from various plant species, such as gums, resins, and mucilage, and combine them with their saliva to create propolis, which is used to strengthen the hive structure, seal off undesired openings on their hives, and protect against bacteria, fungi, other harmful pathogens, and potential predators. It has a somewhat aromatic scent and can range in color from yellow-green to red or dark brown, with lipophilic properties. Although the chemical composition of propolis is variable depending on the nature and environment of each geographic region, plant sources, bee species, and time of collection, its high flavonoid content makes it a valuable tool for treating a variety of illnesses. Propolis has been used in traditional medicine around the world for its healing properties to treat wounds, burns, and skin infections. Its history of use goes back to ancient times, when its preservative abilities helped the Egyptians preserve their dead, and Greek and Roman doctors employed propolis as an antiseptic. Propolis was included in the London pharmacopoeias of the 17th century as an approved medication. With the advancement of laboratory equipment and technological innovations in recent decades, more investigations have focused on discovering more bioactive ingredients in propolis, their mechanisms of action, and their potential to treat both acute and chronic health issues. Propolis is widely used as a natural remedy today and can be found either pure or mixed with other natural ingredients in cosmetic, food, and pharmaceutical products. Propolis's many biological and pharmacological characteristics have been researched in the past few years throughout in vitro and in vivo experimental models as well as human clinical research to determine its health benefits and safety, as well as explore its components and their potential mechanisms of action. This review intends to provide insight into propolis, a fascinating natural product, through studying its history, techniques of harvesting and extraction, chemical

composition, and pharmacological qualities as evidenced by scientific research supporting its role as a valuable natural remedy.

2. Aim of the diploma thesis

The goal of research on propolis is to comprehend its possible therapeutic uses and advantages for human health. Its potential application in treating a range of medical conditions, including infections, ulcers, and inflammation, will be investigated. Its antimicrobial, anti-inflammatory, antioxidant, and immune-boosting qualities will also be examined, and its active ingredients along with their mechanisms of action will be discussed in order to maximize its use in wellness and healthcare products and help to understand how the substance interacts with the body and affects different physiological functions. Alternative antimicrobial agents are becoming more and more necessary as antibiotic resistance poses a serious threat to world health. Propolis is a good option to fight bacteria that are resistant to drugs because it has shown antibacterial action against a variety of infections. Examining propolis can provide important details about its antibacterial properties and their application as a substitute for traditional antibiotics, as well as Its various uses and applications, such as its potential as a natural ingredient in mouthwash and toothpaste, are also intended to be inspected in this review. Additionally, a part of this review aims to gather information about the history, collection and extraction methods, metabolism, and physical properties of propolis. The overall goal of this research is to increase our understanding of this natural material and its possible uses to benefit human health and wellbeing by collecting information from previously published clinical trials and scientific studies using bibliographic databases such as PubMed, Web of Science, and Google Scholar to provide valuable evidence to support the use of propolis as a complementary or alternative therapy in modern healthcare practices.

3. Theoretical part

3.1. Importance of propolis

Several beneficial products, which include honey, beeswax, royal jelly, pollen, and propolis, are produced by honeybees. Propolis comes from two Greek words: pro, which means entrance, and polis, which means city (1). Bees produce propolis from different plant sources by collecting buds or resinous substances such as mucilage, gums, and resins of different plant species like poplar, palm, alder, beech, pine, conifer, and birch and mixing it with the beta-glycosidase enzyme of their saliva to form propolis that is used by honeybees to seal the unwanted opening on their beehives for desired airflow, to help to keep the optimal inner temperature (35°C), protect against predators, mold, or bad weather and rain, repair their hives, strengthen the beehive, and make the interior hive's environment aseptic as an immune defense (2-4).

For thousands of years, propolis has been valued for its stated curative properties as a natural ingredient that fights disease, particularly in folk medicine to deal with a variety of illnesses. In 17th-century London pharmacopoeias, propolis was included as an official medication (5). Because propolis has an extensive range of bioactive chemicals, it is becoming more considered as an alternative to synthetic medication nowadays. Propolis has been shown to possess antiviral, antibacterial, antiseptic, and anti-inflammatory potential with no toxicity (6).

Propolis chemical components have been the topic of numerous investigations to determine their potential in treating acute and chronic illnesses. Propolis-containing products for wound healing and oral hygiene are currently among the most popular propolis-containing products. Propolis products are commercially available in the forms of capsules, mouthwashes, creams, throat lozenges, powders, tablets, lip balms, and more (7).

3.2. Geographical origin of propolis and composition

Of all the propolis types, green propolis has been the most researched, described, and utilized in medicine (8). The most significant botanical source of resin that these bees use is the plant Baccharis dracunculifolia from the Asteraceae family, which gives propolis its deep green hue. According to Park et al. there are nearly 78 bioactive components in green propolis, including flavonoids, triterpenes, sesquiterpenes, phenylpropanoids, and other phenolic chemicals(9).

The source of propolis in New Zealand, Europe, North America, and non-tropical regions of Asia is mainly a poplar tree known as Populus nigra. Egyptian propolis was also found to contain constituents of a poplar tree as well as esters of caffeic acid and long-chain fatty alcohols like tetradecanol, hexadecanol and dodecanol. Russian birch propolis obtained from Betula verrucosa has shown the presence of flavonols and flavones as well as Brazilian propolis obtained from Baccharis dracunculifolia leaf resin including diterpenes, lignans, prenylated derivates of p-coumaric acid, acetophenone and flavonoids (which are distinct from poplar nigra propolis) (10).

Brazilian propolis contains a higher content of artepillin C than caffeic acid phenethyl ester (CAPE). Some constituents like sesquiterpenoids including germacren d, ledol and spatulenol are only found in tropical regions. Clusia rosea is the source of Cuban propolis which contains polyisoprenylated benzophenone, which is distinct from European and Brazilian propolis (11).

3.3. History of propolis

While propolis early discovery and use go back to ancient Egypt and Greeks, where they used propolis as a preservative to prevent dead bodies from decomposition and in perfume production, its potential to cure diseases was discovered first by Greek scientists and doctors, and one of the first medical uses of propolis was to treat wounds by Hippocrates (4). Propolis was used as an antipyretic by the Incas. It was recommended by Greek and Roman doctors for topical therapy of cutaneous and mucosal lesions, as well as for use as an antiseptic and healing substance for mouth hygiene (12). Propolis was also used by ancient Arabs and Persians as a medicine to cure various diseases as well as a cleansing agent. Folk medicine used propolis to heal burns and bed sores and disinfect the mouth (13). “The History of Plants” (1597) by John Gerard expresses the use of propolis in making ointments to treat inflammation and bruises (14). Propolis used by physicians could be seen to treat wounds, lung infections, and malnutrition. It was also utilized in a number of Soviet facilities to treat tuberculosis, respiratory infections, and to restore appetite during World War II. Propolis was also popular among Europeans for its antimicrobial properties between the 17th and 20th centuries (10). Nicolas Vauquelin did the first study on propolis chemical composition in the early 19th century. He was the first to create propolis tincture using alcohol and diethyl ether for its purification (15).

3.4. Chemical composition

In recent decades, with the advancement of laboratory equipment and technological innovations like magnetic resonance spectroscopy, gas chromatography, mass spectroscopy, high-performance liquid chromatography, thin layer chromatography, gas chromatography, and mass spectroscopy, more ingredients of propolis have been identified for the first time. According to the latest research, there have been more than 500 bioactive molecules discovered in propolis, which are mainly secondary metabolites of plants (16).

The chemical composition of propolis is variable depending on the nature and environment of each geographic region, plant sources, bee species, and time of collection. One of the main components of propolis is plant resin, which accounts for 50% of the total composition. Some resin sources are populous, pinophyte, Pinus, Betula, Salix, Alnus, Quercus, and Palmae species (resins are not only used by honeybees but also other bee types to build and repair their nests), 30% of wax,10% of essential oils, 5% of pollen, and 5% of organic compounds including flavonoids, phenolic compounds, polyphenols, terpenes, terpenoids, coumarins, steroids, amino acids, and aromatic acids, and alcohols are the important organic compounds (17). Additionally, propolis is a source of vitamins like Thiamin (B1), Riboflavin (B2), Pyridoxin (B6), Ascorbic acid(C), Tocopherol(E) and electrolytes such as Magnesium (Mg), Calcium (Ca), Potassium (K), Sodium (Na), Copper (Cu), Zinc (Zn), Manganese (Mn), and Iron (Fe) (16).

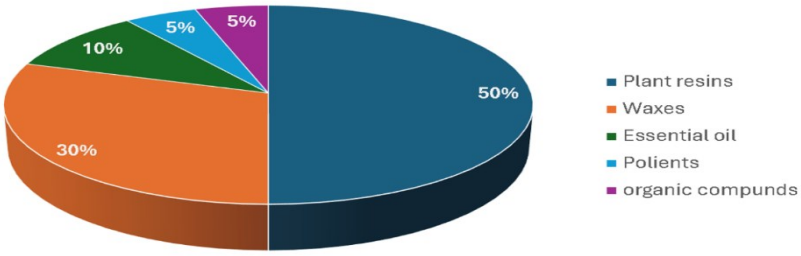

Figure 1. Chemical composition of propolis (17).

Phenolic compounds are secondary plant metabolites important for plant protection in stressful situations like pathogens or cold. They are divided into flavonoids and non-flavonoids. Flavonoid itself is further subdivided into flavones, flavans, flavonones, flavanols, flavononols, flavan-3-ols, isoflavones, and chalcones. Non-flavonoids are categorized as phenolic acids, lignans, stilbenes, tannins, and terpenoids (18).

