REPUBLIC OF TÜRKİYE
BINGOL UNIVERSITY
INSTITUTE OF SCIENCES

INVESTIGATION OF CHEMICAL CONTENT AND BIOLOGICAL ACTIVITIES OF NORTHERN IRAQI PROPOLIS

Ph.D. THESIS

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BİNGÖL-2025

ACKNOWLEDGEMENT

First of all, I would like to express my deepest gratitude to my thesis supervisor, Assoc. Prof. Dr. Adnan Ayna, for his boundless support, invaluable guidance, and constant encouragement during the duration of this study. His expertise, considered remarks and constructive criticisms have been highly influential in shaping the course and merits of this thesis. I am eternally grateful for the honor of being under his supervision. I also extend my sincerest gratitude to the distinguished members of my thesis committee, Assoc. Prof. Dr. Bülent Kaya and Assoc. Prof. Dr. Fatma Caf, for their valuable time, insightful suggestions and helpful comments in preparing and defending this work. Special appreciation should be given to Assoc. Prof. Dr. Yusuf Temel for his generous assistance and technical guidance on purification of GST and enzyme inhibition experiments. His contribution significantly enhanced the experimental section of this work. I also thank the entire membership of the Kaya Laboratory, whose cooperative spirit and learning culture made the research journey fruitful and delightful too. I particularly thank Zeynep and Esra for their selfless support, help and friendship during the lab work. I would also like to thank Prof. Dr. Fethi Ahmet Özdemir for his kindness in donating the microbial strains used in the antimicrobial tests, which were crucial in the biological evaluation of the samples. I also extend my sincerest thanks to BUBAP (Bingöl University Scientific Research Projects Coordination Unit, Project Number: BAP-FBE.2022.002) for their financial and institutional assistance, which was fundamental to the realization of this project. Finally, I would like to extend my heartfelt appreciation to my family members for their unlimited love, tolerance and unwavering confidence in me. Their moral support and tolerance have been a source of encouragement that has lasted the entire duration of this academic endeavor. To all those who assisted me in this work-thank you.

FARHAN JAAFAR TAHER NERWAY

BİNGÖL 2025

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LIST OF SYMBOLS AND ABBREVIATIONS

EEP	Ethanolic Extract of Propolis
WEP	Water Extract of Propolis
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
GAE	Gallic Acid Equivalent
QE	Quercetin Equivalent
GC-MS	Gas Chromatography–Mass Spectrometry
LC-MS/MS	Liquid Chromatography–Tandem Mass Spectrometry
UHPLC	Ultra High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
FCR	Folin–Ciocalteu Reagent
GSH	Glutathione
CDNB	1-Chloro-2,4-dinitrobenzene
KH_2PO_4	Potassium dihydrogen phosphate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
H_3BO_3	Boric acid
$NaCH_3COO$	Sodium acetate
SDS-PAGE	Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis
SDS	Sodium Dodecyl Sulfate
APS	Ammonium Persulfate
TCA	Trichloroacetic acid
CAPE	Caffeic Acid Phenethyl Ester
ROS	Reactive Oxygen Species
CAT	Catalase
GPX	Glutathione Peroxidase
SOD	Superoxide Dismutase

MAPEG	Membrane-Associated Proteins in Eicosanoid and Glutathione metabolism
ACE2	Angiotensin-Converting Enzyme 2
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
M/z	Mass-to-charge ratio
Ppm	Parts per million
Ppb	Parts per billion
UV	Ultraviolet
Na_2CO_3	Sodium carbonate
$NaNO_2$	Sodium nitrite
$AlCl_3$	Aluminium chloride
RT	Retention Time
IC_{50}	Half-maximal inhibitory concentration

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INVESTIGATION OF CHEMICAL CONTENT AND BIOLOGICAL ACTIVITIES OF NORTHERN IRAQI PROPOLIS

ABSTRACT

This study provides a comprehensive investigation of the chemical composition and biological activities of propolis samples collected from four geographically distinct regions in Northern Iraq, namely Erbil, Mosul, Duhok and Sulaymaniyah. With advanced chromatographic and spectrophotometric methods, both ethanol and water extracts were evaluated for phytochemical content, antioxidant potential, enzyme inhibitory activity, antimicrobial activity and photoprotective activity. LC-MS/MS profiling revealed significant regional and solvent-based variation in phenolic and flavonoid composition. Ethanol extracts had consistently higher concentrations of the major flavonoids galangin, chrysin, and naringenin, with Mosul and Sulaymaniyah samples having the most enriched profiles. GC-MS analysis also revealed a broad range of fatty acids, alkanes, and phenolic esters, with Duhok's rich content of palmitic acid and Mosul's unique organoselenium compounds. ICP-MS elemental analysis showed significant diversity in macro- and trace elements, with high concentrations of calcium, magnesium, and iron. Toxic elements such as lead were present at trace levels within acceptable safety levels. Biological tests showed that ethanol extracts possessed superior antioxidant activity, expressed as high total phenolic and flavonoid content, DPPH radical scavenging, and total antioxidant capacity. Sulaymaniyah and Duhok ethanol extracts showed the most activity. Besides, ethanol extracts exhibited significant antimicrobial activity against *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, *Bacillus subtilis* and *Candida albicans*, where Erbil and Sulaymaniyah samples were the most effective. Sun protection factor (SPF) investigation indicated that Mosul ethanol extract exhibited the highest SPF value (17.9 at 20 ppm), confirming the UV-protective activity of propolis. The study also evaluated propolis inhibitory activity on human erythrocyte Glutathione S-Transferase (GST). The most active GST inhibition was that of Sulaymaniyah ethanol extract (IC_{50} = 5.52 µg/mL), which correlated with its high content of flavonoids. The dual role of propolis as both an antioxidant and as an enzyme modulator indicates its medicinal potential as well as the need for careful use in health facilities. Overall, the findings validate Northern Iraqi propolis's chemical and biological richness, warranting its application in nutraceutical, pharmaceutical and cosmetic formulations. The study highlights the contribution of extraction protocols and geographic origin in standardizing propolis product quality and efficacy.

Keywords: Propolis, glutathione s-transferase, LC-MS/MS, sun protection factor

KUZEY IRAK PROPOLİSİNİN KİMYASAL İÇERİĞİNİN VE BİYOLOJİK AKTİVİTELERİNİN ARAŞTIRILMASI

ÖZET

Bu çalışma, Kuzey Irak'taki Erbil, Musul, Duhok ve Süleymaniye olmak üzere coğrafi olarak farklı dört bölgeden toplanan propolis örneklerinin kimyasal bileşimi ve biyolojik aktivitelerinin kapsamlı bir araştırmasını sunmaktadır. Gelişmiş kromatografik ve spektrofotometrik yöntemlerin kullanımıyla, hem etanol hem de su özütleri fitokimyasal içerik, antioksidan potansiyel, enzim inhibitör aktivitesi, antimikrobiyal aktivite ve fotokoruyucu aktivite açısından değerlendirildi. LC-MS/MS profillemesi, fenolik ve flavonoid bileşiminde önemli bölgesel ve çözücü bazlı varyasyon olduğunu ortaya koydu. Etanol özütleri, galangin, krizin ve naringenin gibi başlıca flavonoidlerin daha yüksek konsantrasyonlarına sahipken; Musul ve Süleymaniye örnekleri en zengin fitokimyasal profillere sahipti. GC-MS analizi ayrıca, Duhok'un zengin palmitik asit içeriği ve Musul'un benzersiz organoselenyum bileşikleri ile geniş bir yelpazede yağ asitleri, alkanlar ve fenolik esterler ortaya koydu. ICP-MS element analizi, yüksek kalsiyum, magnezyum ve demir konsantrasyonlarıyla makro ve eser elementlerde önemli çeşitlilik gösterdi. Kurşun gibi toksik elementler, kabul edilebilir güvenlik seviyeleri içinde eser seviyelerde mevcuttu. Biyolojik testler, etanol özütlerinin, yüksek toplam fenolik ve flavonoid içeriği, DPPH radikal temizleme ve toplam antioksidan kapasitesi olarak ifade edilen üstün antioksidan aktiviteye sahip olduğunu gösterdi. Süleymaniye ve Duhok etanol özütleri en fazla aktiviteyi gösterdi. Ayrıca, etanol özütleri *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, *Bacillus subtilis* ve *Candida albicans*'a karşı önemli antimikrobiyal aktivite gösterdi; Erbil ve Süleymaniye örnekleri en etkiliydi. Güneş koruma faktörü (SPF) araştırması, Musul etanol özütünün en yüksek SPF değerini (20 ppm'de 17,9) gösterdiğini ve propolisin UV koruyucu aktivitesini gösterdi. Çalışma ayrıca propolisin insan eritrosit Glutatyon S-Transferaz (GST) üzerindeki inhibitör aktivitesini de değerlendirdi. En aktif GST inhibisyonu, Süleymaniye etanol özütüydü (IC_{50} = 5,52 µg/mL). Propolisin hem bir antioksidan hem de bir enzim modülatörü olarak ikili rolü, tıbbi potansiyelinin yanı sıra sağlık alanında dikkatli kullanılması gerektiğini gösterir. Genel olarak, bulgular Kuzey Irak propolisinin kimyasal ve biyolojik zenginliğini doğrulayarak, farmasötik ve kozmetik formülasyonlarda uygulanmasını garanti eder. Çalışma, propolis ürün kalitesini ve etkinliğini standartlaştırmada ekstraksiyon protokollerinin ve coğrafi kökenin katkısını ortaya koymaktadır.

Anahtar kelimeler: Propolis, glutatyon s-transferaz, LC-MS/MS, güneş koruma faktörü

1. INTRODUCTION

1.1. General Overview

Due to the extraordinary diversity of chemicals present in nature, natural products are thought to be a rich source of bioactive molecules and have significant medical and therapeutic potential. Numerous research have concentrated heavily on examining the chemical constituents of natural products and evaluating their biological potential, particularly for the development of therapeutics for a range of acute or chronic disorders. Propolis, a resinous product collected by bees from plant buds and exudates, has long been known for its diverse biological properties and medicinal value. Bees mostly use propolis to seal their hives preventing pathogens from entering and maintaining hive hygiene. The composition of propolis varies depending on the geographical location and types of plants but it generally consists of resins, beeswax, essential oils, pollen and a range of bioactive compounds including flavonoids, phenolic acids, terpenes and vitamins (Kasote et al., 2022; Ghallab et al., 2025). These compounds contribute to its potent antioxidant, antimicrobial, anti-inflammatory and anticancer activities. Propolis has been widely used in traditional medicine for centuries to treat a variety of diseases from infections to wound and continues to attract scientific attention due to its potential therapeutic effects (Selatino, 2022). Numerous studies have demonstrated its ability to modulate immune responses, protect against oxidative stress and exhibit cytotoxic effects against various cancer cell lines.

1.2. Physical Properties of Propolis

There are many distinct physical characteristics of propolis, but several stand out. These include color, texture, flavor, melting point, density, solubility, brittleness, stickiness and transparenc (Chuttong et al., 2023). Propolis' color might change based on its processing and botanical origins. Dark brown to greenish-brown, reddish-brown, or even black are typical color ranges. It is solid at room temperature but when heated, it can become softer and more malleable (Chuttong et al., 2023). When chilly, it may become brittle. The smell

of propolis is distinctive and pleasant; it's sometimes called resinous or balsamic. Depending on the sorts of plants the bees collected resin from, the aroma may differ (Papp et al., 2021). Due to the inclusion of numerous plant resins and bioactive substances, propolis has a harsh taste (Irigoiti et al., 2021). While propolis cannot be dissolved in water, it may be dissolved in ethanol, acetone and other organic solvents like propylene glycol. It is ideal for the creation of tinctures and extracts due to its solubility (Bankova et al., 2021). Lower temperatures can cause propolis to become brittle. For different uses, this makes it simpler to shatter or crush into smaller pieces. The adhesive qualities of propolis are well known and can be used to seal gaps and cracks in beehives (Chuttong et al., 2023). Additionally, because of its stickiness, it works well in several topical applications. Propolis is often opaque when it is raw, but when it is dissolved in solvents or turned into tinctures, it can turn translucent or transparent. It's vital to remember that the physical characteristics of propolis might change based on its botanical source, location, and collection and processing methods (Alcivar-Saldaña et al., 2024).

1.3. Chemical Constituents of Propolis

Propolis is complex, and its chemical make-up varies depending on the type of bee, where it comes from, and the plants that influence the bee's metabolic processes. Based on their place of origin and chemical makeup, several types of propolis have a range of biological characteristics, such as antioxidant, antimicrobial and anticancer activities. Its content generally consists of water, flavonoids, phenolic acids, terpenes and terpenoids, essential oils, waxes, resins, amino acids, proteins, minerals, vitamins and other aromatic and nonaromatic compounds (Table 1.1; Figure 1.1) (Kasote et al., 2022). Flavonoids are one of the most important groups of compounds in propolis due to their potent anti-oxidant, anti-inflammatory and anticancer activities. They also significantly contribute to the antimicrobial properties of propolis and are often responsible for its color. Some common flavonoids found in propolis include pinocembrin, galangin, chrysin, quercetin and apigenin (Cui et al., 2022). Phenolic acids are another significant group of compounds in propolis contributing to its antimicrobial, anti-inflammatory and antioxidant activities. They can also have positive effects on blood circulation and metabolic health. Key phenolic acids found in propolis include caffeic acid, ferrulic acid, p-coumaric acid and benzoic acid (Nichitoi et al., 2024). Terpenes are a large and diverse group of natural compound with

significant biological activities. Some notable terpenes and terpenoids in propolis include triterpenoids, pinene and limonene (Isidorov et al., 2024). Propolis contains essential oils which contribute to its distinctive aroma and enhance its biological activities. Some of the essential oils found in propolis include eugenol, cineole and linalool (Boulechfar et al., 2022). Waxes and resins are also significant part of propolis's composition providing its sticky texture and contributing its preservative properties. Propolis contains both long chain fatty acids and esters which are derived from bee's secretions and plant exudates. Propolis contains a variety of amino acids (glutamic acid, aspartic acid, proline etc.), vitamins (B vitamins such as B1, B2, B6, Vitamin C and vitamin E), minerals (calcium, magnesium, potassium, iron, zinc etc.) although in relatively small amounts (Zullkiflee et al., 2022).