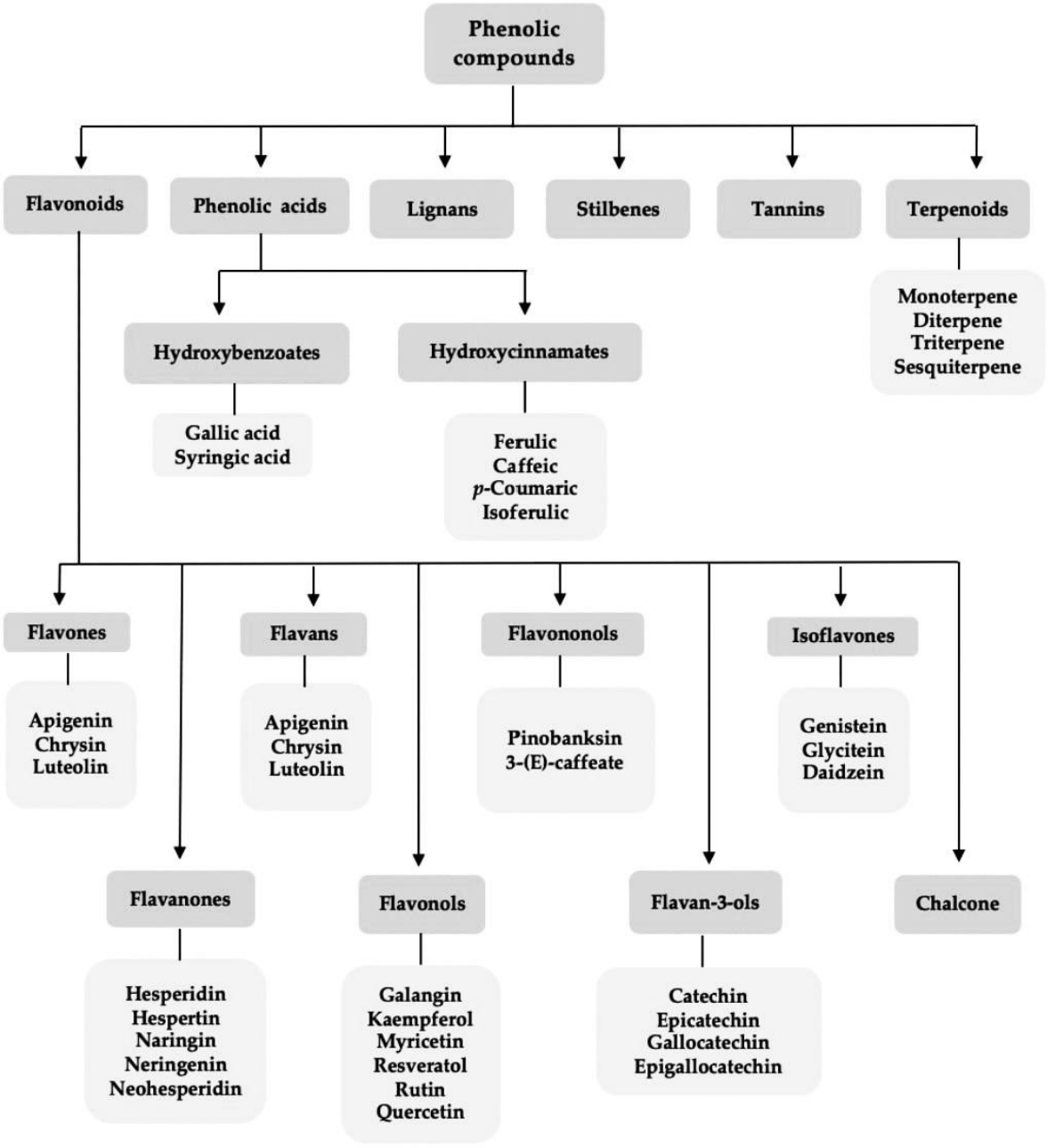

Figure 2. Classification of phenolic compounds (18).

Table 1. Propolis composition (4).

Group Compounds	***Constituents***
Flavonoids, flavones, flavanones & flavonols	Alnusitol, Alnusin, Alpinetin,3-acetyl pinobanksin, Pinostrobin, Pinocembrin, Chrysin, Islapinin, Ermanin, Sakuranetin, Acacetin, Rhamnocitrin, Pinobanksin, Betuletol, Isorhamnetin, Pectolinarigenin, Isosakuranetin, Quercetin- 3,30-dimethyl ether, Luteolin, Naringenin, Kaempferide, Rhamnazin, Tectochrysin, Galangin, Apigenin, Kaempferol, Rhamnetin, Quercetin, Rutin, Catechin.
Benzoic acid and its derivates	Gentisic acid, Trans-coniferyl benzoate, Phenylmethyl ester of benzoic acid, Protocatechuic acid, Trans-p-coumaryl benzoate, Benzoic acid, Salicylic acid, Gallic acid, Phenylmethyl ester of salicylic acid.
Cinnamic acid & its derivates, Cinnamyl alcohol, waxy acids	Cinnamylidene acetic acid, Isoferulic acid, Cinnamic acid methyl ester, Ferulic acid, Cinnamic acid, Cinnamyl alcohol, Cinnamic acid ethyl ester, Caffeic acid, Hydrocaeffic acid, Archid acid, Behenic acid, Cerotic acid, Lauric acid, Linoleic acid, Lignoceric acid, Montanic acid, di-caffeoylquinic acid, tri-caffeoylquinic acid.
Amino acids	Proline, Histidine, Hydroxyproline, d-amino butyric acid, Arginine, Isoleucine, Leucine, Pyroglutamic acid, Alanine, b-alanine, Asparagine, Aspartic acid, Cystine, Threonine, Tryptophane, Tyrosine, a-amino butyric acid, Cystein, Lysine, Methionine, Ornithine, Glutamic acid, Glycine, Sarcosine, Serine, Valine, Phenylalanine.
Benzaldehyde derivatives	Caproic aldehydes, Protocatechualdehyde, Isovanillin p-hydroxybenzaldehyde, Vanillin.
Chalcones, Dihydrochalcones, Nicotinic acid and Pantothenic acid	20,60,a-trihydroxy-40-methoxy chalcone, Pinobanksin-3-acetate chalcone, 20,6,dihydroxy-40methoxydihydro chalcone, Naringenin

Group Compounds	***Constituents***
	chalcone, Alpinetin chalcone, Sakuranetin chalcone, 20,40,6-trihydroxydihydro chalcone, Pinostrobin chalcone, Pinocembrin chalcones, Pinobanksin chalcones.
Esters	Benzyl benzoate, Benzyl-trans-4- coumarate, Tetradecenyl caffeate, Tetradecenyl caffeate (isomer)b, Tetradecanoyl caffeate, 3- Methyl-3-butenyl isoferulate, Cinnamyl caffeate, Tetradecyl caffeate, 3-Methyl-2-butenyl isoferulate, 3-Methyl-3- butenyl caffeate, 2-Methyl-2-butenyl caffeate, Methyl palmitate, Cinnamyl-trans-4-coumarate, Phenylethyl-caffeate, Hexadecyl caffeate, Stearic acid methyl ester, Phthalate ester, 3-Methyl-2-butenyl caffeate, Ethyl palmitate, Benzyl caffeate.
Terpene, Sesquiterpene, alcohol & derivatives:	Geraniol, Neroledol, b-bisabolol, Guaiol, Farnisol, Dihydroeudesmol, a-Acetoxybetulenol.
Sesquiterpene & Triterpene hydrocarbons:	b-patchoulene, b-bisabolene, Squalene, b-bourbonene, Copaene, Calarene, Calamenene, Caryophyllene, Patchoulane, Selenene, Aromadendrene.
Sterols & steroid hydrocarbons:	Cholestrilene, Cholinasterol, Stigmasterol, b-dihydrofucosterol, Lanosterol, Cholesterol.
Aliphatic hydrocarbons	1-octadecene, Pentacosane, Eicosine, Tricosane, Eicosane, Heneicosane.
Alcohol, ketones, phenols and heteroaromatic compounds	Benzyl alcohol, Hexadecanol acetate, Coumarine, Pterostilbene, Xanthorrhoeol, Scopoletol, Acetophenone, p-acetophenolacetophenone, Dihydroxy-acetope9i9inone, Methylacetophenone, Hept-5-en-2-one, 6-methylketone, Acetophenone, p-acetophenolacetophenone, Dihydroxy-acetope9i9inone,

Group Compounds	***Constituents***
	Methylacetophenone, Hept-5-en-2-one, 6-methylketone.
Aliphatic acids & aliphatic esters, other acids and derivates	Acetic acid, Angelic acid, Butyric acid, Crotonic acid, Fumaric acid, Isobutyric acid, Methylbutyric acid, Isobutyl acetate, Isopentyl acetate, Isopentinyl acetate, Phenylmethyl ester of 14-methylpentadecanoic acid, Ethyl ester of palmitic acid, Myristic acid, Sorbic acid, Butyl-2-methylpropyle ester of Phthalic acid, Stearic acid, Methyl ester of alnustic acid.
Minerals	Sodium, Magnesium, Zinc, Iron, Potassium, Calcium
Sugar:	D-fructose, D-gulose, Talose, D-glucitol, D-ribofuranose, D-glucose, sucrose.
Enzymes	Glucose-6-phosphatase, Acid phosphatase, Adenosine triphosphatase, Succinic dehydrogenase.