Table 1.1. Chemical content of propolis

Component Type	**Examples**	**Functions**
Flavonoids	Pinocembrin, Quercetin	Antioxidant, antimicrobial
Phenolic Acids	Caffeic acid, Ferulic acid	Antimicrobial, anti-inflammatory
Terpenes	Pinene, Limonene	Biological activities
Essential Oils	Eugenol, Linalool	Aroma, enhances biological activities
Waxes	Long-chain fatty acids	Sticky texture, preservative properties
Amino Acids	Glutamic acid, Proline	Nutritional value
Vitamins	B1, B2, C, E	Health benefits
Minerals	Calcium, Magnesium	Supports metabolic functions

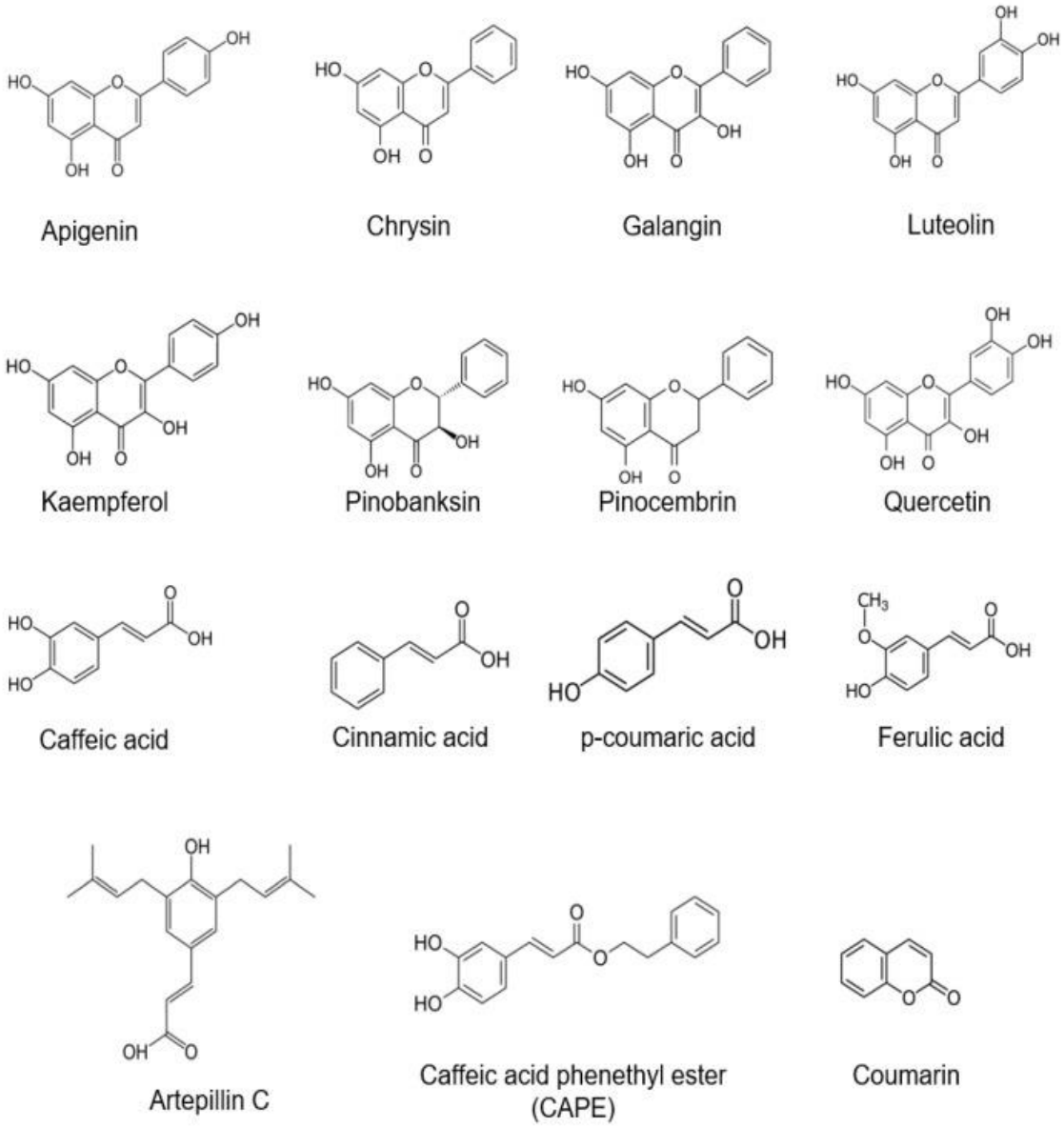

Figure 1.1. Common bioactive compounds in propolis content (Taken from Zulkiflee et al., 2022)

1.4. Biological Activities of Propolis

Propolis is thought to have a wide spectrum of pharmacological effects and has a long history of traditional use in different cultures. Some of the known and speculated pharmacological properties of propolis include antioxidant properties, anti-inflammatory effects, antimicrobial activity, immunomodulatory effects, wound healing, anticancer potential, cardiovascular benefits, anti-diabetic effects, pain relief, skin health and dental health (Table 1.2) (Belmehdi et al., 2023).

Table 1.2. Biological activities of propolis

Pharmacological Effect	Description
Antioxidant Properties	Protects cells from oxidative stress; scavenges free radicals.
Anti-inflammatory Effects	Reduces inflammation and may help in conditions like arthritis and allergies.
Antimicrobial Activity	Exhibits antibacterial, antifungal, and antiviral properties.
Immunomodulatory Effects	Modulates the immune response, enhancing defense against infections.
Wound Healing	Promotes tissue regeneration and accelerates healing processes.
Anticancer Potential	May inhibit cancer cell growth and induce apoptosis in tumor cells.
Cardiovascular Benefits	Supports heart health by improving circulation and reducing cholesterol levels.
Anti-diabetic Effects	Helps regulate blood sugar levels and improves insulin sensitivity.

1.4.1. Antioxidant Properties

Antioxidants are molecules that have the capacity to inhibit or slow down the oxidation of molecules. An antioxidant is a molecule that helps protect cells from damage caused by oxidative stress which results from an excess of reactive oxygen species (ROS) and free radicals in body (Gülçin, 2020). Antioxidants work by neutralizing free radicals preventing chain reactions that can lead to cellular damage. They achieve this by donating electrons without becoming unstable themselves. Some antioxidants also function by chelating metal ions which can catalyze the formation of harmful radicals or by enhancing the body's natural antioxidant defense systems. The antioxidant activity of propolis is primarily attributed to its rich composition of polyphenols, flavonoids and phenolic acids. These bioactive compounds effectively scavenge free radicals, chelate metal ions and enhance the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (Kocot et al., 2018).

1.4.2. Antimicrobial Activity

Propolis exhibits potent antimicrobial activity against a wide range of bacteria, viruses and fungi. Its antimicrobial activity is mainly attributed to its rich flavonoid, phenolic and terpenes all of which interfere with microbial growth by targeting cell membranes, enzymes and genetic material (Zulhendri et al., 2021). Propolis's extensive chemical diversity, extraction techniques, concentration used, collecting season, geography and bee species all play a significant role in how effective it is against microbes. Propolis is effective against both Gram-positive and Gram-negative bacteria though it shows greater

activity against Gram-positive bacteria due to their simple cell wall structure. It has been shown to inhibit growth of S*taphylococcus aureus, Streptococcus mutans, Bacillus subtilis, Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella* species (Cora et al., 2025). The antibacterial effects propolis are linked to the disruption of the bacterial cell membranes, inhibition of bacterial enzymes involved in its metabolism, suppression of biofilm formation and synergistic effects with antibiotics which enhance their efficacy. Additionally, propolis demonstrates potent antifungal activity particularly against pathogenic yeasts and filamentous fungi containing *Candida albicans, Aspergillus niger, Trichophyton* species and *Cryptococcus neoformans (Cerqueira et al., 2022)*. It shows its antifungal activities through multiple mechanisms. These include disruption of fungal cell wall and membrane, inhibition of fungal spore germination and interference with ergosterol synthesis, a key component of fungal cell membrane. Several studies have highlighted potential antiviral effect of propolis against various viruses including influenza virus, herpes simplex virus (HSV-1 and HSV-2), human papillomavirus (HPV) and even coronaviruses. Propolis shows its antiviral activity through blocking viral entry into host cells, inhibiting viral replication and protein synthesis and boosting immune system to combat viral infections (Zulhendri et al., 2021). Owing to its broad spectrum antimicrobial activity propolis has several applications in oral healthcare products to prevent dental caries and periodontal infections, in wound healing formulations, in food preservation as a natural alternative to synthetic preservatives and in pharmaceutical formulations to treat infections.

1.4.3. Anti-Inflammatory Effects

Propolis has been shown to possess anti-inflammatory properties due to its rich composition of flavonoids, phenolics and terpenoids that modulate inflammatory pathways and reduce oxidative stress (Zulhendri et al., 2022). It may help reduce inflammation in the body, which is associated with various chronic diseases. The immune system normally starts an organized process called inflammation in response to microbial infections and/or tissue injury. An effective inflammatory response is one in which the inflammatory processes, which are controlled by anti-inflammatory cytokines and other mediators, attenuate and resolve once the triggers have been removed (Chen et al., 2017). The anti-

inflammatory effects of propolis are attributed to its ability to inhibit the pro-inflammatory cytokines, suppress key inflammatory enzymes and modulate immune system.

1.4.4. Wound Healing

Propolis has been used topically to promote wound healing. It may help in tissue regeneration and accelerate the healing process. Propolis is regarded as a great option for burn treatment since it enhances skin cell proliferation, activation, and growth capability and is well tolerated with infrequent allergic reactions and no toxicity (Oryan et al., 2018). According to quantitative and qualitative investigations of collagen types I and III expression and degradation in the wound matrix, certain results indicate the therapeutic efficacy of propolis (Yang et al., 2022). This suggests that propolis may have a favorable biochemical environment favoring re-epithelization.

1.4.5. Anticancer Potential

Studies have extensively investigated the potential anticancer properties of propolis, particularly attributed to its antioxidant and anti-inflammatory effects. Numerous studies have demonstrated that propolis and its bioactive compounds exhibit anticancer activities showing effectiveness against various human cancer lines including oral cavity, stomach, cervix, colon, leukemia, skin, breast and prostate (Valivand et al., 2024; Ghallab et al., 2025). Most of these studies have focused on how propolis extracts from different regions influence the development, multiplication, and metastasis of cancer cells *in vitro*. Several bioactive compounds present in propolis extracts have been linked to anticancer effects with chrysin and caffeine acid phenethyl ester (CAPE) being the most extensively studied. Among these, CAPE is considered one of the key constituent responsible for biological activities of propolis in cancer research (Barış et al., 2024). The ability of cancer cells to trigger apoptosis and cell cycle arrest, the primary mechanisms behind propolis's anticancer properties, was examined in certain studies to assess the effectiveness of anticancer therapy. Propolis's wide range of chemical constituents has a significant impact on its anticancer properties. Flavonoids, one of propolis's active constituents, have chemopreventive actions against the majority of carcinogenesis. Apigenin, caffeic acid, CAPE, ferulic acid, galangin, luteolin, myricetin, pinocembrin, and quercetin are some of

the other active components of propolis that have been shown to have anticancer and antiproliferative qualities (Elumalai et al., 2022).

1.4.6. Cardiovascular Benefits

Propolis's effects on the cardiovascular system have been linked in the literature to its anti-inflammatory, immunomodulatory, antioxidant, antihypertensive, antiangiogenic, and antiatherosclerosis effects (Chavda et al., 2024). Chronic inflammation is a major factor in cardiovascular diseases. Studies suggest that propolis modulates inflammation related pathways through inhibition of pro-inflammatory cytokines reducing vascular inflammation. Propolis can also lower blood pressure throughpromotion of nitric oxide generation enhancing vasodilation. Additionally, propolis helps to regulate lipid metabolism via a reduction in total cholesterol and triglyceride that could help preventing atherosclerosis and coronary artery diseases (Khoshandam et al., 2023).

1.4.7. Coronavirus Disease

Propolis may help COVID-19 patients with their therapy and symptoms, according to certain clinical research. The reason for this is that propolis may contain significant active phytochemicals such as alkaloids, flavonoids, lipids, phenols, polyphenols, saponins, and tannins, all of which have a strong correlation with the pharmacological characteristics of propolis (Ali and Kunugi, 2021). Some studies suggest that propolis may inhibit viral entry and replication by interfering with viral enymes that contain RNA polymerase (Yosri et al., 2021). Additionally some components of propolis such as artepillin C have shown potential in blocking viral attachment to ACE2 receptors that Sars-Cov-2 uses to enter human cells (Shahinozzaman et al., 2020).

1.4.8. Anti-Diabetic Effects

There is some evidence to suggest that propolis may be helpful in managing diabetes primarily owing to its rich biochemical content. These bioactive chemicals contribute to the regulation of glucose metabolism, insulin sensitivity, and pancreatic function (Tsuda and Kumazawa, 2021). Propolis has been shown to reduce level of hyperglycemia through

enhancing glucose uptake and regulating carbohydrate metabolism. Propolis has also been shown to protect pancreatic β cells from oxidative stress and apoptosis preserving insulin secretion.

1.5. Glutathione –S-Transferase

A family of enzymes known as glutathione S-transferase (GST) is essential to the detoxification of several endogenous and exogenous substances in living organisms, including humans (Aloke et al., 2024). The primary function of these enzymes is to conjugate glutathione, a significant antioxidant molecule, to different electrophilic chemicals, increasing their water solubility and facilitating their excretion from the body. This procedure aids in shielding tissues and cells against harm brought on by toxins and reactive substances.

1.5.1. The Mechanism of GST Catalysis

The catalytic mechanism of GST involves two key binding sites that include the G site where glutathione (GSH) binds and the H site which accommodates the hydrophobic substrate. After binding, GST stabilizes GSH in its reactive thiolate form through interactions with active site residues such as tyrosine, serine or cysteine. The activated thiolate anions then performs nucleophilic attack on electrophilic center of substrate forming glutathione-substrate conjugate. This conjugate is subsequently released from the enzyme and further processed by cellular detoxification pathways. Through this mechanisms GST plays a crucial role in metabolizing xenobiotics, mitigating oxidative damage and maintaining cellular homeostasis (Aloke et al., 2024).

1.5.2. GST Classes

GSTs are classified into several cystosolic, mitochondrial and membrane associated classes based on their structure, catalytic properties and evolutionary origin. The cytosolic GSTs include Alpha, Mu, Pi, Sigma, Omega, Theta and Zeta classes each with distinct substrate specificities and tissue distribution (Figure 1.2). Alpha, Mu and Pi GSTs are primarily involved in detoxification and drug metabolism. Alpha GSTs also playing a role in

antioxidant defence mechanism. Sigma GSTs are important in ptostaglanding metabolism while theta GSTs involve in xenobiotic degradation. Zeta GSTs are unique in their involvement in tyrosine catabolism, while Omega GSTs are crucial in thiol metabolism and oxidative stress response. The mitochondrial GSTs (Kappa GST) structurally differ from cytosolic GSTs and are involved in mitochondrial redox balance (BK et al., 2022). Additionally, membrane associated GSTs (MAPEG) including enzymes such as leukotriene C4 synthase play a significant role in lipid metabolism and inflammatory response. Together these diverse GST classes contribute to cellular detoxification, redox homeostasis and metabolism of endogenous and exogenous compounds highlighting their critical role in cellular protection and biochemical regulation.

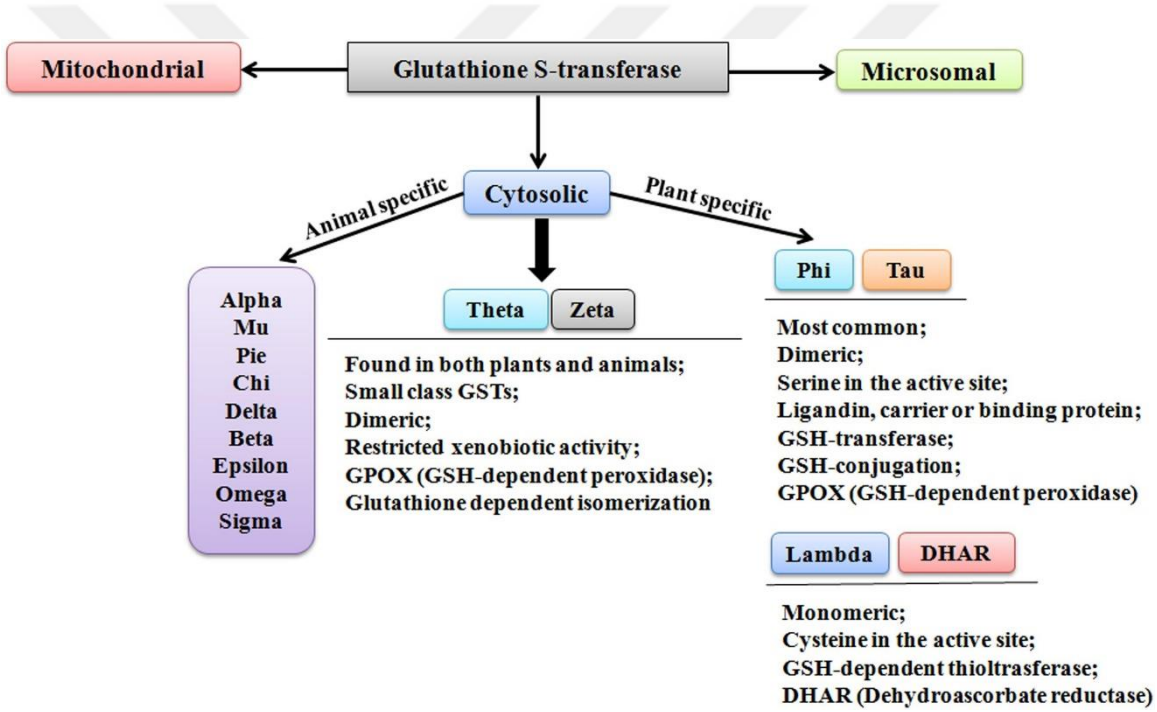

Figure 1.2. Different classes of glutathione S-transferase (Taken from Kumar et al., 2018)

1.5.3. Polymorphisms in GST

Polymorphisms in GSTs significantly influence their enzymatic activity affecting detoxification capacity, drug metabolism and susceptibility to diseases such as cancer, neurodegenerative disorders and cardiovascular anomalies (Grussy et al., 2023). The most well studied polymorphism occur in GSTM1, GSTT1 and GSTP1 genes. GSTM1 and GSTT1 polymorphisms are characterized by gene deletions leading to a complete loss

enzyme activity. GSTP1 polymorphisms result in an altered enzyme structure leading to reduced catalytic efficiency and substrate specificity. These genetic variations influence individual responses to environmental toxins, drugs and oxidative stress making GST polymorphism important biomarkers for personalized medicine, cancer risk assessment and pharmacogenetics (Nakanishi et al., 2022).

1.5.4. Functions of GST

GSTs perform diverse functions that are essential for detoxification, cellular protection and biochemical regulation. Their major role is to catalyze the conjugation of GSH to electrophilic compounds facilitating the detoxification and elimination of xenobiotics, carcinogen and reactive oxygen species (ROS). This helps protect cells from oxidative damage and chemical induced toxicity. GSTs also involved in drug metabolism influencing the pharmacokinetics and effectiveness of various therapeutic agents, including chemotherapy drugs (Mazari et al., 2023). Additionally, GSTs regulate cellular redox homeostasis by neutralizing lipid peroxidation products and maintaining glutathione balance, thus preventing oxidative stress-related diseases. Some GST isoforms, such as GSTP1, have non-enzymatic roles in signaling pathways, including the regulation of apoptosis and cell proliferation by modulating proteins like JNK (c-Jun N-terminal kinase). Furthermore, GSTs contribute to the metabolism of endogenous compounds, such as prostaglandins and steroid hormones and play a role in the degradation of environmental pollutants (Mazari et al., 203). Overall, GSTs are crucial for maintaining cellular integrity by detoxifying harmful substances, regulating oxidative stress and influencing key cellular processes.

1.5.5. GST Inhibition in the Treatment of Human Diseases

Inhibition of GST has emerged as a viable therapeutic strategy against the treatment of many human pathologies, such as cancer, neurodegenerative disorders and drug resistance diseases (Singh and Reindl, 2021; Aloke et al., 2024). GSTs are often overexpressed in cancer cells, where they contribute to chemotherapy resistance by detoxifying anticancer drugs and inhibiting apoptosis. Inhibition of GSTs by selective inhibitors can restore drug sensitivity and enhance chemotherapy efficacy. As examples, ethacrynic acid and TLK117

are known GST inhibitors that have been investigated for their capacity to sensitize tumors to the chemotherapeutic agents such as cisplatin and doxorubicin (Huang et al., 2023). Beyond oncology, inhibition of GSTs is also desirable in neurodegenerative disease such as Parkinson's and Alzheimer's disease, where GSTs play a role in the neutralization of oxidative stress-related injury. Inhibition of specific GST isoforms could modulate pathways of oxidative stress and promote neuronal survival (Khan et al., 2022).

1.6. Aims of The Study

Although numerous studies have extensively documented the biological activities of propolis collected from various regions around the globe, an exceedingly broad literature gap is present regarding the chemical makeup and biological potential of Northern Iraqi propolis. Because of the unique environmental conditions, plant flora, and geographical variations that make up the propolis, it is necessary to conduct an exhaustive investigation of its prospective bioactive character. Therefore, the primary objectives of this Ph.D. thesis are to characterize the chemical content and examine the biological activities of propolis samples collected from different regions of Northern Iraq (Duhok, Sulaymaniyah, Erbil and Mosul). The study also aims to establish the antioxidant, antimicrobial, sun protection and enzyme inhibitory activities of the samples, obtaining valuable information about their therapeutic applications. Employing state-of-the-art analytical techniques for chemical characterization and rigorous bioactivity screening, this research seeks to make a contribution to the growing body of research on propolis and its pharmacological potential, and to clarify the local variations that can influence its efficacy and applications.

2. LITERATURE REVIEW

Literature in propolis has greatly shed light on its bioactivity, chemical composition, and potential uses and directed toward its significance as a natural bioactive compound with far-reaching pharmacological importance. Chemical constituents of propolis are to be critically appraised herein. Moreover, this section will review the reported biological activities of propolis, including antioxidant, antimicrobial, and enzyme inhibitory activities and address the mechanisms responsible for these activities. Through critical examination of the existing knowledge, this section will seek to establish a firm basis for comprehending the significance of studying propolis from various locations and its possible applications in various fields.

A group of researchers studied the chemical composition and biological activity (antibacterial, antifungal, antiviral, antioxidant and cytoprotective activities) of propolis extracts collected from various regions of Poland. The study revealed that the content of phenols (116.16-219.41 mg GAE/g EEP) and flavonoids (29.63-106.07 mg QE/g EEP) of the propolis extracts were correlated with their geographic origin. Chromatographic analysis further confirmed the existence of significant amounts of epicatechin, catechin, pinobanksin, myricetin, and vanillic and syringic acids in the samples. Caffeic acid phenethyl ester was present in all the propolis samples. The biological activities of the propolis extracts also varied according to their geographical origin. The extracts possessed moderate DPPH free radical scavenging activity (29.22% to 35.14%) and relatively low ferrous iron chelation activity (9.33% to 32.32%). Antimicrobial activity of the propolis extracts was inconsistent against pathogenic bacteria and possessed poor antifungal activity. Furthermore, out of the five propolis samples assayed for antiviral activity, just two were active against human papillomavirus (HPV) with the level of activity once more related to the samples' geographic region (Wozniak et al., 2022).

Sarikahya et al. (2021) evaluated chemical composition, antiviral and antioxidant activity of Turkish propolis samples from 39 districts. They quantified phenolic, flavonoid, and triterpene content using LC-HRMS, with diosmetin, rhamnocitrin, isosakuranetin,

naringenin, chrysin, 3-O-methyl-quercetin, and acacetin being prominent flavonoid phenolic components and caffeic acid as the primary detected phenolic acid. The major triterpene compounds were hederagenin and oleanolic acid. Volatile compounds of the propolis samples were characterized by GC–MS, with α-pinene, limonene, and β-pinene being dominant in most of the samples. Antioxidant/phenolic activity of the propolis samples were determined by DPPH, CUPRAC, FRAP and Folin methods and was found to possess total phenolic content ranging from 0.96 to 13.53 mmol TR/g. The antioxidant activities (TAC) by CUPRAC and FRAP methods were ranging from 0.71 to 8.24 and 0.21 to 2.27 mmol TR/g, respectively. Most of the propolis extracts exhibited DPPH scavenging activities more than 80%, and their activities were comparable or better than the positive controls. Another study analyzed Turkish propolis from different regions aimed to determine its qualitative and quantitative composition in both water and ethanolic extracts. HPLC-DAD analysis revealed that the water extract predominantly contained phenolic acids, including caffeic acid (204.00 mg/mL), trans-cinnamic acid, chlorogenic acid, and caffeoylquinic acids, which contribute to its antioxidant activity. In contrast, the ethanolic extract exhibited a rich flavonoid profile, with chrysin (641.33 mg/mL), caffeic acid phenethyl ester (CAPE) (630.67 mg/mL), pinocembrin (572.67 mg/mL), galangin (534.11 mg/mL), and naringenin (372.39 mg/mL), along with kaempferol, trans-cinnamic acid, caffeic acid, myricetin, and quercetin (Bozkuş et al., 2021). A study analyzing 19 Turkish propolis determined their phenolic profiles, antioxidant and antimicrobial activities. Antimicrobial activity was tested against *S. aureus* (ATCC 6538), *P. aeruginosa* (ATCC 15442), *E. coli* (ATCC 11229), and *C. albicans* (ATCC 10231). HPTLC fingerprinting revealed that O-type propolis, primarily derived from *Populus nigra* L., was the dominant type in Turkey. Notably, a novel 3-O-methylquercetin (3MQ)-rich propolis type was identified for the first time, with principal component analysis (PCA) distinguishing it from O-type propolis. Antioxidant activity studies indicated that O-type propolis exhibited superior antioxidant effects, with quercetin, caffeic acid, caffeic acid phenethyl ester (CAPE), and galangin contributing significantly to its bioactivity. Propolis extracts demonstrated moderate antimicrobial activity against tested microorganisms, with minimum inhibitory concentration (MIC) values ranging from 128 to 512 μg/mL. These findings highlight the chemical diversity and biological potential of Turkish propolis (Degirmencioglu et al., 2019).

Nalbantsoy et al. (2022) reported first comprehensive study of the composition of propolis samples from seven Cyprus regions, in terms of their chemical composition and biological activities. LC-HRMS was employed for the determination of the secondary metabolite composition, with the finding that the primary flavonoids that were detected were isosakuranetin, naringenin, rhamnocitrin, diosmetin, chrysin and acacetin. Interestingly, the primary compounds found to be present in the ethanol-water extracts were verbascoside, a phenylethanoid glycoside, and chlorogenic acid. GC-MS scanning for volatile compounds indicated that the extracts of propolis contained α-pinene as the predominant compound.

In Machado et al. (2021), 44 samples of Brazilian propolis from the Paraná and Santa Catarina States, southern Brazil, were analyzed to identify the regional trend of the propolis samples. The chemical compounds of these samples were determined and quantified by comparing their chemical patterns against authentic standards. The results showed that Brazilian Propolis from Southern Brazil is mainly characterized by caffeoyl-quinic acids (11.14 to 21.45 mg/g), p-coumaric acid derivatives (6.27 to 12.17 mg/g), and flavonols (9.35 to 23.55 mg/g). The remaining compounds are benzoic acid derivatives (3.18 to 7.45 mg/g) and dihydroflavonols (0.17 to 4.25 mg/g). These findings contribute to the understanding of the chemical constitution that defines the regional identity of propolis of this part of Brazil.

Studies on Jordanian propolis from Al-Ghour have analyzed its chemical constituent, antioxidant activity and xanthine oxidase inhibitory effects. HPLC-PDA identified key polyphenols including pinocembrin (2.82%), chrysin (1.83%), luteolin-7-O-glucoside (1.23%) and caffeic acid (1.12%) along with β-tocopherol (2.01 µg/g). Antioxidant activities assessed via DPPH scavenging (IC_{50}: 6.13-60.5 µg/mL) and xanthine oxidase inhibition (IC_{50}: 75.11-250.74 µg/mL) were attributed to these compounds (Naik et al., 2021).

Propolis from northwest Greece has been characterized through chemical profiling and biological evaluation. Most samples exhibited a European type composition rich in phenolic acids, flavonoids and chalcones. The European-type propolis showed strong antioxidant activity (86–91% inhibition at 200 µg/mL), along with antibacterial (MIC:

0.56–1.95 mg/mL) and moderate antifungal activity (MIC: 1.13–2.40 mg/mL), particularly in terpene-rich Mediterranean samples (Pyrgioti et al., 2022).

A study analyzing 158 Australian propolis samples revealed a total phenolic and flavonoid content (TPC and TFC) reaching up to 181 mg gallic acid equivalent (GAE/g) and 145 mg quercetin equivalent per gram (QE/g) respectively. Within the control samples, Brazilian red propolis had the highest TFC with 122.3 mg GAE/g extract while the Uruguayan poplar and Brazilian green propolis contained 75 and 57 mg QE/g extract on average, respectively indicating that some Australian propolis exhibited stronger antioxidant activity than Uruguayan and Brazilian propolis as it was also evident in DPPH assays (Tran et al., 2022).

Green, red and brown propolis are the three primary types of Brazilian propolis, which are known for their diversity. Due to variations in botanical sources and geographic locations, each type exhibits notable differences in chemical composition and biological activities. The most researched kind of propolis in Brazil is green propolis, which is mostly derived from *Baccharis dracunculifolia*. It is distinguished by high concentrations of caffeoylquinic acids and prenylated phenylpropanoids, including baccharin and artepillin C. Green propolis also contains significant amounts of the volatile compounds nerolidol and spathulenol. Because of its anti-inflammatory, antimicrobial and antioxidant qualities, this kind of propolis is a useful natural product for both medical and cosmetic uses. Red propolis, which is mostly obtained from *Symphonia globulifera* and *Dalbergia ecastaphyllum*, is abundant in polyprenylated benzophenones, pterocarpans, chalcones, and isoflavonoids. These substances support its unique biological properties, such as its anti-inflammatory and antioxidant properties. Despite being less researched than green propolis, red propolis' distinct chemical profile makes it highly promising for therapeutic uses. The second most common type of propolis in Brazil is brown, which has significant medical and economic value. Terpenes, phenolic acids, and flavonoids such as galangin, pinocembrin, and chrysin are among its varied chemical composition. Sesquiterpenes like β-caryophyllene and humulene, which are common in samples from various regions, are found in the volatile oil of brown propolis (Ribeiro et al., 2023; Righi et al., 2013).

The primary organic components of propolis from New Zealand have been identified and measured through the use of GC-MS and HPLC analysis. Because of their low response

factors in GC-MS, HPLC was chosen for flavonoid analysis, revealing TFC of 30–40 mg/mL. The propolis from New Zealand is notable for having a high percentage (~70%) of dihydroflavonoids, such as pinocembrin, pinobanksin, and pinobanksin 3-acetate. GC-MS analysis of non-flavonoid components revealed low concentrations of fatty acids (0.25–0.78 mg/mL), mostly cinnamic acids and their esters, and aromatic compounds (3–7.5 mg/mL) (Markham et al., 1996). The distinctive native vegetation and the presence of plant species from the Northern Hemisphere have an impact on the composition of propolis in New Zealand. Propolis from Te Urewera, a region with a predominance of native flora, has a unique chemical profile rich in diterpenoids like isocupressic acid, acetyl isocupressic acid, manool, torulosal, communic acid and ferruginol, whereas propolis from areas with poplar and other introduced species resembles propolis from the Northern Hemisphere. These diterpenoids are consistent with Mediterranean-type and some Brazilian propolis, according to GC-MS and NMR analysis. Studies on bioactivity showed that it had antimicrobial effects against *S. aureus*, strong antioxidant qualities (TEAC: 1481 mg trolox g^{-1}), and inhibited mitochondrial dehydrogenase activity and superoxide production (IC_{50}: 262 µg/mL). These results demonstrate the endemic propolis of New Zealand's bioactive potential (Manley-Harris et al., 2025).

Another study assessed the antimicrobial activity and chemical composition of 70% ethanolic extracts of Nepalese propolis, derived from *Apis mellifera* L. and *Trigona sp*. HPLC-DAD-MS/MS analysis revealed that both extracts shared a similar composition, predominantly consisting of flavonoid aglycones (mainly neoflavonoids and isoflavonoids) and pterocarpans. The highest antibacterial activity was observed against *Helicobacter pylori*, *S. aureus* and *Shigella flexneri* (Okińczyc et al., 2020).

Ethanol extracts of propolis from China and the US were examined for their chemical makeup and antifungal properties. Thirty of the 49 compounds found by UPLC-Q-TOF-MS were shared by the two samples. Pinocembrin, pinobanksin-3-O-acetate, galangin, chrysin, pinobanksin, and pinobanksin-methyl ether were the main components of EEP-C and EEP-A. With a minimum inhibitory concentration (MIC) of 0.8 mg/mL, both extracts demonstrated antifungal activity against *Penicillium notatum* (Xu et al., 2019).

Seven propolis samples from various Moroccan regions were examined in a study that assessed their biological activities, phytochemical compositions, and physicochemical characteristics. Hydration (3.3–5.2%), pH (4.1–5.5), soluble (66.1–75.4%) and insoluble (23.8–33.7%) materials, ash content (1.6–2.3%), conductivity (1.5–2.5 mS/cm), organic matter (97.70–98.4%), wax (19.7–51.5%), resin (46.8–75.2%), and balsam (1.5–3.1%) were among the physicochemical parameters that were measured. Proteins (1.7–6.2 g/100 g) and total carbohydrates (1.5–2.0 mg Glc eq/g) were also measured; the most common minerals were calcium, sodium, potassium, and magnesium. Moroccan propolis demonstrated inhibitory activity against α-glucosidase (IC_{50}: 90.99–876.24 μg/mL) and α-amylase (IC_{50}: 195.09–963.79 μg/mL), suggesting possible uses in the treatment of metabolic diseases such as diabetes (Laaroussi et al., 2021). Another study investigating four ethanol extracts of Moroccan propolis focused on their chemical composition, antibacterial activity, and antioxidant potential. Ultra-High-Performance Liquid Chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis identified over 100 compounds, primarily flavonoids, with several flavonoid glycosides. Notably, trigonelline, an alkaloid, was detected in propolis for the first time. Antibacterial activity, assessed using well diffusion and microdilution methods, revealed strong inhibition, with inhibition zones ranging from 10.00 ± 0.00 to 22.5 ± 2.12 mm. Minimum inhibitory concentration (MIC) values varied from 0.15 to >5 mg/mL, while minimum bactericidal concentration (MBC) values ranged from 1.25 to >5 mg/mL. Antioxidant capacity was evaluated using reducing power, β-carotene bleaching, and DPPH scavenging assays. The IC_{50} values for DPPH scavenging ranged from 67.61 to 140.04 μg/mL, while β-carotene bleaching assay results showed a relative antioxidant activity between 68.40 ± 3.90% and 100.92 ± 4.24% (Belmehdi et al., 2021).