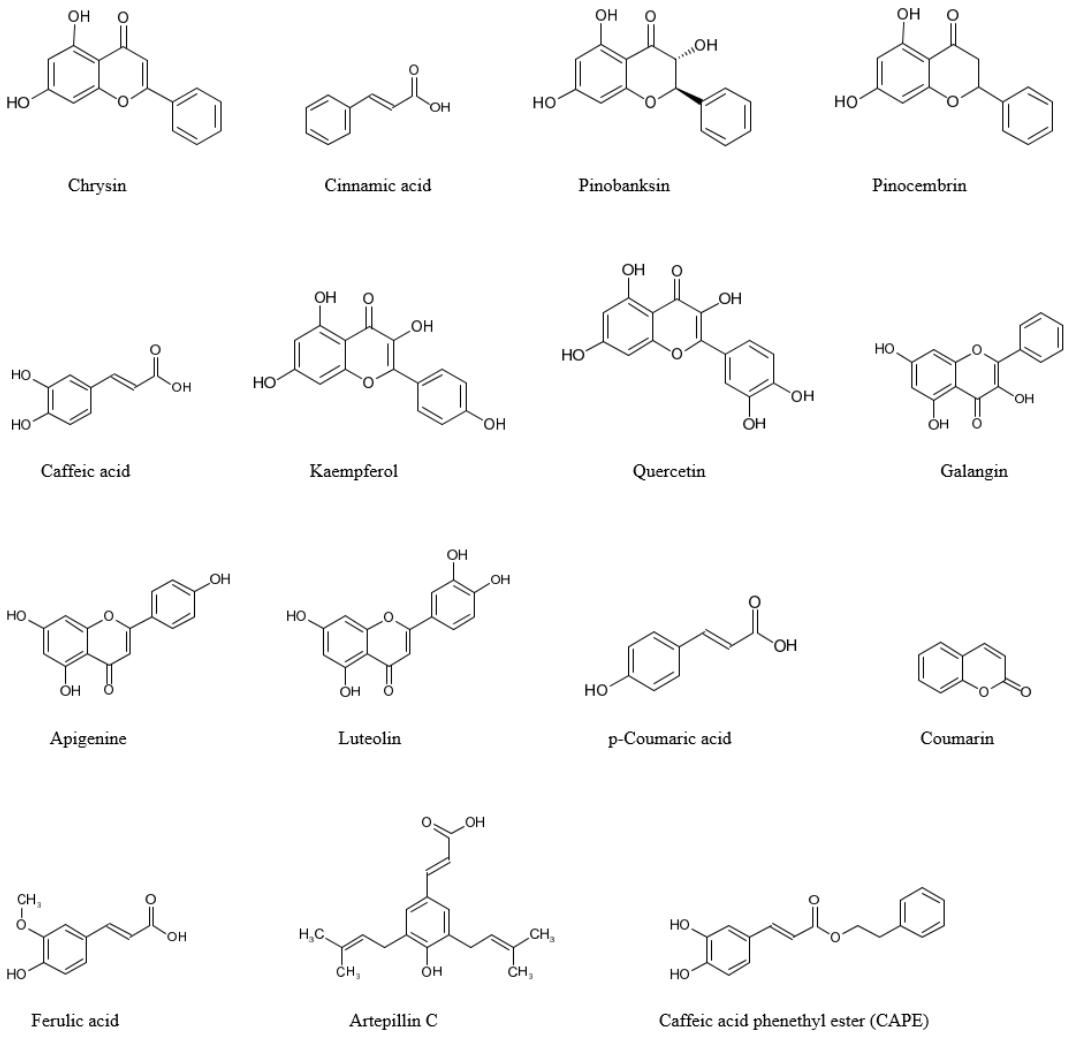

Figure 3. Chemical structures of main bioactive components of propolis (18).

3.5. Physical appearance

Propolis, or bee glue, has a lipophilic nature that is hard and fragile while soft and adhesive, with gum-like properties when heated. Depending on the nature and environment of different locations and the age of propolis, its color may vary from yellow-green to red or dark brown with a mellow aromatic scent. The main constituent of propolis is resin, which has a key role in propolis appearance and color depending on different plant species (1). Its color is variable depending on geographic regions and plant sources. The melting point is between 60 °C and 70 °C, while some types reach a melting point of 100 °C. It's commercially extracted by solvent extraction method

using ethanol, methanol, chloroform, ether, and acetone and could be commercially in the form of toothpaste, lozenges, mouth rinses, creams, gels, cough syrups, wine, cake, powder, soap, chewing gums and tablets, candies, shampoos, chocolate bars, skin lotions, and antiseptic mixtures. It is also used for the preservation of meat. The approximate colony collection per year is 150–200 g (4).

3.6. Propolis harvesting methods from beehive

Recent studies revealing propolis benefits as a natural supplement has caught the attention of pharmaceutical companies in producing supplements containing propolis, which in turn is a great financial benefit to beekeepers as well (19). It varies how much propolis can be extracted from a single hive; some hives are limited to approximately 100 grams, while others could reach up to 900 grams per season. In general, propolis production could be stimulated in honeybees by certain techniques. For instance, when Brazilian beekeepers attach wood slats to the hive boxes' edges with a 4 cm spacing between them, bees are then encouraged to fill the slats with propolis to seal the unwanted spaces (20).

Scraping is an easy way to get propolis without disturbing the hive. An abandoned or failed beehive or, during the cleaning of honey super, propolis can be collected. Propolis obtained by scratching will contain contamination such as wax and wood pieces, dead bees, etc. On the other hand, flavonoids, one of the most important ingredients in propolis that exerts pharmacological effects, are prone to form chelates with metal ions such as lead. Propolis collected by the scratching method shows four times higher levels of chelate formation compared to the trapping method of harvesting; thereby, using propolis traps provides a safer collection (21).

The ideal season to produce propolis is in the fall. The autumn is the ideal time to install traps because the bees are working hard to seal any holes and fissures in their house before winter arrives. As a result, more propolis is produced (22).

Propolis traps are typically thick plastic sheets with 1.6 mm holes all the way through them. Although various materials could be used to make a trap, it's important to use materials that cannot be chewed by bees with optimal gaps. Honeybees are encouraged to deposit more propolis and less wax to seal the holes at this width (23). The first step is to place the trap over the top frame of the honey super and close the colony lid, consequently more light and air enter the trap which stimulate the honey bees to act faster on sealing the holes by producing more propolis (24). This

could be done by making some holes on the top and sides of the honey super where the trap is placed (25). Wilson et al. define genetic, environmental factors, and colony strength as the main influences on propolis quantity and quality. Some colonies fill the propolis trap within a few weeks, while others don't fill the trap holes more than half. Once the mesh is covered with propolis and ready to be collected, it's placed in a bag and stored in the freezer, where it sometimes gets frozen to become fragile and non-sticky during propolis removal from the trap (26).

3.7. Extraction methods and technological aspects of extraction

Due to impurities such as waxes and dangerous materials, the strong smell and poor water solubility of raw propolis, its use in pharmaceutical, food or cosmetic products require farther processing and purification steps. Therefore, organic solvents are used to extract the bioactive components and to remove impurities (27). Primary phenols like flavonoids, phenolic acids, and their esters are among the propolis extracts, which exert many biological activities, including antimicrobial and antioxidant properties. The composition and activity of propolis extracts are not only influenced by plant sources and geographical regions, but also by methods of extraction. Water or ethanolic solutions are typically used to extract propolis (23). To generate wax-free extracts rich in polyphenolic components, ethanol extraction is suitable. On the contrary, water extraction is used to obtain extracts rich in water-soluble constituents (28).

Solvent extraction is the most commonly used technique for obtaining a propolis extract. Nevertheless, ultrasonic extraction is gradually taking the place of this method due to its proven effectiveness in extracting phytochemicals like phenolic compounds (29).

In the earlier days, hot reflux extraction and maceration were frequently employed methods for obtaining propolis extract (28). The process of soaking crushed propolis in ethanol, glycerol, or water yields propolis extracts as poly (vinylidene fluoride) membranes that could be employed to reduce their solvent with the least possible loss of bioactive ingredients in the extract (30).

3.7.1. Ethanol extraction

Traditionally, raw propolis was extracted with the solvent extraction method (SE) using different ethanolic and water mixtures, also known as the maceration method. In this method,

usually 25–60% v/v aqueous ethanol is added to raw propolis at a mass (of propolis) to volume (of solvent) ratio of 1:5–1:10 at room temperature for 7–10 days in a closed vessel, leading to the production of propolis tincture. The optimal maceration time is 10 days, but it could be extended up to 20–30 days, which will result in a minor increase in the polyphenol content of the extract. After maceration, the liquid is passed through a simple filter to separate it from the undissolved propolis, from which a brownish liquid is obtained that could be used in the pharmaceutical, cosmetic, or food industries to produce propolis-containing products. The strength of the produced extract is defined by the drug-to-extract ratio (DER), which represents the mass ratio of raw propolis to the mass of the final liquid extract obtained by the SE method. For example, if 1 kilogram of liquid extract is manufactured from 100 g of raw propolis, then the DER of such extract is 1:10. The efficiency analysis of propolis extract against microorganisms and antioxidant activity suggests that the extract obtained by 60–80% aqueous ethanol extract exerts higher anti-microbial and antioxidant activity compared with 50% and 90% ethanolic extracts. Advantages of this method are low wax content, rich bioactive content of the extract, non-toxicity, and ease of solvent removal if needed (31). On the contrary, it has some disadvantages, like the strong odor of ethanol and alcohol unsuitability for some population groups (pregnancy, children...) (28).

3.7.2. Water extraction

There is limited data about aqueous propolis extraction. The advantages of this method are its low production expense and the absence of ethanol in the extract composition; however, the intense propolis flavor and lower phenolic content of this method cannot be ignored. The extract of this method is prepared by mixing propolis and distilled water in a ratio of 1:10. The suspension is then heated at 40 °C until dissolution. After cooling and filtration, the filtrate is evaporated at 40 °C in the oven to obtain the extract (28).

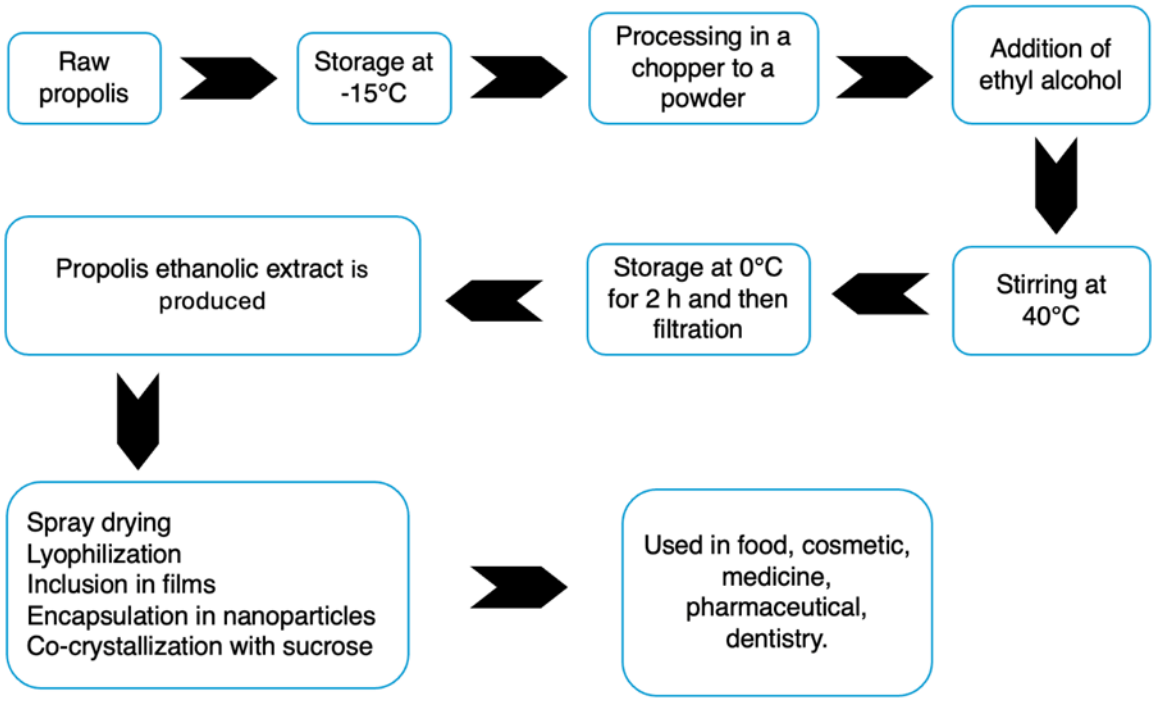

Figure 4. Process of ethanolic extraction (28).