The chemical composition, total phenolic and flavonoid content, antioxidant potential, and antimicrobial activity of propolis samples from different parts of Balochistan were all examined. Steroids, carbohydrates, flavonoids, coumarins, cardiac glycosides, quinones, anthraquinones, terpenoids, tannins, and phlobatannins were all found in different amounts by phytochemical analysis. The range of the TFC was 0.15 ± 0.09 to 0.66 ± 0.33 mg QE g^{-1}, and the range of the TPC was 2.93 ± 1.25 to 6.02 ± 2.87 mg GAE g^{-1}. Propolis sample had antioxidant potential (IC_{50} value between 27.07 ± 0.73 mg/mL and 84.43 ± 2.07 mg/mL), according to an assessment of antioxidant capacity using DPPH radical

scavenging activity. Antibacterial activity was evaluated against *Escherichia coli*, *S. aureus*, *P. aeruginosa*, and *K. pneumoniae*, with an inhibition zone ranging from 7.46 ± 1.50 mm to 20.33 ± 1.52 mm. Antifungal testing indicated that propolis inhibited *Aspergillus niger* by 82%, while it was highly active against *Aspergillus parasiticus* (80% inhibition) (Akbar et al., 2022).

Baltas et al. (2016), described the inhibitory effects of ethanolic propolis extracts (EPE) on the enzymes urease, xanthine oxidase (XO), and acetylcholinesterase (AChE) were diverse, but the lower the inhibition values (IC_{50}: 1/4 0.074 to 1.560 mg/mL), the higher the phenolic content. The EPE has shown to be an excellent source of inhibitors that may be utilized as natural inhibitors to help people's health. The monoamine oxidase (MAO) enzyme activity was suppressed by propolis, pollen, and honey, in 50% order but propolis had stronger MAO inhibition than pollen and honey is due to the presence of more phenolic compounds in its composition (Yildiz et al. 2014). In a different study, Izol and Turhan described antidiabetic, antiepilepsy, anticholinergic, and antiglaucoma properties of Bitlis propolis. The enzyme inhibition results are as follows: hCA I exhibited an IC_{50} value of 7.19 µg/mL, hCA II demonstrated an IC_{50} of 8.15 µg/mL, AChE showed an IC_{50} of 5.17 µg/mL, BChE presented an IC_{50} of 7.50 µg/mL, and α-glucosidase displayed an IC_{50} of 5.72 µg/mL (Izol and Turhan, 2024). In the analyzed propolis sample, a total of 31 phytochemicals were identified, with acacetin being the most abundant compound, detected at a concentration of 23.604 mg/g. The presence of these phytochemicals is associated with the antioxidant properties of propolis. Mineral analysis revealed the presence of 18 elements, indicating a composition rich in minerals contributing to the salt content. The TPC was determined to be 215.14 mg GAE/g, while the TFC was measured at 79.11 mg QE/g. Antioxidant activity assessments yielded the following results: Fe^{3+} reduction capacity of 0.940 µg/mL, CUPRAC of 1.183 µg/mL, FRAP of 0.963 µg/mL, DPPH• scavenging activity with an IC_{50} value of 16.7 µg/mL, and ABTS radical scavenging activity with an IC_{50} value of 8.01 µg/mL.

In vitro and *in vivo* studies using an ethanol extract of Brazilian green propolis (EEP-B55) and drugs revealed that EEP-B55 inhibited the activities of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 in a concentration-dependent manner and that artepillin C, kaempferide, dihydrokaempferide, isosakuranet after treating rats with 5-fold the

recommended daily dosage of EEP-B55, the IC_{50} values of artepillin C for each CYP were roughly 33-fold greater than their Cmax in the blood. These demonstrate that at the recommended daily consumption of EEP-B55, artepillin C has no significant detrimental effect on liver CYP enzyme activity (Naramoto, et al. 2014).

A study conducted by Zhang, et al. (2015), on glucosidase and sucrase enzymes demonstrated that the 75% ethanol extracts of propolis (75% EEP) exhibited the highest inhibitory effect through a non-competitive inhibition mechanism. In contrast, a mixed inhibition mode was observed with 50% EEP, 95% EEP, and 100% EEP; whereas a competitive inhibition mode was observed with water extracts of propolis (WEP) and 25% EEP. In addition, WEP had the higher inhibitory efficacy against maltase. These findings suggest that propolis extracts have potential applications as nutraceuticals to control postprandial hyperglycemia.

The sun protection factor (SPF) of the ethanolic extracts of the Lithuanian propolis and its plant precursors was evaluated spectrophotometrically (Stanciauskaite et al., 2022). The results indicated that the SPF values of 10 µg/mL extracts ranged from 2.010 to 4.851, with the highest SPF observed in the propolis extract (4.851) and the lowest in the pine buds extract (2.010). Additionally, the SPF values of standard compounds, including quercetin, p-coumaric acid, and salicin, were assessed, revealing that p-coumaric acid exhibited the highest SPF at 8.921. Extracts rich in p-coumaric acid, such as those from poplar buds and propolis, demonstrated nearly twice the SPF compared to extracts from birch and pine buds. Furthermore, p-coumaric acid exhibited a higher SPF than quercetin, highlighting its potential as an effective natural UV-protective compound.

3. METHODOLOGY

3.1. Material Collection

The propolis samples were collected from different regions of Northern Iraq (Duhok, Sulaymaniyah, Erbil and Mosul). Propolis samples were collected directly from beekeepers rather than purchased from markets. Propolis was obtained from permanent beekeepers. The geographical locations of the collected samples are presented in Table 3.1.

Table 3.1. The geographical locations of the collected samples

	Locations	Coordinate	Altitude
1	Erbil	N 36°23’18.6792” E 44°12’07.6356”	1063.0m
2	Mosul	N 36°48’53.6796” E 42°17’07.6956”	406.0m
3	Duhok	N 37°00’11.25”, E 43°31’46.6356”	1220.0m
4	Sulaymaniyah	N 35°43’12.3888” E 45°34’12.5724”	1170.0m

3.2. Instruments Used in the Study

The instruments used to examine the biological activity of propolis samples and determine their chemical composition and the functions provided by each device in this study are summarized in Table 3.2. These instruments play a critical role in various processes, from sample preparation to analysis stages. Each device provides an accurate evaluation of the physical, chemical and biological properties of propolis, increasing the reliability of the data obtained.

Table 3.2. The instruments that are used in the study

N	Instrument name	Use for
1	Weighing scale	To accurately measure the mass of propolis samples and reagents.
2	Laboratory oven	To dry propolis samples
3	Vacuum	To facilitate solvent evaporation during extraction.
4	Vortex shaker	To mix small volumes of solutions thoroughly.
5	Orbital shaker	To ensure uniform mixing during extraction and sample preparation.

Table 3.2. (Continue): The instruments that are used in the study

6	Autoclave	To sterilize equipment and materials used in the study.
7	Water bath	To maintain a constant temperature for extraction
8	Spectrophotometer	To measure absorbance for antioxidant, SPF and enzyme inhibition assays.
9	Centrifuge	To separate liquid and solid phases by centrifugal force.
10	Mixer	To homogenize samples and reagents.
11	µL pipet, mL pipet	To accurately transfer small volumes of liquids.
12	pH-meter	To measure and adjust the pH of solutions used in assays.
13	LC-MS/MS	To analyze the chemical composition of propolis, identifying and quantifying bioactive compounds.
14	GC/MS	To analyze volatile and semi-volatile compounds in propolis, providing information on its chemical profile.
15	ICP/MS	To determine the elemental composition and quantify trace metals in propolis samples.

3.3. Solutions Used in The Study

3.3.1 Preparation of Agar and Broth Solutions Preparation Antimicrobial Activity Determination

Agar Preparation: To prepare the agar medium, 17 g of agar nutrient was dissolved in 1 L of distilled water and mixed thoroughly until fully dissolved. The solution was subsequently autoclaved to ensure sterility and then poured into petri dishes.

Nutrient Broth Preparation: For the preparation of nutrient broth, 8 g of nutrient broth powder was dissolved in 1 L of distilled water and vortexed until completely dissolved. The solution was then autoclaved and used as a culture medium for microbial studies.

3.3.2. Solutions for Enzyme Activity Measurement

Enzyme activity buffer solution (0.1M KH_2PO_4, pH 6.5): A total of 0.68 g of KH_2PO_4 and 0.014 g of EDTA were dissolved in 30 mL of distilled water. The pH was adjusted to 6.5, and the final volume of the solution was brought to 50 mL using distilled water. Glutathione (GSH) solution (20mM): This solution was prepared by dissolving 0.03 g of GSH in 3 mL of distilled water followed by adjusting the final volume to 5 mL with distilled water. 1-Chloro-2,4-dinitrobenzene (CDNB) solution (25 mM): To prepare this solution, 0.025 g of CDNB was dissolved in 5 mL of 95% ethanol.

3.3.3. Preparation of Affinity Chromatography Solutions

1. Regeneration buffer solution 1: The buffer was prepared by dissolving 1.545 g of boric acid (H_3BO_3) and 7.31 g of NaCl in 200 mL of distilled water. The pH of the buffer was adjusted to 8.5, then the volume was completed to 250 mL with distilled water.
2. Regeneration buffer solution 2 (acetate buffer): 2.05 g of $NaCH_3COO$ and 7.31 g of NaCl were dissolved in 200 mL of distilled water. The pH was adjusted to 4.5 and the final volumewas adjusted to 250 mL by distilled water.
3. Equilibration solution; This solution was consisted of 10 mM KH_2PO_4 and 150 mM NaCl at pH 7.4. The preparation involved dissolving 0.68 g of KH_2PO_4 and 4.38 g of NaCl in 400 mL of distilled water adjusting the pH to 7.4, then bringing the total volume to 500 mL.
4. Washing solution: 0.136 g of KH_2PO_4 and 0.745 g of KCl were dissolved in 80 mL distilled water. The pH was adjusted to 8.0 and then the final volume of the solution was completed to 100 mL.
5. Elution solution: This buffer was prepared by dissolving 0.181 g of Tris and 0.092 g of GSH dissolved in distilled water. The pH was adjusted to 9.5 and the final volume was completed to 30 mL.

3.3.4. Preparation of SDS-PAGE Solutions

1. 1M Tris-HCl (pH 8.8): 12.11 g of Tris was dissolved in 80 mL of distilled water and the pH was adjusted to 8.8. The final volume of the solution was completed to 100 mL.
2. 1M Tris-HCl (pH 6.8): After dissolving 12.11g of Tris-HCl in 80 mL of distilled water and adjusting the pH to 6.8, distilled water was added to bring the volume of the solution to 100 mL.
3. 30% acrylamide and 0.8% bisacrylamide solutions: The solution was prepared by dissolving 15 g of acrylamide and 0.4 g of bisacrylamide in 50 mL of distilled water.
4. 10% sodium dodecyl sulphate (SDS) solution: 1 g of SDS was dissolved in 10 mL of distilled water.
5. 10% ammonium persulfate (APS) solution: 1 g of APS was dissolved in 10 mL of distilled water.

6. Running buffer: It was prepared by dissolving 1.51 g of Tris and 7.51 g of glycine in 450ml of distilled water, adding 5 mL of 10% SDS, adjusting the pH of the solution to 8.3, and then adding distilled water to bring the volume of the solution to 500ml.
7. Sample buffer: This buffer consists of 0.65 mL of 1 M Tris-HCl (PH: 6.8) with 1 mL of 10% SDS, 1 mL of 100% glycerin and 1 mL of 0.1% bromthymol blue to make a 10 mL solution volume. Before use, 50 µl of β-mercaptoethanol was added per 950 µl of sample solution.
8. Gel fixation solution: It was used to stabilise the proteins in the gel, was prepared by mixing 50% isopropanol, 10% TCA, and 40% distilled water.
9. Gel staining solution: 50 mL of methanol, 10 mL of acetic acid, and 40 mL of distilled water were combined with 0.1 g of Coomassie Brilliant Blue R-250mL.
10. Gel washing solution: This was prepared by mixing 10 mL of acetic acid, 40 mL of distilled water and 50 mL of methanol.
11. 0.1% bromthymol blue solution: 16 mL of 0.01 M NaOH was used to dissolve 0.1 g of bromthymol, and distilled water was added to bring the total volume to 100 mL.

3.3.5. Preparation of Bradford Reagent for Protein Quantification

Coomassie Brilliant Blue solution: This reagent was prepared by dissolving 100 mg of Coomassie Brilliant Blue G-250 in a mixture of 100 mL of 95% phosphoric acid and 50 mL of 95% ethanol. The final volume of the solution was adjusted to 1000 mL using distilled water and the solution was stored in the dark environment.

3.4. Extraction of Propolis

Propolis extraction was carried out sequentially using hexane, ethanol and water to obtain different fractions enriched with specific bioactive compounds. The multi step extraction approach aimed to first remove non polar compounds such as lipids and waxes using hexane, followed by the extraction of polyphenols and flavonoids with ethanol and finally the recovery of water soluble compounds.

3.4.1. Hexane Extraction of Propolis

To extract propolis using hexane, the raw propolis was first crushed and grained using a grinding device to maximize the extraction surface area. Next, 10 g of propolis from each region was accurately weighed, placed in appropriate containers and transferred to a vacuum apparatus to minimize the risk of solvent exposure. 100 mL of hexane was then added to each container which was securely sealed by parafilm and aluminum foil to prevent solvent evaporation. The extraction process was conducted in an orbital shaker for 24 hours at 40°C and 150 rpm. After incubation, the solutions were filtered under a vacuum system using paper filters and the filtrates were transferred to an oven at 37°C to facilitate the evaporation of the residual hexane. The solid residues remaining on the filter papers were carefully collected and stored for the subsequent procedure. This extraction approach was employed to effectively remove lipids and waxes from propolis thereby enhancing the purity of the extracted compounds.

3.4.2. Ethanolic Extracts of Propolis

Ethanolic extracts of propolis (EEP) were obtained with minor modifications to the methods described by Paviani et al. (2009). For this process, 10 g of crude propolis that was previously used for hexane extraction was combined with 100 mL of ethanol (Merck, Darmstadt, Germany) in a sealed container. The containers were tightly closed with parafilm and aluminum foil to minimize solvent evaporation. The extraction was carried out at 37°C under continuous stirring for 24 hours. Following extraction, the mixture was filtered using paper filters were to remove insoluble materials. The filtrate was then subjected to vacuum evaporation at 37 °C to remove the ethanol yielding a dry EEP. The remaining residues on the filters were carefully collected and stored for subsequent aqueous extraction phase.

3.4.3. Water (Aqueous) Extracts of Propolis

For the preparation of aqueous propolis extracts (WEP), the residues remaining from the EEP were collected and transferred into appropriate containers. 100 mL of distilled water was added to each container and the openings were securely sealed with aluminium foil

and parafilm to prevent evaporation. Subsequently, the samples were incubated in an orbital shaker at 150 rpm for 24 hours at 37°C to facilitate the extraction process. Following incubation, the solutions were filtered through paper filters to separate insoluble materials. The filtrates were transferred to fresh containers and subjected to controlled heating at 37°C to evaporate any remaining water. The final product, consisting of the water soluble components of propolis, was collected and stored for further analysis.

3.5. Chemical Content of Propolis

3.5.1. Fatty Acid Analysis by GC MS

The lipid extraction from propolis was performed using the method by Hara and Radin, (1978). For this purpose, 10 g of propolis was homogenized in 10 mL of hexane (with as ratio of 3:2) for 30 sec at 10 krpm. The homogenized mixture was then centrifuged at 5 krpm for 10 minutes and the supernatant was carefully collected, filtered and transferred into tubes for further processing. Since fatty acids require derivatisation for gas chromatography (GC) analysis, methyl esterification was performed following the procedure outlined by Christie (1990). For the derivatisation process, the previously prepared lipid extract was placed into 30 mL screw-capped tubes to prepare the methyl ester. 5 mL of 2 % methanolic sulfuric acid was added and vortexed thoroughly. This mixture was then incubated in an oven at 50 °C for 15 hours to allow complete methylation. After incubation the tubes were cooled to ambient temperature and 5 mL of 5% NaCl solution was added before vortexing again. The fatty acid methyl esters (FAME) formed in the reactions were extracted with 5 mL of hexane and the hexane phase was removed from the top with a pastor pipette and was treated with 5 mL of 2% KHCO3 and allowed to stand for 1-2 hours. The solvent containing the methyl esters was then evaporated under nitrogen stream at 45 °C. The residual fatty acids were dissolved in 1 mL of hexane, transferred into amber colored GC vials and prepared for GC-MS analysis.

The analysis was conducted using an Agilent 7890A / 5975C model GC-MS apparatus (Agilent Technologies,USA) and SGE Analytical BPX90 100 m x 0.25 mm x 0.25 mm column (Australia) was used. The temperature program was set as follows: the initial temperature of 120 °C was increased to 252 °C at a rate of 4 °C/min, followed by an

isothermal hold at 250 °C for 19 minutes resulting in a total run time of 45 minutes. The autosampler was rinsed with hexane 5 times before and after sample injection. Injection volume 1 µL in split mode (1:10) with a solvent delay time of 10 minutes. The carrier gas was hydrogen with a constant flow rate of 1 mL / min. Additional gas flow setings included a hydrogen flow rate of 35 mL/min, dry air flow rate of 350 mL/min and automatic nitrogen flow regulation by system.

3.5.2. Phenolics and Flavonoids Analysis by UHPLC-Orbitrap®-HRMS/MS

Ultra-high-performance liquid chromatography coupled with Orbitrap high-resolution mass spectrometry (UHPLC-Orbitrap®-HRMS/MS) analysis were conducted using an Exactive Plus Orbitrap mass spectrometer which was equipped with a Phenomenex® Gemini® column (100 mm × 2 mm i.d., NX-C18 110 Å 3 µm particle size). The mobile phase consisted of solvent A, prepared as 2% (v/v) glacial acetic acid in ultrapure water (GFL 2004/Human Power 1) and solvent B, and composed of 99.9% pure methanol (Sigma–Aldrich) for liquid chromatography-mass spectrometry (LC–MS) applications. The total experiment duration was set to 20 minutes with the gradient elution program structured as follows: 100% A from 0–2.00 minutes, transitioning to 98% B and 2% A from 2.01–13.00 minutes maintained from 13.01–15.9 minutes and reverting to 100% A from 15.90 to 19.00 min. The volume of the injection was 20.0 µL, the flow rate of the mobile phase was maintained at 0.3 mL/min, and the column temperature was regulated at 30 °C. The Orbitrap HRMS operated in both positive and negative ionization modes (Full MS/AIF) utilizing a heated electrospray ionization (HESI) interface. The ionization parameters were configured as follows: a sheath gasous flow rate of 35, an auxiliary gasous flow rate of 7, a spray voltage of 3.5 kV, a capillary heat of 350 °C, an auxiliary gas temperature of 350 °C, and an S-lens RF level of 50. Mass spectrometric data were acquired within a scan range of 60-800 m/z, with a resolution of 17,500; an ACG target of 3×10^6; a maximum injection time of 2 ms; and a collision energy (CE)/step CE of 25 V.

To construct calibration curves for the analysis of phenolic compounds content using the UHPLC-Orbitrap®-HRMS/MS analysis (in thesis it will also be mentioned as LC–MS), the standard solutions were prepared at concentrations of 10-20-40-60-80-100-200-300-400-

500 ng/mL each injected in triplicate. The propolis extract solutions were diluted to a concentration of 1 mg/mL prior to analysis. Prior to the injections, the solutions were filtered through a 0.22-micron PTFE syringe filter and a 10-mL syringe. 1.5 mL aliquot of the filtrate was then transferred into a vial and injected into the instrument. Data acquisition and processing were performed using Xcalibur software (version 2.1.0.1140) while system operation was managed via TraceFinder 3.2 software (Thermo Scientific).

3.5.3. Mineral Analysis by ICP-MS

Elemental Analysis of the raw propolis samples were conducted using an ICP-MS NexION® 2000 instrument (PerkinElmer® Inc., USA) equipped with a quartz nebulizer, cyclonic spray and an integrated auto-sampler. The method was developed utilizing a washing solution composed of 1% HCl prepared in ultrapure water. Approximately 0.2 gram of propolis accurately weighed and subjected to digestion in a microwave system. The digestion process involved the addition of 10 mL of HNO_3 to each sample followed by a microwave-assisted decomposition.

For the calibration, solutions were prepared by diluting commercially available multi-element standards in a 1% HNO3-ultra pure water matrix with specific concentrations detailed in Table 3.3. Additionally, prior to the analysis, ICP-MS calibration experiments were performed for ensuring accuracy and reliability. The internal standards, including 100 part per billion (ppb) 45Sc, 89Y and 209Bi were utilized to monitor and control elemental analysis precision.

Table 3.3. Calibration standards used in ICP-MS

Analytes	Std1 (ppb)	Std2 (ppb)	Std3 (ppb)	Std4 (ppb)	Std5 (ppb)	Internal standart
Ag, Al, As, Ba, Be, Bi,Cd, Co, Cr, Cs, Cu, Ga, In, Li, Mn, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn	0,5	1	5	25	50	
Ca, Fe, Mg, K, Na	12,5	25	125	625	1250	^{89}Y

Table 3.4. Working conditions for ICP-MS

Parameters	
Nebulizer	MEINHARD® plusGlassType C
Spray chamber	Glasscyclonic (baffled), 4 °C
One-Piece torch	w/2.5mm QuartzInjector
Injector	2.0 mm i.d.
Nebulizer flow	Optimizedfor< 2% oxides
RF power	1600 W
Cones	Ni
Replicates	3
Dwell time	50 ms
Aerosol dilution	Set to 2.5x
Sample delivery rate	350 µL/min
Rinse time	45 saniye
Nebulizer gas flow rate	0,93 L/min
Deflector voltage	−12 V,
Analog stage voltage	−1750 V
Pulse stage voltage	1100 V,
Discriminator threshold	26,
Sample tubing (Orange-Yellow)	Flared PVC PumpTubes 0.51mm/0.89mm
Internal standard tubing(Orange-red)	Flared PVC PumpTubes 0.19mm/0.91mm
Peristaltic pump rate	35 rpm
Alternating current (AC) rod offset	−4,

3.6. Total Polyphenol Content Determination (TPC)

For the preparation of 50% Folin-Ciocalteu Reagent (FCR), 50 ml of FCR was diluted with distilled water to a final volume of 100 mL. 2% Na_2CO_3 solution was prepared by dissolving 2 g of Na_2CO_3 in distilled water and adjusting the total volume to 100 mL. The Folin-Ciocalteu method was employed for the determination of total phenolic compounds in propolis extracts. To perform the assay, 100 µL of the propolis extract was aliquoted. Following the addition of 100 µL of FCR, the mixture was incubated for 5 minutes. Subsequently, 100 µL of Na_2CO_3 (20g/100mL) was added and the mixture was pipetted thoroughly. The total volume of 300 µL was then maintained at 25 °C for 90 minutes in the dark. The absorbance was measured at 760 nm in the spectrophotometer. Solutions other than the extract were used for control samples. 3 replicate tubes were prepared for each extract sample (Kaya, 2020). The total polyphenol content was expressed as ppm gallic acid equivalent.

3.7. Total Flavonoid Content Determination (TFC)

15% $NaNO_2$ solution was prepared by dissolving 15 g of $NaNO_2$ in distilled water bringing the total volume to 100 mL. 4 % NaOH solution was prepared by dissolving 4 g of NaOH in distilled water to achieve a total volume of 100 mL. 10 % $AICI_3$ solution was prepared by dissolving 10 g of $AICI_3$ in distilled adjusting the total volume to 100 mL. For the standard quercetin solution; 10 mg of quercetin was dissolved in 10 mL of methanol. The total flavonoid content was determined using the method outlined by Estevinho et al. (2008). 10 µl of the aliquot of extract solutions were transferred into separate tubes. To each sample, 40 µL of distilled water and 6 µL of $NaNO_2$ (150 g/L) solution were added and vortexed for 6 minutes. Then, 3 µL of $AICI_3$ (2.5 g / 25 mL) was added to this mixture, vortexed again and samples were incubated for 5 minutes. Subsequently, 40 µL of NaOH (4 g / 100 mL) solution and 100 µL of distilled water were added, vortexed, and the absorbance was measured at 415 nm. For the control samples, solutions other than the extract were placed in the tubes and 3 replicate tubes were prepared for each extract sample. The total flavonoid content was expressed as ppm quercetin equivalent.

3.8. Determination of Total Antioxidant Capacity

The total antioxidant capacity was evaluated using the method developed by Dinis et al. (1994), which expresses the results as ascorbic acid equivalent. For each sample, 25 µL of the extract solution was transferred vials and 250 µL of reagent solution (comprising 28 mM sodium phosphate, 0.6 M H_2SO_4 and 4 mM ammonium molybdate) solution was added and mixed. The mixture was incubated in a water bath at 95 °C for 90 minutes and their absorbance was measured at 695 nm. For the control samples, solutions other than the extract were placed in the wells and three replicate tubes were prepared for each extract sample. The total antioxidant capacity was expressed as α-tocopherol equivalent (α-tocopherol ppm extract).

3.9. Radical Scavenging Action (DPPH)

0.1 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 200 mL of methanol. To evaluate the scavenging activity of propolis extracts on DPPH radical, Hatano et al.

(1988) approach was used. This method is a based on the ability of the prepared extracts to donate protons or electrons, leading to reduction and the purple color of the DPPH solution. 50 µL of the extract solutions were taken for each sample in turn and 200 µL of DPPH solution was placed on it and vortexed. The mixture was then incubated in the dark for 60 minutes. After incubation, the absorbance was measured at 517 nm. The scavenging activity of DPPH was calculated using following equation, which represents the percent inhibition of the DPPH radical.

% of Inhibition DPPH• = (ADPPH•-ASAMPLE) / (ADPPH•) X 100

Here, ADPPH is the absorbance of the DPPH and ASAMPLE is the absorbance value measured in the presence of the extract.

3.10. Metal Chelating Activity

The metal chelation assay was conducted done by preparing the 5 mM ferrozine solution. Specifically, 0.0492 gram ferrozine was dissolved in 40 mL of methanol with vortexing. Similarly, 2 mM $FeCl_2$ solution was prepared by dissolving, 0.0160 gram of $FeCl_2.4H_2O$ was in 40 mL methanol. For the determination of metal chelating activity, the method described by Wenli et al. (2004) was employed. In this method, 50 µL of the extract solution was mixed with 160 µL of distilled water and 5 µL of $FeCl_2$ (2 mM) solution. After waiting for 30 seconds, 20 µL of ferrozine solution (5 mM) was added and the mixture was vortexed. After incubation at room temperature for 10 minutes, absorbance was measured at 562 nm. Metal chelating activity was expressed as EDTA equivalent (µg/mL).

3.11. Evaluation of Propolis's Antimicrobial Efficacy

Effects of 0.5, 1, 2 and 4 mg/mL propolis extracts were tested on Gram-positive (*Listeria monocytogenes* NCTC 5348, *Bacillus subtilis* IM622), Gram-nagative (*Salmonella typhimurium* NRRLE 4413, *Escherichia coli* ATCC 25922) and yeast (*Candida albicans* ATCC 25922) by disc diffusion assay. For that purpose, 8 grams of Nutrient Broth was dissolved in 1 liter of distilled water. Similarly, 17 grams of nutrient agar were dissolved

in 1 liter of distilled water and autoclaved. Subsequently, the materials were transferred to a sterile cabinet where they were sterilized with UV rays. 9 mL of nutrient broth were added into glass tubes and stock bacteria stored at 4 °C were transferred into the media using disposable loops. The medium was then incubated at at 37 °C for 24 hours. The following day, the bacterial concentration was adjusted to match the McFarland 0.5 standard. After adjusting McFarland to 0.5, 25 µL were bacteria and yeast were spread onto agar. 0.5, 1, 2 and 4 mg/mL of samples were aseptically impregnated into 6 mm discs placed on agar plates. The antimicrobial effects of propolis on this microorganisms were evaluated by comparing their effects to that of streptomycin which served as positive control. The antibiotic zones were measured by image J.

3.12. Purification and Enzyme Inhibition studies

Human blood was supplied fresh from Bingöl State Hospital Blood Center and stored at 4°C until used in experiments.

3.12.1. Preparation of Hemolysate

12 mL of fresh blood was placed in a test tube and centrifuged at 4°C for 25 minutes at 3000 rpm. The plasma was then removed and red blood cells were retained. The red blood cells solution was transferred to eppendorf tubes and cold water (1 part blood to 5 parts cold water) was added in order to lyse the cells and release their contents resulting hemolysis of the cells. The tubes were then centrifuged for 1 hour at 3500 rpm at 4°C. The supernatant located at the top of solution was collected and prepared for further analysis for purification step.

3.12.2. Purification of GST

The enzyme was purified from human blood erythrocyte hemolysate by GSH-agarose affinity column chromatography. Initially, the hemolysates was prepared from the red blood cells and the column was equilibrated with a buffer containing 10 mM phosphate (pH 7.4) and 150 mM NaCl. The hemolysate was subsequently loaded onto the column and washed with the same equilibration buffer to remove unbound proteins. GST was then

eluted with 50 mM Tris-HCI buffer (pH 9.0) and 10 mM GSH. The eluted fractions were collected in 1 mL volume. The purity of the collected fractions was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The presence of the GST bands was visualized by Coomassie Brilliant Blue (R- 250) stain. Bradford method was used to measure the protein concentration.

3.12.3. Quantitative Protein Determination

The protein concentrations in homogenate and purified protein were determined by Bradford assay with bovine serum albumin (BSA) employed as the standard protein. This colorimetric method is based on binding of Coomassie Brilliant Blue G-250 dye to proteins which results in shift in dye's absorbance maximum. Negatively charged Coomassie Brilliant Blue G-250 forms a complex by binding to the positively charged protein. The absorbance was measured at 595 nm, and protein concentrations were calculated using a standard curve generated from known concentrations of BSA. The procedure followed in quantitative protein determination was as follows: Initially, a standard BSA was prepared at a concentration of 1 mg/mL. Aliquots of 10, 20, 40, 60, 80 and 100 µL of BSA standard solutions were transferred into separate tubes. The final volume in each tubes was adjusted to 100 µL by distilled water. Subsequently, 4.9 mL of Bradford reagent was added into each tube and mixed thoroughly. These mixtures were incubated at room temperature for 10 minutes to allow color development. Absorbance measurements were performed at a wavelength of 595 nm, with a blank sample consisting of 100 µL of buffer and 4.9 mL of Bradford reagent. A standard calibration curve was constructed by plotting the measured absorbance values against the known BSA concentrations. For sample analysis, tubes were prepared using 100-fold diluted homogenate and pure enzyme. These were treated identically to standards mixed with Bradford reagent vortexed and incubated for 10 minutes. Absorbance values were recorded at a wavelength of 595 nm in a spectrophotometer. Each sample was measured in triplicate and the average absorbance value was used to determine the protein concentration based on a standard graph.

3.12.4. GST Enzyme Activity Assay

In vitro GST enzyme activity was measured spectrophotometrically at 25°C using GSH and CDNB as substrates. The reaction was monitored by measuring the increase in absorbance at 340 nm which corresponds to the formation of the GSH-CDNB conjugate. For spectrophotometric measurement, the assay medium contains 200 mM potassiumphosphate buffer (pH 8.0), 1 mM GSH and 0.5 mM CDNB. The enzymatic reaction was started with the addition of the enzyme. The absorbance was measured at 340 nm at 1 second intervals for 3 minutes. The rate of increase in absorbance was used to calculate GST activity using following formula:

$$EU = \frac{\Delta abs/3}{9.6} \times \frac{VT}{VE} \times DF$$

EU: Enzyme unit per 1 mL
Abs: change in absorbance
3: total time (min)
9.6: extiniction coefficient of 1mM DNB-SG at 340 nm
VT: total volume of the cuvette
VE: enzyme volume
DF: dilution factor

3.12.5. *In vitro* Enzyme Inhibitory Effects of Propolis extracts on Glutathione-s-Transferase

To assess the inhibitory effects of water and ethanol extracts of propolis collected from different part of Northern Iraq on GST activity, a concentration range of 0 to 200 µg/mL was tested. The propolis extracts were introduced into the reaction mixture to achieve different concentrations of propolis in a total reaction volume of 1 mL. To determine IC_{50} values, enzyme activity assays were conducted at constant GSH and CDNB concentration and varying concentrations of propolis extracts. Enzyme activity measured in the absence of propolis extracts were considered as 100%. The percentage of residual enzyme activity

was plotted against the propolis concentrations. IC_{50} values were calculated from dose-response curves using nonlinear regression analysis.