3.7.3. Glycerol/glycol extraction

It is proven that the solvent used for extraction influences the effectiveness of the antimicrobial activity of the extract. Four propolis extracts were used in industries: ethanol, glycerine, propylene glycol, and oil solutions, in which all the extracts showed similar antimicrobial properties. The extract obtained from glycerine as a solvent has a little selectivity against Gram-positive bacteria, while the oil extract has a wider range of antimicrobial activity. Ethanol and propylene glycol extracts show more activity against yeasts (32).

Glycerine extract is prepared by forming a suspension from powdered propolis and glycerine, heating at 40 °C at room temperature for 7 days with occasional manual shaking, and finally being filtered. Non-ethanolic solvents and higher extraction temperatures allow efficient extraction of propolis as well as the production of extracts with low wax content, which are rich in biologically active compounds. Using polyethylene glycol (PEG) as an extraction solvent permits more efficient extraction of various active ingredients from propolis in comparison to the water extraction method. Extraction at 70 °C using a PEG and water mixture, or PEG, olive oil, and

water, is the key to obtaining active ingredients with poor solubility in water. On top of that, PEG can dissolve waxes to form a glue-like solution that could be used for coating pharmaceuticals. Some advanced extraction methods including Ultrasound, Microwave and carbon dioxide extractions are also used in obtaining propolis extraction. Their advantage over previously mentioned methods are being less time-consuming, require less solvent and higher yield of propolis extract (33).

3.7.4. Ultrasound extraction (UE)

This method allows selective removal and recovery of active ingredients and efficiently assists the ethanolic extraction with the use of ultrasonic energy and an ultrasonic bath. UE produces bubbles that allow better mixing of raw propolis with solvent, resulting in higher solvent penetration and complete extraction (34). In this method, dried propolis is crushed, homogenized, and sieved through a 2 mm mesh. Mixed with ethanol (96%) in a ratio of 1:5 for one hour in an ultrasonic bath at 35 °C. The extract is then concentrated using a rotary evaporator at 45 °C until a solid residue (35).

3.7.5. Microwave extraction

This method provides a rapid extraction compared to ethanolic and even ultrasound extractions, and less solvent is required, but it has a lower extraction selectivity, in which the final extract contains a higher amount of wax and a lower amount of active ingredients caused by degradation during the extraction process (36).

3.7.6. Supercritical Carbon dioxide extraction

Carbon dioxide is another suitable solvent for the extraction of lipophilic ingredients due to its non-toxicity, inert properties, and ease of removal from the final product. Time and pressure are the most important factors that affect the production yield and composition of the extract. Using the supercritical carbon dioxide method, an extract rich in antioxidants could be established. However, the extraction of flavones and phenolic constituents from propolis was not sufficient for this method (37).

3.8. Digestion and metabolism

Propolis is made up of lipids, waxes, and resins with a large molecular weight, leading to poor bioavailability and absorption. As polyphenolic compounds are important for the therapeutic effects of propolis, its bioavailability is also measured by its polyphenol contents (38). Polyphenol molecules are naturally occurring exogenous antioxidants. When taken in conjunction with food, they can either be absorbed unaltered or undergo hydroxylation, methylation, sulfation, and glucuronidation as metabolization processes (39). Its poor bioavailability might arise from digestive defects, poor transcellular absorption in the intestine, a high metabolism rate, and rapid excretion (40). Apart from this, intestinal flora and its enzymatic activity are also involved in polyphenol absorption, as they must be hydrolyzed before absorption. Polyphenols' bioavailability varies according to the kind of food consumed. Alcohol boosts their absorption, while proteins inhibit their absorption due to bond formation. Most polyphenols that are consumed are broken down by the bacterial microbiota in the gut (41).

Unabsorbed polyphenols are then broken down into smaller phenolic fragments with better bioavailability by microflora enzymes, which exert similar antioxidative activity to the parent compound (42). Regardless of the poor absorption of polyphenols, their plasma concentration was significant due to the high content of polyphenols in propolis (43). Additionally, according to Pandareesh et al. the blood-brain barrier and systemic elimination are other factors influencing propolis phenolic constituent distribution and bioavailability in the body; however, not all the phenolic constituents are impermeable to the blood-brain barrier, as a recent in vivo study on rats found caffeic acid phenethyl ester (CAPE) to be crossing the blood-brain barrier (38). Polyphenols passage through the blood-brain barrier is proportional to their lipophilicity, having fewer polar phenols or metabolites, for example O-methylated polyphenols are capable of reaching the brain better than sulphated derivatives (44).

A study of the urinary excretion rate of phenolic compounds by Alkhaldy et al. revealed different results between participants. As a result, it's assumed that aging, renal function, and type of propolis are factors influencing polyphenol excretion (42).

3.9. Health benefits of polyphenol

Phenolic compounds are mainly known for their antioxidant properties, acting as free radical scavengers by binding to radical oxygen species (ROS), for instance, superoxide or hydrogen peroxide (45). Phenolic compounds also show affinity toward metals, leading to chelate formation and exerting even higher antioxidant activity than phenolic compounds alone. An example of such interaction is the kaempferol-bound zinc complex with highly effective radical scavenging properties and anti-cancer activity (46, 47). Besides that, phenolic compounds cause improvements in nuclear factor erythroid 2-related factor 2 (NRF2), Kelch-like ECH-associated protein 1 (Keap1), and the antioxidant response element (ARE) pathway, which plays an important role in antioxidant and detoxification enzyme transcription, thereby inhibiting radical-generating enzymes like NADPH and activating the expression of antioxidant enzymes like dismutase and catalase (48).

The mechanism of the anti-cancer effect of phenolic compounds could be explained by regulating the phosphatidylinositol 3-kinase (PI3k), Ak transforming (AKT), and mammalian target of rapamycin (mTOR) pathway, which is a known cell growth pathway involved in cancer activity(49). Additionally, they inhibit nuclear factor kappa B (NF-kappa B), cyclooxygenase 2 (COX-2), tumor necrosis factor alpha (TNF-alpha), and interleukin (lL-1 and lL-6) involved in inflammation (50).

Chrysin, a flavone-based compound, appeared to have antioxidant and anti-inflammatory properties by suppressing nuclear factor kappa B (NF-kappa B), reducing tumor necrosis factor-alpha (TNF-alpha), and interleukin beta (lL-beta) production, inhibiting cyclooxygenase-2 (COX-2) and prostaglandin E2 (PG-E2) (51). Moreover, its anti-cancer mechanism is associated with the activation of Notch 1 leading to the inhibition of tumor growth and angiogenesis, reduced inflammation, and cell apoptosis (52).

Galangin flavonol is another beneficial flavonoid present in propolis. Its anti-inflammatory mechanism is based on the inhibition of nuclear factor kappa B (NF-kappa B) and the cellular phosphatidylinositol 3 kinase (PI3K)/AK transforming (AKT) pathway (53). Galangin and quercetin were shown to induce apoptosis in gastric carcinoma cell lines (54). Ethanolic extract of propolis shows positive antibacterial properties in which there was an inhibition zone around galangin in vitro. Galangin targets the bacterial membrane of the cytoplasm by changing membrane permeability and depletion of potassium, leading to bacterial cell death (55).

The pinocembrim cardioprotective mechanism can be explained by expressing glycolytic enzymes through hypoxia-inducible factor 1-alpha (HIF-1-alpha) that influence myocardium glycolysis, a positive mechanism toward heart ischemia (56).

The phenolic acids of hydroxycinnamate derivates like caffeic acid, p-coumaric acid, and ferulic acid express many similar effects to flavonoids and other constituents of propolis. While affeic acid exerts some anti-cancer effects, it can decrease the apoptosis of cancer cells by increasing cell-regulating proteins (survivin and BCL2) and its antioxidant properties (57).

Caffeic acid phenethyl ester (CAPE) is known for its anti-inflammatory, antioxidant, wound healing, anti-cancer, and anti-microbial effects (58). However, these effects are concentration-dependent (59). It inhibits the nuclear factor kappa-light-chain enhancer of activated B cells (NF-kappa B) and Phosphatidylinositol 3 kinase (PI3K)/protein kinase B (Akt) pathways, as well as the regulatory effect on the mitogen-activated protein kinase (MAPK) signaling pathway responsible for proliferation, differentiation, and apoptosis (60).

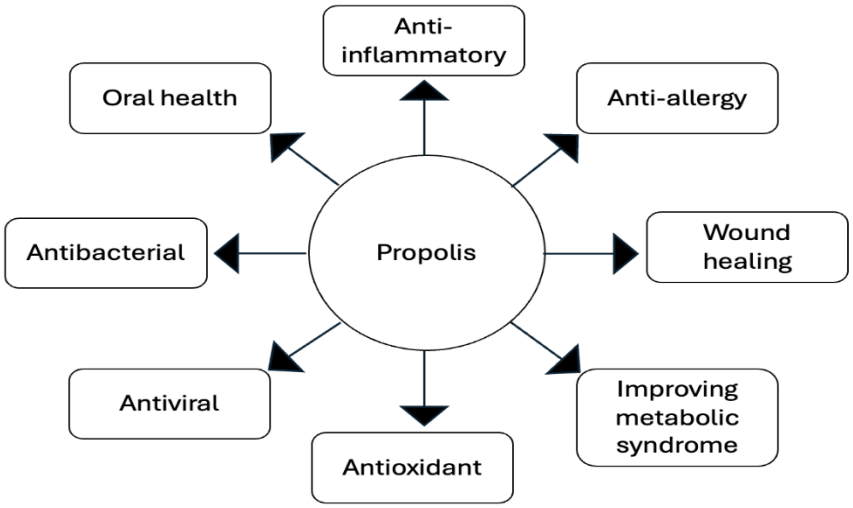

Figure 5.Biological activities of propolis (18).