3.13. Photoprotective Effects of Propolis

The sun protection factor (SPF) of water and ethanol extracts of Northern Iraqi propolis was assessed according to Mansur et al. with minor modifications. Through the investigation, each extract was diluted with 96% ethanol (v/v) to a concentration of 0.5, 1, 5, 10 and 20 ppm (µg/mL). The absorption spectrum of the test samples was obtained in the range of 290–320 nm. A 1 cm quartz element was used for the study. Absorbance data were obtained from 290 to 320 nm in 5 nm increments. 96% ethanol (v/v) was used as a blank.

SPF values were calculated according to Mansur et al. equation [17]:

$$CF \times \Sigma EE\ (\lambda) \times I\ (\lambda) \times Abs\ (\lambda)$$

EE (λ)—erythemal effect spectrum; I (λ)—solar intensity spectrum; Abs (λ)—absorbance of extract; CF—correction factor (= 10).

3.14. Statistical Analysis

Results were expressed standard deviation of three measurements. These data were calculated using Graphpad Prism 6. The fatty acid composition of propolis samples were analyzed statistically using multivariate and univariate method. Principal Coordinates Analysis (PCO) based on the Bray-Curtis similarity matrix were employed for GC-MS data analysis.

4. RESULTS AND DISCUSSION

4.1. Chemical Content of Propolis

4.1.1. Phenolics and Flavonoids Analysis by LC/MS-MS

The phenolic profiles of EEP and WEP samples collected from four different geographical zones of Northern Iraq were determined by chromatographical analysis. The analysis revealed significant variations in the types and concentrations of phenolic compounds depending on both the extraction solvent and the region of collection (Table S1-S8).

In the ethanol extract of Erbil propolis, galangin (4639.7 ppm) and chrysin (3339.9 ppm) were identified as the predominant flavonoids followed by naringenin (1911 ppm) and diosmetin (1695.5 ppm). Other phenolics such as apigenin, isorhamnetin and sakuranetin were also present in considerable concentrations ranging from 729.8 to 1027.5 ppm. In contrast, the water extract of Erbil propolis was rich in caffeic acid (1309.5 ppm) and naringenin (936 ppm) along with notable quantities of propionic acid, coumaric acid and 3,4-dihydroxybenzoic acid (Table 4.1).

The ethanol extract from Mosul exhibited the highest concentrations of galangin (4981.6 ppm), chrysin (3767 ppm) and naringenin (2374.5 ppm) indicating a strong flavonoid content. Other major contituents included diosmetin (1909.6 ppm) and isorhamnetin (1129.8 ppm). The water extract, while still containing naringenin (1899.7 ppm) and caffeic acid (2280.5 ppm), showed relatively lower levels of other biomolecules such as sakuranetin, galangin and benzoic acid (Table 4.1).

Duhok propolis ethanol extract showed high levels of galangin (3740.4 ppm), chrysin (3654.8 ppm) and naringenin (2374.5 ppm). Caffeic acid (335.8 ppm), caffeic acid phenyl esters (666.3 ppm) and apigenin were also quantified. The water extract of Duhok propolis had relatively lower concentrations of compounds, with naringenin (657.9 ppm) and caffeic acid (602.9 ppm) being the major phenolics. Several phenolic acid such as ellagic acid,

chlorogenic acid and protocatechuic acid were detected in minor concentrations (<50 ppm) (Table 4.1).

Ethanol extract from Sulaymaniyah propolis contained substantial amounts galangin (4560. 1 ppm), chrysin (4381.5 ppm), diosmetin (2414.3 ppm) and naringenin (2177.3 ppm) consistent with the other ethanolic samples. In the water extract, however, most of the phenolics were either present in very low amounts or not detected. Ellagic acid (41.3 ppm), caffeic acid (41.2 ppm) and benzoic acid (7.5 ppm) were the most prominent phenolics (Table 4.1).

Table 4.1. The phenolic profiles of ethanol and water extracts of propolis samples collected from four different geographical zones of Northern Iraq

Erbil (EEP)			**Erbil (WEP)**		
Compound	RT	Concentration (ppm)	Compound	RT	Concentration (ppm)
Galangin	12.29	4639.7	Caffeic acid	7.26	1309.5
Chrysin	12.15	3339.9	Naringenin	10.50	936
Naringenin	10.49	1911	Propionic acid	7.64	266.539
Diosmetin	11.39	1695.5	Coumaric acid	8.31	231
Caffeic acid phenyl ester	11.97	1605	3,4-Dihydroxybenzoic acid	4.33	148.837
Apigenin	11.25	1027.5	Quinic acid	1.21	130.064
Isorhamnetin	11.16	797.5	4-Hydroxybenzoic acid	6.26	126.637
Sakuranetin	11.68	729.8	Galangin	12.30	73.538
Caffeic acid	7.22	444.3	Ferulic acid	8.55	44.191
Genkwanin	12.37	331.252	Diosmetin	11.40	36.636
Acacetin	12.37	329.1			
Quercetin	10.44	324.79			
Coumaric acid	8.26	276.803			
Mosul (EEP)			Mosul (WEP)		
Compound	RT	Concentration (ppm)	Compound	RT	Concentration (ppm)
Galangin	12.29	4981.612	Naringenin	10.51	1899.67
Chrysin	12.15	3767.023	Caffeic acid	7.19	2280.506
Naringenin	10.49	2633.49	Coumaric acid	8.26	385.873
Diosmetin	11.39	1909.592	Propionic acid	7.57	281.343
Isorhamnetin	11.20	1129.778	4-Hydroxybenzoic acid	6.15	181.51
Caffeic acid phenyl ester	11.97	1085.365	Ferulic acid	8.54	142.888
Apigenin	11.25	936.278	Quinic acid	1.21	139.072
Caffeic acid	7.21	705.473	Sakuranetin	11.84	137.526
Coumaric acid	8.26	389.408	Benzoic acid	8.95	95.828
Sakuranetin	11.85	389.347	Galangin	12.30	88.791
Quercetin	10.44	361.968			
Genkwanin	12.39	296.709			
Acacetin	12.39	294.798			

Table 4.1.(Continue): The phenolic profiles of ethanol and water extracts of propolis samples collected from four different geographical zones of Northern Iraq

Duhok (EEP)			**Duhok (WEP)**		
Compound	RT	Concentration (ppm)	Compound	RT	Concentration (ppm)
Galangin	12.29	3740.424	Naringenin	10.50	657.929
Chrysin	12.15	3654.83	Caffeic acid	7.19	602.862
Naringenin	10.49	2374.517	Propionic acid	7.57	127.499
Diosmetin	11.37	1624.519	Quinic acid	1.21	125.373
Apigenin	11.25	909.448	4-Hydroxybenzoic acid	6.15	68.84
Caffeic acid phenyl ester	11.98	666.342	Homovanillic acid	7.36	49.372
Caffeic acid	7.21	355.846	Ellagic acid	9.43	41.869
Isorhamnetin	11.18	346.091	Chlorogenic acid	7.07	36.397
Genkwanin	12.39	279.176	Ferulic acid	8.53	26.966
Acacetin	12.39	277.353	Protocatechuic acid	4.29	26.92
Sakuranetin	11.87	226.937			
Propionic acid	7.54	185.805			
Gallic acid	0.79	175.462			
Sulaymaniyah (EEP)			Sulaymaniyah (WEP)		
Compound	RT	Concentration (ppm)	Compound	RT	Concentration (ppm)
Galangin	12.28	4560.148	Ellagic acid	9.43	41.337
Chrysin	12.16	4381.523	Caffeic acid	7.24	41.176
Diosmetin	11.39	2414.312	Benzoic acid	8.86	7.476
Naringenin	10.49	2177.271	3-hydroxybenzoic acid	6.95	2.556
Isorhamnetin	11.22	1032.504	Galangin	12.30	2.428
Apigenin	11.27	934.707			
Caffeic acid	7.19	548.693			
Genkwanin	12.39	377.056			
Acacetin	12.39	374.746			
Sakuranetin	11.87	363.751			
Propionic acid	7.57	283.415			
Quercetin	10.44	218.317			
Caffeic acid phenyl ester	11.97	204.979			

The phenolic profile of propolis samples analyzed in this study revealed a clear influence of both extraction solvent and geographical original on quantitative and qualitative distribution of bioactive compounds. Ethanol extracts consistently yielded higher total phenolic content and a broader spectrum of flavonoids compared to water extracts supporting findings from previous studies (Bozkuş et al., 2021; Sarikahya et al., 2021). For instance, Turkish propolis is notably rich in chrysin, naringenin and galangin which matches data of this thesis suggesting similar botanical origins or environmental influences (Sarikahya et al., 2021). Among the zones studied, Mosul EEP stood out with the highest concentrations of major flavonoids including galangin (4981.6 ppm), chrysin (3767 ppm) and naringenin (2633.5 ppm) indicating a rich flavonoid composition that aligns with O-type Populus-derived Turkish propolis reported by Degirmencioglu et al. (2019). This flavonoid dominance in ethanolic extracts parallels previous findings where compounds such as chrysin, CAPE, pinocembrin and galangin were characteristic of Turkish and Brazilian propolis (Bozkuş et al., 2021; Ribeiro et al., 2023). Interestingly, Duhok EEP was notable for its relatively high concentration of CAPE (666.3 ppm), a compound widely

recognized for its potent bioactivity and abundance in Mediterranean and Brazilian green propolis (Bozkuş et al., 2021; Righi et al., 2013). CAPE was either absent or present in negligible amounts in water extracts highlighting again the solvent dependency of polyphenolic extraction. The inter-zonal variation observed in the phenolic content of both ethanol and water extracts also emphasizes the influences of botanical source and geographical conditions on the chemical composition. Compared to Brazilian propolis, particularly green and brown types that are distinguished by high caffeoylquinic acids, p-coumaric acid derivatives and flavonols, the Northern Iraqi propolis demonstrates a stronger tilt towards flavonoid agylcone (galangin, chrysin) than towards hydroxycinnamic acid derivatives. This might point to different plant resins being the primary source suggesting a different ecological niche and resin source profile (Ribeiro et al., 2023; Righi et al., 2013). In relation to Jordanian Propolis, which is also Middle Eastern, the similarity in presence of chrysin, pinocembrin and caffeic acid (though present in different ratios) indicates that Northern Iraqi samples are likely collected from flora with richer flavonoid content given the notably higher concentrations observed in EEP collected from Northern Iraq (Naik et al., 2021). In comparison with New Zealand (Markham et al., 1996), Chinese (Xu et al., 2019) and American propolis, which prominently feature pinocembrin, pinobanksin and their acetates, the Northern Iraqi samples seem relatively lower in these compounds but richer in chrysin, galangin and naringenin underscoring regional chemical diversity and possibly differing bioactivities. The chemical composition of Northern Iraqi propolis aligns with and expand upon previous findings notably those reported by Sulaiman et al. (2011). Their findings emphasized the predominance of phenolic acids and their esters followed by flavones, flavonols and flavanones. Notably, compounds such as CAPE, pinobanksin and myricetin were detected underscoring the complex polyphenolic nature of Iraqi propolis.

4.1.2. Fatty Acid Profile

Several fatty acids, hydrocarbons, esters, alcohols, phenolic compounds and other bioactive molecules were found in the hexane extracts from Erbil, Mosul, Duhok, and Sulaymaniyah, according to the GC-MS analysis (Table 4.2). Numerous saturated and unsaturated fatty acids, such as myristic acid, lauric acid, palmitic acid, oleic acid (cis and trans isomers), and linoleic acid (cis and trans isomers), were found to be common among

the four regions based on fatty acid profiling. The consistent presence of palmitic acid in all four regions suggests that it is abundant and stable in a variety of environmental settings. All samples had common levels of oleic acid (cis-9) and linoleic acid (cis,cis-9,12), underscoring their importance as necessary unsaturated fatty acids. Higher fatty acid diversity was seen in Mosul and Sulaymaniyah, including less prevalent fatty acids like arachidic acid, eicosanoic acid methyl ester, and docosanoic acid methyl ester. The Erbil and Mosul samples did not contain the 20:1 (cis-11) omega-9 fatty acid and cis-13-eicosenoic acid found in Duhok.

Tetracosane, pentacosane, heptacosane, and octacosane were among the long-chain alkanes found in all samples. These substances might support long-term stability and hydrophobic qualities. Particularly common was tetracosane, which was present in all four regional extracts but was more intense in Sulaymaniyah. Mosul and Duhok had higher levels of icosane, nonadecane, and heneicosane, which may indicate that the environment has an impact on alkane biosynthesis. Fatty alcohols, including 1-heptacosanol, 1-hexacosanol, and n-tetracosanol-1, were confirmed to be present. These alcohols might have antibacterial properties or act as precursors to wax. Significant levels of 1-heptacosanol and n-tetracosanol-1 were found in Erbil and Duhok. While they were present in several samples, esters such as octacosyl acetate and methyl esters of benzenepropanoic acid were more prevalent in Erbil. All extracts contained phenolic derivatives, including 2,4-di-tert-butylphenol and benzenepropanoic acid. These substances, which are well-known for their antioxidant qualities, were particularly prevalent in Sulaymaniyah and Mosul propolis.

Some substances were exclusive to particular areas. Different derivatives such as docosyl trifluoroacetate and nonadecyl trifluoroacetate were seen in Duhok, indicating a distinct metabolic profile. The rare organoselenium compound N,N-diethyl-1-benzoselenophen-2-amine was found in Mosul, which made it noteworthy. 3,4-dihydroxyphenylglycol, a significant antioxidant metabolite, was only found in Erbil.

Table 4.2. Fatty acid content of Northern Iraqi propolis

Erbil	Mosul	Duhok	Sulaymaniyah
3,4-Dihydroxyphenylglycol	Palmitoleic acid		Pentane, 3-methyl-
Myristic acid Lauric acid Palmitic acid Capric acid	Myristic acid Lauric acid Palmitic acid Capric acid Arachidic acid Heneicosylic acid	Myristic acid Pentadecanoic acid Palmitic acid Stearic acid	Myristic acid Lauric acid Palmitic acid Arachidic acid
(-)-(3R,4S,5R)-5-Hydroxy-3,4-(isoropylidenedioxy)-1-[3-(2-trimethylsilyl-1,3-dithian-2-yl)propyl]pyrrolidin-2-one	Phenol, 2,4-bis(1,1-dimethylethyl)	Hentriacontane	2-Naphthalenemethanol
N,N-Diethyl-1-benzoselenophen-2-amine	Icosane Tetracosane Nonadecane, 9-methyl-	Heneicosane Nonadecane	2,4-Di-tert-butylphenol
Cadinene	Tetracosane	n-Tetracosanol-1	Tricosane
Docosane Eicosane Icosane	Benzenepropanoic acid, 3,5-bis(1,1 dimethylethyl)-4-hydroxy-, methyl ester	Eicosane	Eicosane
Phenol	Eicosanoic acid, methyl ester	Heptadecane	Heptadecanoic acid, methyl ester
3-(4-N,N-Dimethylaminophenyl) propenoic acid	Docosanoic acid, methyl ester	Phenol, 2,4-bis(1,1-dimethylethyl)	
Oleic acid (trans-9 omega)	Oleic acid (trans-9 omega	Oleic acid (trans-9 omega	Oleic acid (trans-9 omega
Oleic acid (cis-9 omega)	Oleic acid (cis-9 omega)	Oleic acid (cis-9 omega)	Oleic acid (cis-9 omega)
Linoleic acid (cis,cis - 9,12) (omega 6)	Linoleic acid (cis,cis - 9,12) (omega 6)	1-Heptacosanol	Linoleic acid (cis,cis - 9,12) (omega 6)
Linoleic acid (trans,trans - 9,12) (omega 6)	Linoleic acid (trans,trans - 9,12) (omega 6)	1-hexacosanol	Linoleic acid (trans,trans - 9,12) (omega 6)
Alpha-linolenic (all cis - 9,12,15) (omega 3)	Alpha-linolenic (all cis - 9,12,15) (omega 3)	n-Tetracosanol-1	Benzenepropanoic acid, 3,5-bis(1,1 dimethylethyl)-4-hydroxy-, methyl ester
		Cyclotetracosane	1-Tetracosene
Pentacosane	Pentacosane	Pentacosane	cis-13-Eicosenoic acid
Heneicosane	20:1 (cis - 11) (omega 9)	2,4-Di-tert-butylphenol	2-Heptadecenal
		(18S,19S)-18,19-Dihydroxy-1,4,7,10,13,16 hexaoxocycloencosane	Cyclopentadecane 1-Heptacosanol
Benzenepropanoic acid, 3,5-bis(1,1 -dimethylethyl)-4-hydroxy-, methyl ester	1-Nonadecene	Phenol, 2,5-bis(1,1-dimethylethyl)	17-Pentatriacontene
Nonadecane, 9-methyl-	1-Docosene	Nonadecane, 9-methyl-	n-Tetracosane
Tetracosane	Tetracosane	Tetracosane Heptacosane	Tetracosane
Octacosane	1-Heptacosanol	Decanoic acid, methyl ester	Octacosyl acetate
Hexadecane, 1-iodo-	Cyclotetracosane	Nonadecyl trifluoroacetate	Myristic acid, methyl ester
	1-Tetracosene	Heneicosyl pentafluoropropionate	
		Tricosyl trifluoroacetate	
		Eicosyl pentafluoropropionate	
		Docosyl trifluoroacetate	

The fatty acid composition of propolis samples were also analyzed statistically using multivariate and univariate method (Table 4.3.). The results demonstrated significant regional variation in both the presence and concentration of individual fatty acids. Among the detected fatty acids, palmitic acid (C16:0) was dominant in all samples with the highest level observed in Duhok propolis (47.71%) significantly exceeding levels in Erbil (36.86%), Mosul (36.76%) and Sulaymaniyah (15.58%) samples. Duhok showed a distinctive fatty acid profile with elevated levels of palmitic acid and other saturated fatty acids. Oleic acid (C18:1) was also abundant particularly in Mosul (22.26%) and Erbil (20.72%) while its concentration was significantly lower in Duhok (11.11%) and Sulaymaniyah (5.37%) propolis. This reveals a contrast between the northern (Erbil and Mosul) and southern (Duhok and Sulaymaniyah) regions regarding monounsaturated fatty acid content. Stearic acid (C18:0) was highest in Erbil (4.98%) and Duhok (4.91%) showing no significant difference between them but significantly higher than Mosul (3.84%) and Sulaymaniyah (1.73%) propolis. Erbil had higher levels of linoleic acid (C18:2) and linolenic acid (C18:3) (0.90% and 1.52%), whereas other areas had much lower levels or no linoleic acid at all.