3.10. Pharmacological properties of propolis

3.10.1. Free radicals and oxidative stress

Reactive oxygen and nitrogen species, or ROS and RNS in short, are the sources of free radicals in organisms. Free radicals can lead to protein, nucleic acid, and lipid oxidation within

cells, leading to cell protein aging, mutation, and cancer, as well as risk factors for other disorders like Parkinson's or cardiovascular diseases via membrane destabilization and low-density lipoprotein (LDL) oxidation (61). Studies of both in vitro and in vivo confirm the antioxidant activity of propolis due to its high content of various phenolic compounds, and their mechanisms of action could be explained as follows: regulatory effect on enzymes forming ROS, for instance, xanthine oxidase, forming chelate with metals like copper and iron participating in oxygen metabolism, capturing ROS, and lastly, synergistic effect with other antioxidants. Additionally, flavonoids and polyphenols of propolis directly react with ROS by donating a hydrogen atom reducing ROS reactivity and aggregation (62, 63).

Although the antioxidant effect of propolis has been mostly studied in vitro or in animals, there are some available investigations regarding the antioxidant effect of propolis in humans. According to Mujica et al. 90-day intake of oral propolis supplements has been linked with reduced lipid peroxidation byproducts, thiobarbituric acid reactive substances by 67%, increased glutathione (reduced form of GSH) by 175%, and increased HDL levels compared to the baseline level (64). Furthermore, Zhao et al. investigation on the antioxidant effect of green Brazilian propolis in diabetes type 2 patients indicates an increase in GSH and reduction of carbonyls (an indicator of protein oxidation) serum levels, as well as reduced activity of lactate dehydrogenase (LDH) (65). Meanwhile, glycosylated hemoglobin, insulin, and blood glucose were unchanged. As a result of this study, propolis has been found to positively affect oxidative stress in diabetes type 2 populations, but not diabetes itself (66).

In vitro and in vivo studies of preclinical trials indicated pinocembrin, a flavonol subtype constituent of propolis, to have a protective effect on rats' brains against ischemia-induced oxidation and apoptosis. Another study of Iranian propolis in mice was also done and confirmed the antioxidant and oxygen radical scavenging properties of propolis as a result of improved superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzyme activity and decreased lipid peroxidation (67).

3.10.2. Inflammation

In vitro and ex vivo studies of the anti-inflammatory effect of propolis have approved a positive modulatory effect of propolis on the inflammatory response. Bueno-Silva et al. study on mice demonstrated a reduction in various cytokines (interleukin 1 alpha, interleukin 1 beta,

interleukin 4, and interleukin 6) expression as well as reduced monocyte chemoattractant protein-1 (MCP1), granulocyte-macrophage colony-stimulating factor (GM-CSF), and a number of inflammatory genes expression (68). Additionally, in vivo, studies of the propolis effect on the immune system showed anti-inflammatory outcomes both in mice through reduced transdifferentiation of macrophages leading to anti-inflammatory effects and in goats via a reduction in pro-inflammatory cytokines (69, 70).

Ethanolic and aqueous extracts of propolis (EEP, AEP) from various bee species and geographical regions, both in vitro and in vivo, are still being investigated for a better understanding of their anti-inflammatory effects and the constituents responsible for such properties. Reis et al. study of the anti-inflammatory effect of EEP in mice concluded that the inhibitory effect of EEP on the inflammatory process was similar to that of the non-steroidal anti-inflammatory drug without a negative effect on gastric mucosa or blood, and no toxic effect associated with EEP was observed by the end of the experiment (71). Another in vivo study of 14 different extracts of Brazilian propolis collected from various origins was tested on mice for their anti-inflammatory properties, and only 4 of the extracts exerted anti-inflammatory action similar to indomethacin (72).

According to Park et al. the anti-inflammatory effect of propolis might be associated with the suppression of PG production (73). Krol et al. and Hu et al. suggest the underlying mechanisms of propolis' anti-inflammatory effect are linked to suppression of PG-E2 and nitric oxide (NO), as well as reduced IL-6 levels and an inhibitory effect on macrophage activation, all caused by flavonoids and other constituents of propolis (74, 75). Moura et al. show caffeic acid from aqueous propolis extract to be capable of suppressing cell migration and regulating the inflammation response without affecting the tissue repair mechanism in vivo (76).

In vivo studies of propolis extract administration to mice for a short period of time suggest propolis influences interferon-gamma production as another anti-inflammatory mechanism of propolis (77). As previously mentioned, various animal model studies have proven the anti-inflammatory effect of propolis. According to Raso et al. the above effect is believed to be associated with the presence of flavonoids, particularly galangin and quercetin flavonols, in propolis by inhibiting cyclooxygenase and lipoxygenase activity, reducing PG-E2 level, as well as COX-2 expression and release (78). Additionally, chrysin flavone blocks the expression of COX-2(79).

Artepillin C, a prenylated derivative of p-coumaric acid and an abundant phenolic compound of green Brazilian propolis, showed inhibitory activity against PG-E2, NO, and tumor necrosis factor (TNF) production during peritoneal inflammation in an in vivo study (80). CAPE, another bioactive compound found in propolis, has been widely studied both in vivo and in vitro, and its anti-inflammatory properties are among its other benefits. CAPE involvement in anti-inflammatory processes can be explained by its inhibitory effect on initial and late phases in T-cell receptor-mediated T-cell activation, inhibition of cyclooxygenase 1 and 2, suppression of cyclooxygenase 2 gene expression, and inhibition of nuclear factor-kappa B (NF-kappa B) activation (78, 81, 82).

Mirzoeva & Calder have identified caffeic acid, quercetin, naringenin, and caffeic acid phenethyl ester (CAPE) as anti-inflammatory compounds of propolis, causing inhibition of PG and IL synthesis, as well as suppression of myeloperoxidase activity and tyrosine-protein kinase inhibition of NO production by macrophages (83). Other compounds of propolis have also been verified as anti-inflammatory, such as apigenin, ferulic acid, and galangin (74). Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1 beta (IL-1 beta), interleukin-6 (IL-6), and interleukin-17 (IL-17) which may lead to joint degradation by inducing inflammation and synovitis, a known pathogenesis in rheumatoid arthritis (84, 85). As propolis anti-inflammatory capability was shown to be related to the suppression of pro-inflammatory cytokines in animal models by several studies, further research was conducted to evaluate its effectiveness in rheumatoid arthritis (RA) (86, 87). According to Cheung et al. study of green Brazilian propolis confirms the positive inhibitory effect of propolis on IL-17 production in human CD4 T lymphocytes, and propolis supplementation in control of RA could be beneficial(88).

Table 2. Summary of anti-inflammatory effect of propolis in clinical trials.

Year	***Country***	***Disease type***	***Dosage***	***Duration (day)***	***Effects***	***Source***
2002	Egypt	Mild to moderate asthma	2 ml/day	56	Decreased TNF-alpha, IL-6, and IL-8 Increased IL-10	(89)
2015	Japan	Diabetes mellitus type II	226.8 mg/day	56	No change of CRP, TNF-alpha, IL-6	(90)

Year	*Country*	*Disease type*	*Dosage*	*Duration (day)*	*Effects*	*Source*
2016	China	Diabetes mellitus type II	900 mg/day	126	Decreased TNF-alpha Increased IL-6	(65)
2017	Chile	Healthy individuals	Unspecified	84	No change of CRP	(64)
2017	Iran	Diabetes mellitus type II	1500 mg/day	56	Decreased CRP and TNF-alpha	(91)
2018	China	Diabetes mellitus type II	900 mg/day	126	Increased IL-6	(92)
2018	China	Elderly	66 mg/day	672	Decreased IL-6	(93)
2019	Iran	Diabetes mellitus type II	1000 mg/day	84	Decreased CRP and TNF-alpha No change of IL-6	(94)
2019	Iran	Men with asthenozoospermia	1500 mg/day	70	Decreased CRP and TNF-alpha	(95)
2020	Iran	Breast Cancer	500 mg/day	84	No change of TNF-alpha	(96)
2021	Iran	Healthy individuals	900 mg/day	28	Decreased IL-6	(97)

3.10.3. Bacterial infections

Propolis and some of its constituents have been shown to be effective against bacteria, viruses, mycotic, and protozoa in various studies (98, 99). The major antibacterial property of propolis arises from the presence of phenolic compounds; thereby, EEP shows greater antibacterial activity compared to AEP as there are more phenolic constituents extracted using ethanol than water. Additionally, antibacterial testing of AEP resulted in AEP having no significant antibacterial effect due to its poor phenolic content (100). The Czech Republic's propolis extract was found to have a potent bactericidal influence due to its high phenolic content compared to German and Irish propolis (101). Although many studies suggest the potential antibacterial property of propolis, the main constituents responsible for such an effect and their exact mechanism of action are not known. Data from different sources express that propolis was more effective against gram-positive than gram-negative bacteria. A study of Iranian propolis efficacy against gram-negative (Pseudomonas aeruginosa) and gram-positive (Staphylococcus aureus) bacteria suggested a greater activity of propolis against Staphylococcus aureus as well as an antimicrobial effect of an ethanolic extract of Brazilian propolis against Staphylococcus aureus

and Escherichia coli, and the result indicated a positive bacteriostatic activity against Staphylococcus aureus but no effect against Escherichia coli (102, 103). Additionally, ethanolic and water extracts of Mexican propolis were tested against Salmonella typhimurium, Escherichia coli, Staphylococcus aureus, and Listeria monocytogenes, but no effect was observed with the aqueous extract, concluding that the active components responsible for the antimicrobial effect were water-insoluble. Ethanol extract exerted antimicrobial activity against Staphylococcus aureus and Listeria monocytogenes but had no effect against Salmonella typhimurium and Escherichia coli (104). Thereby, the above results prove the Almuhayawi MS. claim that geographical locations influence the antimicrobial properties of propolis (105).