Fatty acids that were either unique or regionally distinct were noted. Mosul was the only place where capric acid (C10:0) was found (3.42%), and only in Sulaymaniyah (0.18 ± 0.07%) and Mosul (0.23%) was C12:0 (lauric acid) detected. Sulaymaniyah was the only location where hexadecanoic acid (C17:0) was found (0.066%). Erbil had the highest levels of C20:0 and C20:1, which were significantly higher than those in the other three regions (0.88% and 0.65%, respectively).

Table 4.3. Fatty acid composition of propolis from four regions (% of total FA)

FA	Erbil	Mosul	Duhok	Sulaymaniyah
C10:0	-	3.42 ± 0.58^{b}	-	-
C12:0	-	0.23 ± 0.02^{b}	-	0.18 ± 0.07^{b}
C14:0	0.41 ± 0.12^{a}	0.30 ± 0.02^{a}	0.32 ± 0.06^{a}	0.28 ± 0.06^{a}
C16:0	36.86 ± 4.81^{a}	36.76 ± 1.87^{a}	47.71 ± 0.42^{c}	15.58 ± 0.73^{b}
C16:1	-	0.29 ± 0.02^{b}	-	-
C17:0	-	-	-	0.066 ± 0.03^{b}
C18:0	4.98 ± 0.04^{a}	3.84 ± 0.2^{c}	4.91 ± 0.22^{a}	1.73 ± 0.15^{b}
C18:1	20.72 ± 1.21^{a}	22.26 ± 1.74^{a}	11.11 ± 1.42^{c}	5.37 ± 0.54^{b}
C18:2	0.90 ± 0.29^{c}	0.67 ± 0.01^{bc}	-	0.30 ± 0.02^{ab}
C18:3	1.52 ± 0.14^{c}	0.54 ± 0.03^{b}	-	-
C20:0	0.88 ± 0.01^{d}	0.28 ± 0.02^{b}		0.39 ± 0.03^{c}
C20:1	0.65 ± 0.09^{d}	0.46 ± 0.05^{c}		0.21 ± 0.02^{b}

All regions clustered within 80% similarity, according to Principal Coordinates Analysis (PCO) based on the Bray-Curtis similarity matrix (Figure 4.1), with Duhok forming a slightly different group because of its higher palmitic acid content. One characteristic that set Sulaymaniyah apart from other samples was C17:0. The SIMPER analysis indicates that the samples from Duhok showed the greatest similarity, followed by those from Sulaymaniyah and Mosul. Mosul and Sulaymaniyah had the biggest regional differences, mostly because of variations in their C16:0 (47%) and C18:1 (37%) contents. Erbil and Mosul showed the least amount of dissimilarity, with C16:0 (28%), C10:0 (26%), C18:1 (15%), and C18:0 (9%), having the biggest effects. According to these results, minor fatty acids might be used as local indicators of honey origin.

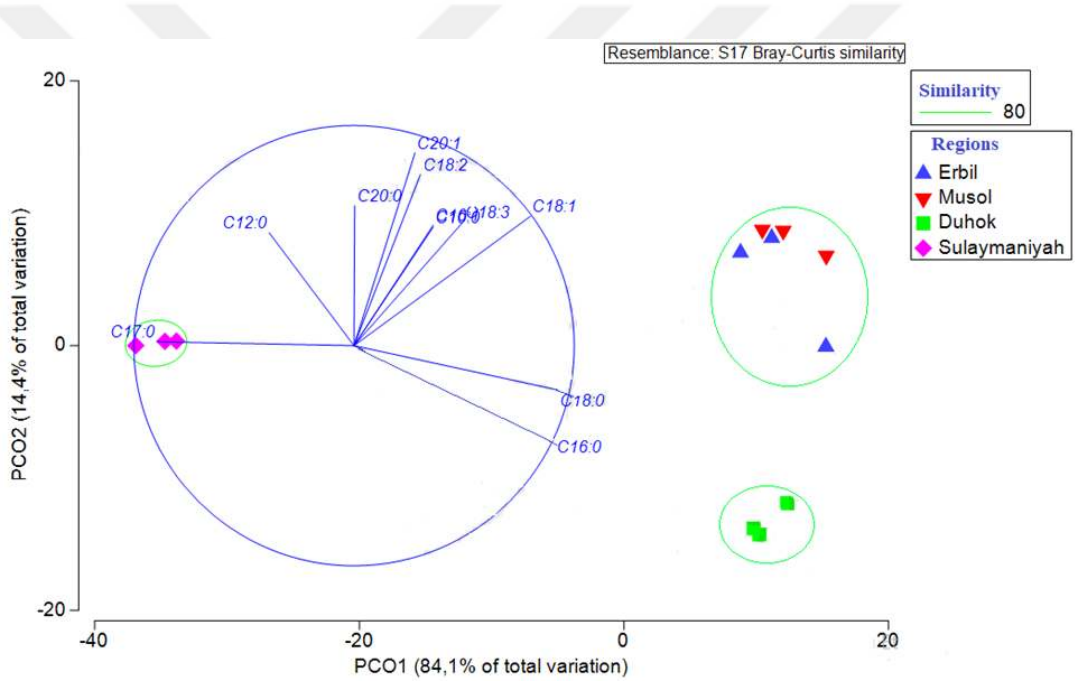

Figure 4.1. Two-dimensional configuration plot of a PCO analysis of a resemblance matrix of fatty acids of the honey at four regions. The lower tringular matrix was created using by Bray-Curtis similarity coefficients. Pearson correlation > 0.55

4.1.3. Mineral Profile

The elemental analysis of propolis samples from four different Iraqi cities was conducted using ICP-MS. The analysis focused on both essential and trace metals and results are expressed in ppb. A total of 29 elements were screened among which 18 were detected in at least one of the samples. Among the major elements, Ca was found in high

concentrations across all samples with the highest level in Sulaymaniyah propolis (2458611 ppb) followed by Mosul propolis (2245407 ppb), Erbil (1482999 ppb) and the lowest in Duhok (315756 ppb). K also showed notable variation with Erbil propolis having the highest concentration (1518143 ppb), while Duhok had a significantly lower levels.

For essential trace elements, Mg concentration ranged from 46071 ppb in Duhok propolis to 701051 ppb in Mosul propolis. Fe levels were particularly high in Duhok propolis (525248 ppb) and lowest in Sulaymaniyah propolis (284614 ppb). Mn concentrations followed a similar pattern highest in Mosul propolis (26957 ppb) and lowest in Sulaymaniyah propolis (15200 ppb). Cu and Zn were consistently present in all samples except for Zn which was undetectable in Duhok propolis. The highest Cu concentration was observed in Sulaymaniyah propolis (3513 ppb) while Zn was most abundant in Erbil propolis (8450 ppb).

Regarding the presence of toxic elements, Pb was detected in all samples with trace quantities with Duhok propolis having the highest levels (5387 ppb). Other potentially toxic elements such as Cd, As, Bi and Tl were not detected in any of the samples. The elements such as Ag, Be, Se and U also were not detected in any of the samples. The number of detected elements (excluding non-detects) varied slightly among regions: Duhok propolis exhibited 13 elements while Erbil, Mosul and Sulaymaniyah propolis had 11 detected elements.

Table 4.4. Mineral analysis of propolis from four regions

Elements (ppb)	*Erbil*	*Mosul*	*Duhok*	*Sulaymaniyah*
Ag 107	NF	NF	NF	NF
Al 27	767391.01	856407.50	452621.51	553178.26
As 75	NF	NF	NF	NF
Ba 138	7544.19	9528.15	17906.89	3785.61
Be 9	NF	NF	NF	NF
Bi 209	NF	NF	NF	NF
Ca 43	1482999.11	2245407.44	315756.25	2458611.52
Cd 111	NF	NF	NF	NF
Co 59	494.25	633.39	315.10	334.38
Cr 52	2039.32	3297.45	1888.62	2809.90
Cs 133	NF	NF	NF	NF
Cu 63	2422.93	2834.81	2306.62	3513.04
Fe 57	413629.79	440306.05	525248.81	284614.70
Ga 69	1696.42	2198.25	3893.30	910.63
In 115	NF	NF	NF	NF
K 39	1518143.00	1073545.89	334767.88	794962.55
Li 7	351.93	598.95	NF	369.07
Mg 24	454895.91	701051.27	46071.84	509915.94
Mn 55	19226.58	26957.23	16526.01	15200.79
Na 23	NF	NF	NF	NF
Ni 60	2271.10	3588.56	1021.88	2933.62
Pb 208	3490.97	3634.49	5387.73	862.18
Rb 85	1372.06	1237.03	963.11	606.77
Se 82	NF	NF	NF	NF
Sr 88	24358.67	13312.51	4796.88	4840.26
Tl 205	NF	NF	NF	NF
U 238	NF	NF	NF	NF
V 51	1386.03	1642.38	804.58	910.97
Zn 66	8450.49	7201.13	NF	2738.65

The comparative elemental analysis underscore pronounced regional variability in the mineral composition of propolis. While Mosul and Duhok propolis exhibited higher concentrations of several trace and macroelements, Erbil and Sulaymaniyah propolis stood out for specific metal enrichments. These differences likely reflect the complex interplay of local flora, soil composition, climate and environmental exposure.

The elemental analysis of propolis samples collected from different regions of Northern Iraq revealed the presence of some trace element heavy metals notably Co, Cr, Ni, Pb albeit at low concentrations. These results are consistent with prior studies indicating that propolis can serve as a natural bioindicator of environmental contamination due to bee's foraging behavior and exposure to local air, soil and flora (Izol and Turhan, 2024; Besharati et al., 2024; Glevitzky et al., 2025). Despite the detection of these metals, their

concentrations were found to be significantly lower than those of essential macroelements suggesting that the areas from which the propolis was sourced are relatively uncontaminated. The relatively low heavy metal content found across all propolis samples indicates a low level of anthropogenic pollution in the ecosystems surrounding the hives. This aligns with understanding that bees typically forage within a limited radius (less than 1 km) thus reflecting localized environmental conditions (Ohlinger et al., 2024). Notably, while the propolis samples contained detectable levels of toxic elements, the values fell within internationally accepted safety limits for dietary intake suggesting that the propolis remains safe for therapeutic or nutraceutical use (Abdullah et al., 2020). In this study, Al concentrations (e.g., up to ~856,000 ppb) were several orders of magnitude higher than other trace metals. The high Al concentration in Iraqi propolis is likely multifactorial with soil composition, regional dust activity, botanical resin sources and possibly environmental exposure all contributing. As raw propolis is analyzed without prior washing or purification steps, external surface contamination especially from dust or soil particles may significantly elevate Al concentrations. This is a critical consideration when comparing to studies that only analyze only purified or processed propolis.

4.2. Total Phenolic and Flavonoid Content and Total Antioxidant Capacity Analysis

The total phenolic content (expressed in ppm gallic acid equivalent, GAE) (Figure 4.2) and total flavonoid content (expressed as ppm quercetin equivalent, QE) (Figure 4.3) were significantly higher in ethanol extracts compared to water extracts across all regions (Table 4.5). Among ethanol extracts, Duhok propolis exhibited the highest phenolic content (965 ppm GAE) followed by Sulaymaniyah (713 ppm GAE), Mosul (657 ppm GAE) and Erbil (595 ppm GAE) propolis. Similarly, flavonoid content peaked in Sulaymaniyah propolis (1967 ppm QE) with Mosul propolis also showing high levels (1717 ppm QE) indicating a rich polyphenolic profile in these regions. Water extracts showed markedly lower phenolic and flavonoid concentrations. The highest TPC was observed in Mosul propolis (267 ppm GAE) while Sulaymaniyah propolis had the lowest values. The same trend was observed for TFC highlighting the limited solubility of phenolic compounds in water. These results are in agreement with previous studies (Bozkuş et al., 2021; Degirmencioglu et al., 2019) that demonstrated ethanol as a more efficient solvent for extracting flavonoids and phenolics from propolis.