According to Sforcin et al. the mechanism of propolis antibacterial activity could be explained by its influence on organism immunity or direct action on microorganisms. For instance, propolis has the ability to reduce adenosine triphosphate (ATP) production by influencing membrane permeability, leading to interference with bacterial normal activity and mobility; cinnamic acid causes bacterial membrane damage and diminishes key metabolic pathways in bacteria, leading to inhibition of ATPase production, cell division, and biofilm development (106). Moreover, Veiga et al. explain the antibacterial mechanism of propolis, in which artepillin C modulates the NF-kappa B pathway, causing inhibition of the synthesis of PG-E2 and NO (107). Although not all the flavonoid compounds of propolis and their mechanisms of action are known, it's believed that flavonoids are responsible for most of propolis's biological properties. The antimicrobial activity of flavonoids can be explained as inhibiting nucleic acid synthesis in microorganisms by flavonoid's B-ring quercetin binding to the DNA gyrase of Escherichia coli (108, 109). The reason for the higher antibacterial activity of propolis toward gram-positive bacteria is because of the difference in outer membrane structure between gram-positive and gram-negative bacteria. More specifically, the presence of hydrolase enzymes produced in the outer membrane of gram-negative bacteria diminishes the antibacterial effect of responsible constituents (106). Kaempferide is a flavonoid found in propolis with a positive influence in the treatment of Staphylococcus aureus and skin infections and is effective against Enterococcus faecalis, Listeria monocytogenes, and Staphylococcus saprophyticus (110, 111). On top of this, synergistic activity between propolis and antimicrobial agents has been observed, says Dantas Silva et al. (112). This claim was further investigated by Al-Ani et al. in which he found Bulgarian propolis to support

the effect of chloramphenicol against Salmonella typhi as well as synergistic activity between red Brazilian propolis and fluconazole against Candidiasis (101).

Furthermore, pinocembrin and apigenin flavonoids indicated antibacterial activity, with apigenin being more efficient against gram-negative bacteria (113). Cinnamic acid, another abundant component of propolis, was also confirmed to exert antibacterial properties by its destructive influence on the microbial cell membrane (114).

There is evidence concerning propolis ability to modulate bacterial resistance to antibiotics, particularly quercetin modulating bacterial resistance to beta-lactam in penicillin-resistant Staphylococcus aureus and its efficacy against methicillin-resistant Staphylococcus aureus (MRSA)(115, 116). An in vitro-based study described quercetin as a potential propolis constituent against oral microorganisms like *Porphyromonas gingivalis*(117).

In summary, the antibacterial mechanism of propolis is explained as follows (105):

- Inhibition of nucleic acid synthesis
- Inhibition of protein synthesis
- Alteration of microorganism energy production and mobility
- Change of cellular integrity and membrane permeability
- Diminishing normal membrane function
- Reducing biofilms
- Reducing bacterial resistance

3.10.4. Viral infections

As many pathogenic viruses are unresponsive to antiviral agents, natural sources with antiviral properties are being more focused on. In vivo and in vitro studies reveal the potential antiviral activity of propolis against the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the Herpes simplex virus (HSV), and influenza viruses (118, 119). A study of the antiviral effect of propolis showed a positive result in inhibiting virus replication of HSV-1 and HSV-2, similar to acyclovir, using south Turkey's propolis. This effect was approved in the study of Canadian and French propolis as well (119, 120). The study of the EEP of green Brazilian propolis has also confirmed the antiviral effect against acyclovir-resistant HSV in vitro as well as the efficacy of brown Brazilian propolis in reducing epidermal, dermal, and vaginal lesions caused by HSV-2 in vivo (121).

In vitro studies of propolis extract from Brazil and China have indicated propolis activity against the human immunodeficiency virus (HIV), the cause of acquired immune deficiency syndrome (AIDS), by inhibiting reverse transcriptase and viral entry blockage (122).

Governa et al. in vitro study of poplar propolis extract effect on the H1N1 subtype of influenza A virus demonstrated inhibitory activity on viral growth as well as neuraminidase activity. The same antiviral activity was reported using Brazilian propolis extract both in vitro and in vivo by Shimizu et al. (123, 124).

Kai et al. in vivo study suggests inhibition of viral activity due to the prevention of virus binding to host cell receptors and inhibition of viral replication by coumaric acid, apigenin, and kaempferol. (125). In vivo and in vitro studies have indicated that flavonoids and other phenolic compounds are responsible for antiviral activity against various viruses, namely Herpes simplex virus (1&2), Influenza A virus subtype H1N1, Varicella zoster virus (VZV), Canine Distemper virus (CDV), Newcastle disease virus (NDV), Pseudo Rabies virus (PRV), and Human immunodeficiency virus (HIV), and their mood of antiviral action could be explained as following mechanisms, as the exact mechanism of each constituent is not known yet due to component variability of propolis: Inhibition of viral protein-forming complexes, Forming an electron-dense layer on the cell membrane, damaging viral proteins, and causing virus damage within the cell (118). Table 3 demonstrates seven reviews of clinical trials regarding the antiviral effectiveness of propolis in different viruses and trial outcomes regarding the antiviral effect of propolis.

Patients diagnosed with HSV-2 treated with propolis ointment showed significant symptom improvement compared to patients treated with acyclovir and placebo (70). Additionally, a higher healing rate was observed with propolis lotion than placebo in patients with varicella-zoster viral infection, with no signs of adverse effect (126). Dermatological observation in patients with HSV-1 treated with propolis lip balm in various concentrations resulted in pain suppression and an improved healing process (127-129).

Table 3. Clinical trial results of propolis potential in the treatment of viral infections.

Virus type	***Propolis dose / Dosage form***	***Trial outcome***	***Source***
Genital herpes (HSV-2)	Four times daily for 10 days/topical	Symptomatic improvement and skin lesions healing	(70)

Virus type	*Propolis dose / Dosage form*	*Trial outcome*	*Source*
HSV-1	Propolis 3%/topical	Positive influence on healing of skin lesions	(130)
Herpes zoster	Propolis lotion (3 times/day topical) + Acyclovir (400 or 800 mg oral) for 28 days	Pain reduction and lesion healing	(126)
HSV-1	Propolis (0.1%, 0.5% and 1%)/topical as lip balm	Pain reduction and minor healing	(129)
HSV-1	Topical propolis 0.5%, 5 times for 5 days	Symptomatic improvement by reducing pain, swelling and itching	(128)
HSV-1	Topical propolis 0.5%/(0.2 g) 5 times for 5 days	Improvement of herpes labialis.	(127)

Debiaggi et al. expressed flavonoids, namely quercetin and CAPE, as being among the propolis constituents exerting anti-coronavirus activity (131). Quercetin interferes with viral proteins functioning in Middle East respiratory syndrome-related coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and CAPE inhibits an important enzyme for entry and replication of the virus, specifically p21-activated kinase (PAKs)(132, 133). In addition, recent clinical trials investigating Brazilian and Iranian propolis activity against COVID-19 as an adjuvant to pharmacotherapy indicate as follows: Patients aged between 18 and 75 diagnosed with COVID-19 were given propolis extract, and the summary of outcomes was reduced hospitalization time with Brazilian propolis and symptomatic improvement with Iranian propolis (134). On top of the previously mentioned results, the antibacterial potential of propolis cannot be ignored, as COVID-19 increases the chance of secondary bacterial infections (135).

Propolis extract activity against rhinovirus, one of the common cold causes in humans, was studied in clinical trials. Children aged 5 to 12 years old diagnosed with viral tonsillopharyngitis were given a mixture of propolis, royal jelly, and honey for 10 days, which had a positive influence on the upper respiratory tract infection (136). Another clinical trial done by Esposito et al. using an oral spray of propolis in patients aged between 18 and 77 years old with symptoms of upper respiratory tract diseases resulted in symptom improvement after three days of use of the propolis spray (137). Additionally, Di Pierro et al. conducted a clinical study in patients with pharyngitis

caused by viruses who were given pure propolis, which indicated symptom improvement like sore throat and fever associated with viral infection (138).

3.10.5. Wounds and burns

Skin tissue damage can occur due to burns, trauma, cuts, or other diseases leading to wounds, microorganism growth, and infection, causing a longer healing process and more severe skin damage. As mentioned previously, propolis could play a significant role in healing wounds as it inhibits bacterial growth, promotes cell proliferation and healing, has antioxidant and anti-inflammatory properties, and is used in folk medicine as a wound healer and local painkiller, among other benefits (139). Propolis as a natural remedy to treat skin wounds, ulcers, and burning is widely used, and its history of therapeutic properties in treating skin conditions goes back to ancient times. Propolis, being non-toxic with excellent tolerability, is considered a natural option in the management of skin altercations with its capability to influence skin cell activation, growth, and proliferation (140). The study of the propolis effect on skin wounds and collagen production revealed the supporting effect of propolis on skin re-epithelialization by stimulating the expression of transforming growth factor beta (TGF-beta), collagen expression, and extracellular matrix regulation (ECM) (141). TGF-beta takes part in the early phases of wound repair, such as homeostasis and inflammation. ECM monitoring after propolis administration revealed an increase in ECM components at the beginning of the healing phase, and later, its components decreased (142).

Another factor influencing the skin repair process is ROS, in which excessive ROS and oxidative stress negatively impact the healing process; thereby, propolis and its constituents, particularly quercetin flavonoid, as a free radical scavenger, could bring more benefits to injured skin as they inhibit ROS formation, leading to suppression of NF-kappa B activation, eicosanoid production, and oxidative stress to the cell components (143, 144). The antibacterial property of propolis could be another benefit of its use in skin injuries and wounds, as shown in previous studies, to fight against pathogens, leading to an aseptic wound bed.

Diabetic foot ulcers, being a complication of diabetes mellitus, require topical treatments and ulcer management. An in vivo study performed by Mujica et al. indicated that propolis use in diabetic ulcers was associated with an increased rate of wound healing and supporting re-epithelialization in mice (66). This claim is also supported by McLennan et al. in vivo study of the

propolis effect on diabetic wounds (145). A clinical study of propolis effectiveness as an adjuvant in diabetic foot revealed propolis to be beneficial as it promotes healing and reduces local inflammation, on top of this, it was observed that propolis decreased wound bed infection(146). Afkhamizadeh et al. conducted a clinical study on human diabetic foot ulcers using propolis ointment, which resulted in decreased lesion size and healing effects after 4 weeks of use (147).
As the use of renewable, biodegradable, and biocompatible materials has gained attention in healthcare, propolis nano-fiber wound dressing using polyurethane and propolis solution has been developed as a modern alternative. Those nano-materials based on honey and propolis have shown great results as wound dressing both in vitro and in vivo (148).

3.10.6. Metabolic syndrome and its complications

The World Health Organization's definition of metabolic syndrome (MeS) is "a pathologic condition defined by obesity, insulin resistance, hyperlipidemia, and hypertension." High rates of morbidity and mortality associated with MeS have become a major health concern in the world, as two causes are bolder among others in the development of MeS, one being high-calorie and low-fiber fast food intake and the other being low physical activity and a sedentary lifestyle. Uncontrolled MeS eventually leads to other disease development such as kidney failure, cardiovascular diseases, stroke, etc., so having a healthy lifestyle and diet could be considered a preventative measure. Propolis supplementation as a natural source is investigated as a potential adjuvant against MeS and its chronic diseases (149).
A study of AEP and EEP in vivo was found to be effective in reducing glycemia by 18–29% in diabetic rats given propolis extracts for 8 weeks. The hypoglycemic effect, better glucose tolerance, and improved insulin sensitivity in mice were all the key results of Kitamura et al. (150). According to Abo-Salem et al. the hypoglycemic effect of propolis is dose-dependent (151).
Propolis hypoglycemic efficacy was further investigated through clinical trials, resulting in its potential to prevent worsening of uricemia in DM II patients given green Brazilian propolis for 8 weeks. The antioxidant effect was a result of increased GSH levels, decreased oxidative stress, and inflammatory mediators (TNF-alpha), while IL-6 and IL-1 beta levels were elevated and no change in glucose metabolism was observed after 18 weeks of supplementation with propolis in diabetic patients (65). El Sharkawy et al. observed that 25 weeks of 0.4 g/day propolis supplementation in diabetic type 2 individuals reduced glycosylated hemoglobin A1c (HbA1c); a similar result was

shown by Sameni et al. where patients with DM II were given 0.9 g/day for 12 weeks, which resulted in reduced HbA1c and fasting blood glucose. 1 g/day propolis ingestion in DM II patients for 12 weeks was associated with improved glucose metabolism, reduced HbA1c, and preventing 2 hours of postprandial hyperglycemia during the clinical trial (152, 153).

Upon studying the antihypertensive activity of propolis on rats, flavonoids and phenolic constituents, namely caffeoylquinic acids, possessed positive results on decreasing blood pressure in rat models given EEP and AEP, while 25% EEP showed a higher efficacy than 70% EEP (154). Furthermore, Maruyama et al. in vivo study of the antihypertensive effect of EEP observed Dihydrokaempferide, Isosakuranetin, Betuletol, and Kaempferide flavonoids to reduce blood pressure the most in which their activity is dose-dependent (155). Tyrosine hydroxylase is a rate-limiting enzyme in the production of catecholamine, causing sympathetic activation and leading to hypertension. Its activity was reduced in rats with inhibited NO synthase when given 30% EEP (156).

Zhou et al. investigation on sodium chloride-induced hypertension in rats revealed AEP to lower the blood pressure as well as some protective mechanisms such as higher activity of catalase, decreased ROS level, and anti-inflammatory effect (157).

Since individuals with MeS are more likely to acquire kidney disease, there is a definite correlation between the development of chronic kidney disease and MeS (158). Propolis as a potential nephroprotective supplement was studied in diabetic rats and resulted in positive effect. Its nephroprotective effect was linked to the antioxidant capacity of propolis (reduced malondialdehyde, increased GSH, SOD, and catalase activity) in rat's kidney (153). Furthermore, propolis in carbon tetrachloride-induced renal damage in mice showed less glomerulus swelling and restoration of kidney membrane-bound enzymes compared to mice without propolis, as well as reduced kidney cell apoptosis by influencing gene expression of enzymes involved in cell death induction (caspase-9) and regulation (B-cell lymphoma 2) (159, 160). Higher apoptosis and histological changes in the kidney were observed as a result of methotrexate kidney damage in rats without propolis supplementation. The nephroprotective effect of propolis is once again reported by Aldahmash et al. in an in vivo study of gentamicin-induced kidney disease, where propolis led to a decrease in cell death, kidney fibrosis due to collagen accumulation, and nephrotic damage (161).

Silveira et al. conducted a clinical study of propolis effectiveness in patients diagnosed with chronic kidney disease, where participants were given 0.5 g/day propolis extract for a year and confirmed the nephroprotective properties of propolis as a result of decreased inflammatory markers in urine, specifically monocyte chemoattractant protein-1 and proteinuria. Propolis safety in this study was confirmed, as no adverse effects were observed among participants (162).

Low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and cholesterol are the most common causes of atherosclerosis and other cardiac diseases arising from it. Atherosclerosis has a close relation to oxidation and inflammation and consequently intracellular component damage, cellular stress, provoking an inflammatory response, and cell apoptosis (163). In vitro studies by Claus et al. showed reduced LDL oxidation and oxidized LDL-induced apoptosis in the presence of AEP (164). Saavedra et al. observed propolis to reduce macrophage activity and matrix metalloproteinase-9 (MMP-9) gene expression, in which they play a role in ECM component degradation that is involved in atherosclerosis development, as suggested by Galis et al. (165, 166). Results of cardiovascular benefits with propolis treatment in animal models were also positive, such as upregulating gene expression of TIMP-1 (a metalloproteinase inhibitor) and downregulating vascular cell adhesion molecule-1, monocyte chemotactic protein-1, fibroblast growth factor, vascular endothelial growth factor, inhibiting expression of vascular endothelial growth factor-A, and HIF-1-alpha through upregulation of microRNA, preventing heart inflammation, and ventricular remodeling (167, 168).

Studying propolis efficiency in preventing cardiovascular diseases in humans, Mujica et al. clinical trials revealed increased HDL and GSH and reduced lipid peroxidation byproducts, namely thiobarbituric acid reactive substances, upon propolis intake for 12 weeks (66).

Sameni et al. clinical study resulted in a decline in cholesterol and LDL levels with propolis use in patients with DM II (153). On the contrary, Zakerkish et al. clinical observation was that propolis did not influence LDL and cholesterol, but it increased HDL levels in type 2 diabetic patients (94). To conclude the above-mentioned studies, it seems propolis can be beneficial in cardiovascular conditions because of its antioxidant and regulatory impact on lipids.

3.10.7. Oral health and inflammation

As propolis shows potential antibacterial, antioxidant, antifungal, and anti-inflammatory effects, being cost-effective and available as well as a natural remedy with very low risk of adverse

effects and no toxicity, its use in dentistry and oral health was soon brought to attention. Existing microorganisms in the oral cavity, together with other risk factors like tobacco smoking, poor oral hygiene, and corticosteroid inhalation, may result in oral infection and inflammation (169).
The World Health Organization defines dental caries as "a pathological process that occurs locally in the extrasomatic background. It causes the hard tissue in the teeth to break down and the enamel to become decalcified, which ultimately produces a cavity in the teeth". Various bacteria contribute to caries, the most important being Streptococcus mutans and Lactobacillus species (170).

Researchers in an in vivo study of propolis effectiveness against dental caries found an inhibitory effect on Streptococcus mutans, S. cricetus, and S. sobrinus growth with drinking water-mixed propolis in rats approved by Ikeno et al. (171). Following the benefit of propolis in oral caries in animal models, it was tested in humans. Duailibe et al. clinical trial investigation of propolis extract influence on caries resulted in diminished bacterial growth causing dental caries on 20 participants who rinsed their teeth with propolis extract, and their before and after-rinsing saliva as well as saliva samples after daily use of the extract for a week, respectively, showed a decrease in the salivary content of Streptococcus mutans (172). Interestingly, Anauate-Netto et al. performed a randomized clinical study to compare the efficacy of propolis extract and chlorhexidine mouthwash efficiency on oral microorganisms. A 4-week study revealed propolis treatment to be more effective in reducing Streptococcus mutans and Lactobacillus after a saliva test (173). On the contrary, the Ozan et al. study demonstrated that when it comes to caries prevention, propolis is not as efficient as chlorhexidine solutions, but propolis-containing preparations exert a lower cytotoxic effect on gum compared to chlorhexidine (174). Meanwhile, Santiago et al. examined how propolis-containing mouthwash and a commercial mouthwash with chlorhexidine affected tooth plaque, and during the two-week testing period, it was discovered that the mouthwash containing propolis was just as successful as the mouthwash containing chlorhexidine in reducing plaque accumulation on teeth (175).

A similar trial was conducted on 30 children between 5 and10 years old, and salivary examination after 60 seconds of rinsing the mouth with propolis confirmed Streptococcus mutans reduction(176). Tulsani et al. clinical study on potential activity against oral Streptococcus mutans of propolis chewing gum and xylitol chewing gum demonstrated promising results as both gums reduced salivary S. mutans, while propolis gum (from France) showed a better result than xylitol chewing gum (177). Because propolis and its components limit the activity of glucosyltransferase

and/or growth of bacteria, they lower the number of germs that cause dental caries says Park et al. and Koo et al.(73, 178). Additionally, propolis lowers the amount of acid manufactured by bacteria and inhibits F-ATPase, a crucial enzyme linked to S. mutans' ability to withstand acidic pH (179).

Propolis as a potential natural agent was further investigated in preventing other oral health conditions such as gingivitis and dental plaque. After 45 days of propolis rinse twice a day in a phase II clinical study held by Pereira et al. there was a noticeable decrease in both the plaque index and the gingival index when compared to the index values of participants before the use of propolis mouthwash. Also, participants in the propolis mouthwash group showed that it effectively reduced supragingival plaque incidence in comparison with the placebo group (180). Ercan et al. conducted a study to compare the effectiveness of propolis mouthwash and propolis chewing gum on young adults. Where 5 participants were instructed to chew the gum 3 times daily for 20 minutes after main meals, and 5 participants used mouthwash twice a day for 5 days. As a result, the plague index and gingival index revealed propolis mouthwash to be more efficient than gum. Therefore, the effectiveness of propolis extract in lowering dental plaque may depend on the vehicle in which it is employed (181).

3.10.8. Allergic diseases

As allergic disorders such as atopic dermatitis, allergic rhinitis, and bronchial asthma become more common, they pose a growing global health challenge. Clinical therapy for allergy illnesses is hampered by the side effects of current drugs (steroids and antihistamines), patients' perceptions of ineffectiveness, and the high cost of some pharmacotherapies (omalizumab). Additionally, long-term use of medications, relapses, and the persistence of allergic symptoms cannot be tolerated by all patients. For the reasons mentioned, propolis as a potential natural remedy to prevent or treat allergic ailments was taken into consideration. Although propolis's ability to reduce allergies has not been thoroughly investigated, the information that is now available indicates that flavonoids (such as chrysin, kaempferol, galangin, and pinocembrin) and compounds produced from cinnamic acid (such as artepillin C and CAPE) can have some anti-allergic properties. Since propolis from different geographical regions has different compositions and different concentrates, their composition analysis helps to better understand its effectiveness in future studie. Allergic diseases are growing worldwide with mild to severe symptoms such as itchy skin, rashes, runny nose, watery eyes, sneezing, bronchoconstriction, eosinophilic

inflammation, and anaphylactic reactions(182). Allergic reactions arise from the binding of allergens to IgE, leading to the activation of mast cells and basophils following the release of inflammatory and pro-inflammatory agents (183).

EEP collected from Iran inhibits IL-13 and IL-17 production and increases IL-12 production in vitro, where allergy was induced by Aspergillus fumigatus conidia (an allergic inflammation mediator in humans); therefore, data suggests the anti-allergic capability of propolis (184). Tani et al. reported a dose-dependent inhibitory effect of EEP on histamine release as well as a minor inhibition of IL-5 and IL-13 production by green Brazilian propolis; however, the exact constituent responsible for the mentioned effect remains unknown. It is believed that Artepillin C, Bacharrin, and Kaempferide are the contributors due to the higher concentration in green Brazilian propolis (185).

According to Nakamura et al. investigation of Brazilian and Chinese propolis (AEP and EEP) on mast cell degranulation in vitro, Chinese extract showed higher activity against degranulation than Brazilian. Chrysin and kaempferol are the main active ingredients supplying the anti-allergic properties of Chinese propolis, according to an analysis of China's EEP. Brazilian propolis, on the other hand, had lower measurable quantities of chrysin and kaempferol, which may have contributed to its lesser inhibitory effects on mast cell degranulation when compared to Chinese propolis. Despite the origin of propolis extract, Nakamura et al. found EEP to have a higher potency than AEP against allergy (186).

Possible anti-allergic properties of propolis were further tested on asthmatic individuals throughout the clinical studies: 36 males and 10 females. Individuals between 19 and 52 years old with mild to moderate asthma for the last 2 to 5 years were divided into two groups. Group 1 took oral theophylline plus an AEP-containing sachet, and Group 2 took oral theophylline plus a placebo sachet over 8 weeks prior. As a result, group 1 blood levels of IL-6, IL-8, intercellular adhesion molecule-1, TNF-alpha, and PG-E2 were reduced, while IL-10 seemed to increase compared with group 2. Subjects in Group 1 showed improvements in pulmonary ventilatory functioning and a decrease in the frequency and intensity of asthma attacks, cough, dyspnea, wheezing, and, importantly, reduced use of drugs for acute asthma in the propolis group (89).

Additionally, another clinic trial evaluated the Iranian propolis effectiveness, in which 52 participants with newly diagnosed, previously untreated moderate asthma were divided into two groups (propolis and placebo) taking 3 tablets daily for 4 weeks. After the end of 4 weeks,

improvements in asthma symptoms and frequency of attacks, along with improved airway hyperresponsiveness, pulmonary function, disease activity score, and reduced hospitalization during the trial, were observed in the propolis group (187).

3.11. Propolis adverse effects

The World Health Organization (WHO) defines adverse drug effects as "undesirable and unintended responses following administration of a normal dose range in humans for the purpose of prevention, therapy, diagnosis of diseases, or alteration of physiological functions" (188).
Since any chemical compound can cause toxicity and harm, it's crucial to understand its safety. The drug or chemical compound quantity, administration route, organism sensitivity, and duration of exposure are the determinants of toxicity, according to Schulz et al.(189). The same principle applies to honey products and propolis, as they may cause adverse drug reactions (ADRs) such as discomfort, pain, rashes, and death as a result of propolis-induced allergies. The severity of ADRs could be associated with propolis composition and medication history, as polymedication may intensify the ADRs (190).

In general, propolis, as a valuable and potentially useful natural substance in prevention and treating various health conditions, is believed not to manifest serious adverse effects in most cases at usual quantities (191). As natural substances could be as effective as synthetic substances in the restoration of health, it doesn't mean that their use is risk-free (192). Although in the majority of the previously mentioned literature, propolis extracts and propolis-containing products are considered safe as no adverse effects or toxicity were observed, there are some concerns regarding propolis' capability to cause allergic reactions in individuals with a history of allergies with excessive exposure to propolis constituents (6, 169, 189)

Several propolis constituents have been shown to have potential toxic effects, such as benzoic acid, which, when it interacts with vitamin C, forms benzene, which is known as a mutagen that can cause damage to chromosomes as a result of DNA breakdown (193). Bachewar et al. define benzyl benzoate as a compound capable of causing lightheadedness and triggering epilepsy(194). Longstanding exposure to concentrated phenol has been associated with cardiac, hepatic, nephrotic, and pulmonary damage, according to Philip and Marraffa (195).

Among the most reported cases of propolis side effects worldwide, allergic reactions, namely swollen lips, stomatitis, skin rash and redness around the mouth, shortness of breath, and

contact dermatitis upon use of propolis and propolis-containing products, are the main undesirable events, whereas contact dermatitis is the most common ADR limited to the areas where propolis has been applied (196-198)

Allergic manifestation in poplar-derived propolis is assumed to be caused by caffeic acid ester derivatives, while benzyl cinnamate, benzyl salicylate, and 3-methyl-2-butenyl are the triggers in non-polar propolis (199, 200). Orsi et al. describe the mechanism of allergic manifestation arising after exposure to propolis as being caused by mast cell activation and the release of inflammatory mediators because of IG-E release (201). Meanwhile, T cells, NK cells, and NK-T cells are the key players in allergic reactions in contact dermatitis as well (202). According to Celikel et al. study of systemic allergic reaction risk assessment in beekeepers, individuals with a genetic predisposition to allergic reaction development are at higher risk of systemic and anaphylactic reactions (203).

With all that being said, propolis is considered a natural substance with few side effects. As of today, no tissue damage or organ harm has been reported with the use of propolis in the usual doses in any scientific studies until now (204).

4. Conclusion

In conclusion, according to currently available evidence, propolis's potential as a natural compound in the treatment or prophylaxis of various diseases and health conditions is promising. As there are several propolis types with different constituents due to different plant sources, geographical locations, conditions of the environment, and bee species leading to diverse pharmacological activities, the following constituents are considered to be the most pharmacologically important ingredients: Caffeic acid, Apigenin, Artepillin C, CAPE, Chrysin, Naringin, Pinocembrin, Kaempferol, Galangin, Luteolin, Coumaric acid, Quercetin, and Genistein are involved in the health benefits of propolis, such as antioxidant, anti-inflammatory, wound healing, oral hygiene, antiviral, antibacterial, immune system, and metabolic disease regulation, as proven experimentally on animals and/or humans and making propolis a valuable natural ingredient for pharmaceutical companies to explore and incorporate into their product development.

Despite various studies on the therapeutic effects of propolis on both animals and humans, many of those studies lack the chemical examination of constituents, plant sources, and extraction methods, which play an important role in the pharmacological activity of propolis extract. Additionally, the lack of a standardized propolis extract is another issue that would be useful to take into consideration in future studies, but propolis still remains a potentially useful medicinal ingredient that can be used to treat illnesses either as a primary or adjunctive treatment.

As preclinical and clinical evidence points to propolis's potential role in antioxidants, immune system regulation, anti-microorganisms, oral health, and anti-inflammatory activity, more controlled and further research with a larger number of samples and participants, as well as a better understanding of propolis's bioactive ingredients, their exact mechanism of action, appropriate dose, suitable dosage form, effectiveness, and safety in long-term use, are required to ensure its therapeutic value. Additionally, more studies regarding combinations of propolis with synthetic drugs could be done to gain more accurate information about the synergistic effect between synthetic drugs and propolis, as currently available studies are limited in terms of a small number of participants and insufficient clinical studies.

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6. List of tables

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8. Table of abbreviation

Abbreviation	***Definition***	***Abbreviation***	***Definition***
IL	Interleukin	PRV	Pseudo Rabies Virus
MCP-1	Monocyte chemoattractant protein 1	NDV	Newcastle disease virus
CRP	C-reactive protein	CDV	Canine Distemper Virus
PG	Prostaglandin	VZV	Varicella zoster virus
CAPE	Caffeic acid phenethyl ester	TGF	Transforming growth factor
NF-kappa B	Nuclear factor kappa B	ECM	Extracellular matrix
COX	Cyclooxygenase	NKT	Natural killer T cells
NO	Nitric Oxide	NSAID	Non-steroidal anti-inflammatory drug
EEP	Ethanolic extract of propolis	SE	Solvent extraction
AEP	Aqueous extract of propolis	UE	Ultrasound extraction

Abbreviation	*Definition*	*Abbreviation*	*Definition*
MERS-CoV	East respiratory syndrome–related coronavirus	ARE	Antioxidant response element
SOD	Superoxide dismutase	mTOR	Mammalian target of rapamycin
ROS	Reactive oxygen species	PI3K	Phosphatidylinositol 3-kinase
RNS	Reactive nitrogen species	AKT	AK transforming – Protein kinase B
HDL	High-density lipoprotein	TNF	Tumor necrosis factor
LDL	Low-density lipoprotein	MAPK	Mitogen-activated protein kinase
NA	Neuraminidase	vLDL	Very low-density lipoprotein
HIF-1-alpha	Hypoxia-inducible factor 1-alpha	GSH	Reduced form of glutathione
Keap1	Kelch-like ECH associated protein 1	GM-CSF	Granulocyte-macrophage colony stimulating factor
PEG	Polyethylene glycol	RA	Rheumatoid Arthritis
DER	Drug to extract ratio	PAK	p21-activated kinase
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2	DM II	Diabetes mellitus type 2
HIV	Human immunodeficiency virus	MMP-9	Matrix metalloproteinase 9
Ig	Immunoglobulin	NK	Natural killer cells
NRF2	Nuclear factor erythroid 2-related factor 2	GPx	Glutathione peroxide