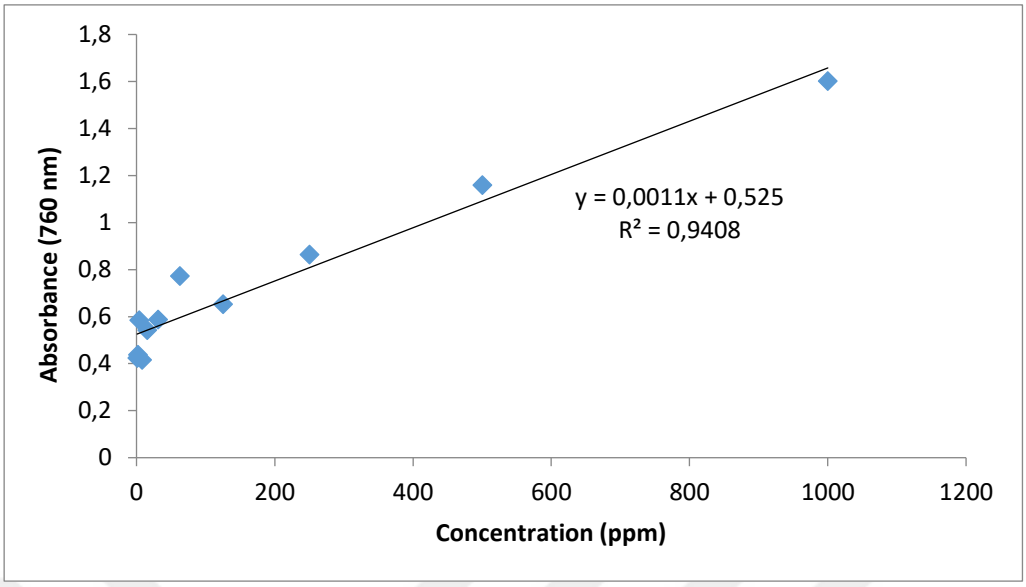

Figure 4.2. Standard calibration curve of gallic acid. The absorbance of gallic acid was measured at 760 nm. The resulting linear regression equation was used to calculate the total phenolic content of samples expressed as ppm GAE

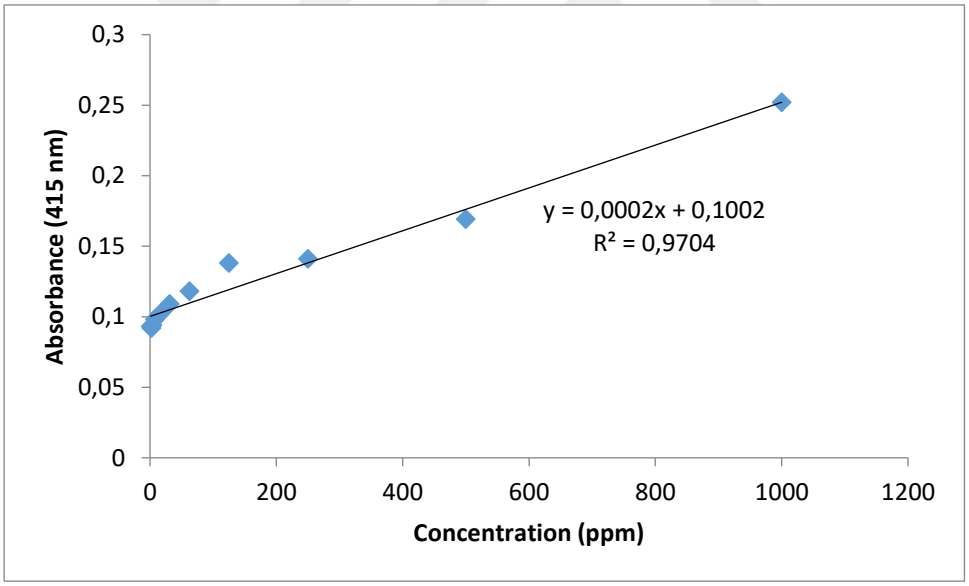

Figure 4.3. Standard calibration curve of quercetin. The absorbance of quercetin was measured at 760 nm. The resulting linear regression equation was used to calculate the total flavonoid content of samples expressed as ppm QE

The total antioxidant capacity expressed as ppm α-tocopherol equivalent (αTE) further supported the superiority of ethanol extracts (Figure 4.4). Duhok EEP exhibited the highest TAC (542 ppm αTE) followed by Sulaymaniyah (377 ppm αTE), Erbil (300 ppm αTE) and Mosul (288 ppm αTE) EEPs. The correlation between high phenolic/flavonoid content and

TAC suggests these compounds play a major role in antioxidant activity consistent with the findings of Sarikahya et al. (2021) and Ribeiro et al. (2023). In contrast, the TAC of WEP was significantly lower with Erbil (47 ppm αTE) and Mosul (54 ppm αTE) propolis showing moderate values and Duhok propolis lowest (13 ppm αTE).

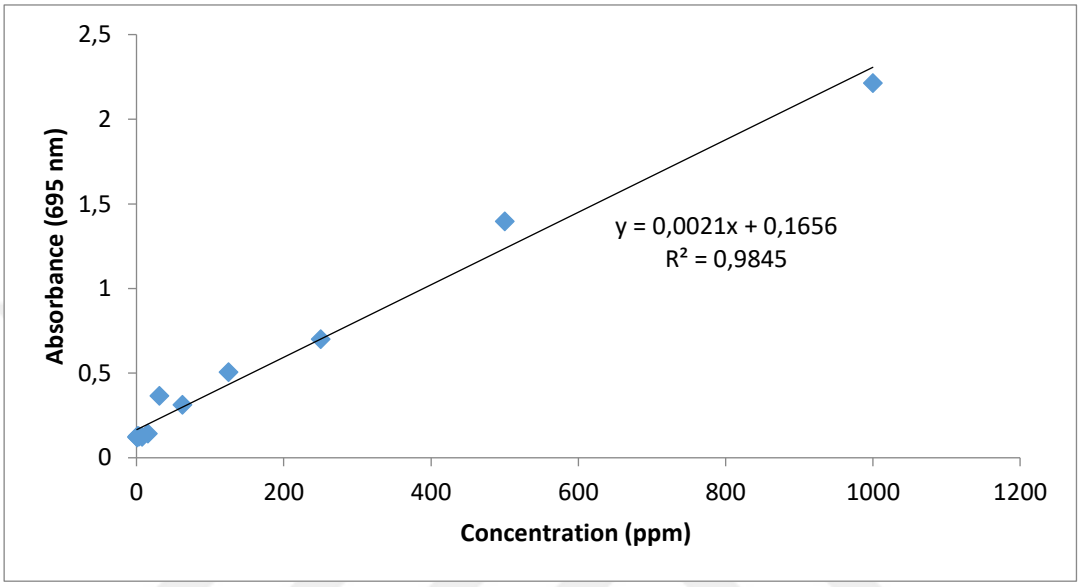

Figure 4.4. Standard calibration curve of α-tocopherol. The absorbance of α-tocopherol was measured at 760 nm. The resulting linear regression equation was used to calculate the total antioxidant capacity of samples expressed as ppm αTE

Table 4.5. Total phenolic, total flavonoid, and total antioxidant content of Northern Iraqi propolis

Ethanol Extract			
Region	Total Phenolic Content (ppm/GAE)	Total Flavonoid Content (ppm/QE)	Total Antioxidant (ppm/αTE)
Erbil	595.75±22	662.33±21	300.03±8
Mosul	657.57±12	1717.33±33	288.76±9
Duhok	965.75±18	1172.33±25	542.41±11
Sulaymaniyah	713.63±31	1967.3±29	377.8±10

Water Extract			
Region	Total Phenolic Content (ppm/GAE)	Total Flavonoid Content (ppm/QE)	Total Antioxidant (ppm/αTE)
Erbil	189.09±5	342.36±11	47.96±3
Mosul	267.87±10	465.66±12	54.47±4
Duhok	180.78±14	132.33±7	13.04±1
Sulaymaniyah	85.75±4	260.66±10	26.69±2

TPC, TFC and TAC values obtained from EEP and WEP strongly correlate with their underlying chemical composition as determined by LC/MS-MS and ICP-MS analyses. EEPs particularly from Duhok and Sulaymaniyah exhibited the highest TPC, TFC and TAC values in line with their rich profiles of bioactive flavonoids such as galangin, chrysin, naringenin and CAPE. In contrast, WEP which primarily contained pehenolic acids like caffeic and chlorogenic acids showed lower values. The results indicated that TFC is higher than TPC. This is an unexpected result since flavonoids are also a class phenolic compounds. One scientific explanation could be high Al content in propolis samples. Al can artificially increase the TFC due to the chemistry of the $AlCl_3$ method which is commonly used for TFC analysis. The TPC, TFC and TAC values obtained in this study for Iraqi propolis samples demonstrated significant variability depending on geographic origin and extraction solvent consistent with previous reports emphasizing the influence of environmental and botanical factors.

4.3. DPPH Radical Scavenging and Metal Chelating Activity

The DPPH scavenging activity of EEP across all regions ranging from 83.63% to 88.3% with Duhok propolis showing the highest activity (Table 4.6). This results supports the previously observed trend in TAC and indicates the presence of potent electron donating antioxidant constituents in Duhok propolis. In the WEP, Mosul and Erpil WEP showed slightly higher DPPH scavenging than Sulaymaniyah while Duhok displayed the lowest activity. While WEP generally had lower activity than EEP, the difference was relatively modest. Although TPC, TFC and TAC of the WEP were relatively low, their DPPH activity was comparable to that of EEP. This may be attributed to the presence of water soluble non phenolic compounds such as ascorbic acid, amino acids or organic acids which are capable of effectively scavenging free radicals. Additionally, even in low concentrations, certain potent hydrophilic phenolics with strong antioxidant properties may significantly contribute to the DPPH activity. Furthermore, synergistic interactions among various hydrophilic constituents in WEP may enhance overall antioxidant activity, despite their individual quantities being insufficient to raise TPC or TFC values.

Table 4.6. DPPH Removal activity and metal chelating activity of Northern Iraqi propolis

Ethanol Extract		
Region	% DPPH Removal Activity	% Metal Chelating
Erbil	84.63±2	22.51±1
Mosul	83.63±3	22.75±1
Duhok	88.3±3	35.15±1
Sulaymaniyah	84.22±4	21.58±1

Water Extract		
Region	% DPPH Removal Activity	% Metal Chelating
Erbil	80.89±4	32.71±2
Mosul	81.59±3	33.69±1
Duhok	77.02±3	18.4±1
Sulaymaniyah	80.06±2	51.92±2

Interestingly, WEP demonstrated higher metal chelating activity in some cases. Sulaymaniyah WEP showed the highest chelating activity (51.92%) followed by Mosul (33.69%) and Erbil (32.71%) propolis. In contrast, Duhok WEP showed the lowest chelating ability (18.4%). In EEP, the highest metal chelation was observed in Duhok propolis (35.15%) significantly outperforming the other regions. EDTA was used as positive control. The metal chelating activity of EDTA was 32.26±2.35% which was lower than WEP of Sulaymaniyah region. These results suggest that both polar and semi polar compounds may contribute to metal ion binding and chelation capacity may not correlate linearly with TPC, TFC or TAC.

The current findings reveal that ethanol extracts of Northern Iraqi propolis from Duhok and Sulaymaniyah, possess notable levels of TPC and TFC which are closely related with strong antioxidant activity. Although the total phenolic (965 ppm GAE) and flavonoid (1967 ppm QE) content of this thesis are numerically lower than the concentrations reported in Australian propolis reaching up to 181 mg GAE/g and 145 mg QE/g respectively they still demonstrate a robust antioxidant profile as evident in both TAC (up to 542 ppm αTE) and DPPH radical scavenging activity (up to 88.3%) (Tran et al., 2022) compared to Brazilian red propolis which showed TPC values around 122.3 mg GAE/g extract and Uruguayan and Brazilian green varieties with average TFC OF 75 and 57 mg QE/g extracts respectively, Iraqi propolis particularly from Sulaymaniyah and Mosul showed a competitive polyphenolic composition. Interestingly, water extract of Iraqi

propolis exhibited lower TPC and TFC values consistent with limited solubility of polyphenols in water. Nevertheless, they showed notable metal chelating activity particularly in Sulaymaniyah (51.92%) indicating that water soluble compounds may also contribute to the antioxidant profile.

4.4. Antimicrobial Activity

The antimicrobial effects of water and ethanol extracts of propolis collected from Erbil, Mosul, Duhok and Sulaymaniyah were evaluated by disc diffusion assay against a panel of Gram-positive (*Listeria monocytogenes* NCTC 5348, *Bacillus subtilis* IM622), Gram-nagative (*Salmonella typhimurium* NRRLE 4413, *Escherichia coli* ATCC 25922) and yeast (*Candida albicans* ATCC 25922) at concentrations of 0.5, 1, 2 and 4 mg/mL (Table 4.7). Against *S. typhimurium,* ethanol extracts exhibited a slight increase in inhibition zones with rising concentrations. The inhibition zones formed by the standard antibiotic used (streptomycin) as positive control was around (10±1.1 mm) across all tested microorganisms. This served as reference point to compare the antimicrobial activity of propolis extracts. The Erbil extract demonstrated the most significant activity increasing from 6.14 mm at 0.5 mg/mL to 7.48 mm at 4 mg/mL. In comparison, Mosul and Duhok samples showed limited activity reaching only 6.78 mm and 6.22 mm respectively at the highest concentration tested. The Sulaymaniyah sample showed a modest increase from 6.16 mm to 6.73 mm. Water based extracts exhibited less activity overall, with inhibition zone for Erbil sample increasing slightly from 6.01 mm to 6.44 mm and the highest values for Sulaymaniyah reaching only 6.11 mm at 4 mg/mL.

In case of *C. albicans*, ethanol extracts from Erbil and Sulaymaniyah displayed higher antifungal activity. Erbil's extract showed an increase from 6.21 mm at 0.5 mg/mL to 7.44 mm at 4 mg/mL, while Sulaymaniyah sample achieved the highest zone 7.79 mm. Water extracts remained mostly inactive at lower concentrations (0.5-2 mg/mL). Only the extracts of Erbil and Sulaymaniyah samples showed slight activities at 4 mg/mL indicating limited water solubility of active antifungal components.

Against *L. monocytogenes*, the ethanol extracts from Sulaymaniyah and Erbil samples displayed more promising results. At 4 mg/mL, inhibition zones reached 6.57 and 6.91 mm

respectively. The Mosul and Duhok samples remained less active with inhibition zones peaking at 6.74 mm and 6.45 mm. Water extracts showed lower activity overall; only the Erbil and Duhok samples exceeded 6 mm at 4 mg/mL reaching 6.34 mm and 6.38 mm respectively.

For *E. coli*, the ethanol extract from Erbil demonstrated the greatest inhibitory effects reaching 6.78 mm at 4 mg/mL. Sulaymaniyah, Mosul and Duhok samples also showed antibacterial effect with inhibition zones of 6.68 mm, 6.51 mm and 6.34 mm respectively. Water extracts were less effective; however, Erbil sample still showed a measurable increase from 6.05 mm at 0.5 mg/mL to 6.6 mm at 4 mg/mL. Notably, Duhok and Sulaymaniyah extracts reached 6.4 mm and 6.49 mm respectively at highest concentrations tested.

Finally, *B. subtilis* was susceptible to both ethanol and water extracts. Ethanol extracts from Erbil and Duhok displayed the highest antibacterial activity producing inhibition zones of 6.58 mm and 6.51 mm at 4 mg/mL while Mosul and Sulaymaniyah samples exhibited more limited effects. Regarding water based extracts, Erbil sample showed the greates activity with a zone of 6.54 mm, followed closely by Duhok (6.26 mm). Other samples presented less notable inhibition remaining around or below 6.2 mm.

Overall, the antimicrobial activity results revealed that EEP generally exhibited stronger inhibitory effects than WEP across all tested microorganisms. Among the samples, propolis from Erbil and Sulaymaniyah showed the most pronounced activity against *C. albicans*, *E. coli* and *S. typhimurium* indicating the presence of bioactive compounds with broad spectrum antimicrobial potential. The increasing inhibition zones with rising concentrations especially in EEP suggest a concentration dependent antimicrobial effects. WEP, on the other hand, showed minimal activity, highlighting the influence of solvent polarity on the extraction efficiency of antimicrobial efficacy of propolis varies by geographic origin, concentration and extraction method with EEP from Erbil and Sulaymaniyah appearing to be the most effective. When compared to the inhibition zones produced by standard antibiotic control, which were around 10±1.1 mm, the ethanol extracts displayed moderate efficiency reaching up to 7.8 mm inhibition zone at 4 mg/mL,

whereas the water extracts showed limited or negligible activity. This may highlight the potential of ethanol based propolis extractions for antimicrobial applications.

Table 4.7. Inhibition zones (mm) of ethanol and water extracts of propolis from different regions against various microorganisms and different concentrations

Region	**Extract**	**Bacterial** Strain	**Antibiotic inhibition zone/mm**	**Propolis Concentration (mg/mL) and inhibition zone (mm)** 0,5	1	2	4
Erbil	Ethanol	*Salmonella*	10.682±0.056	6.140±0.02	6.460±0.036	6.480±0.091	7.475±0.0662
Mosul	Ethanol	*Salmonella*	9.540±0.051	6.038±0.014	6.071±0.034	6.171±0.044	6.782±0.065
Duhok	Ethanol	*Salmonella*	9.626±0.165	6.017±0.0110	6.082±0.037	6.099 ±0.052	6.220±0.050
Sulaymaniyah	Ethanol	*Salmonella*	10.120±0.130	6.156±0.041	6.238±0.022	6.441±0.010	6.731±0.0211
Erbil	Water	*Salmonella*	10.266±0.074	6.008 ±0.005	6.050±0.015	6.081±0.006	6.445±0.083
Mosul	Water	*Salmonella*	10.526±0.027	6.025±0.034	6.085±0.023	6.124±0.65	6.169±0.027
Duhok	Water	*Salmonella*	9.717±0.0115	6.015±0.016	6.068±0.042	6.069±0.024	6.141±0.001
Sulaymaniyah	Water	*Salmonella*	9.783±0.221	6.007±0.0005	6.032±0.018	6.046±0.020	6.115±0.038
Erbil	Ethanol	*Candida*	11.009±0.266	6.214±0.044	6.309±0.050	6.651±0.047	7.438±0.110
Mosul	Ethanol	*Candida*	10.369±0.355	6.167±0.021	6.255±0.041	6.520±0.107	7.263±0.167
Duhok	Ethanol	*Candida*	11.130±0.319	6.164±0.048	6.326±0.042	6.334±0.021	6.465±0.052
Sulaymaniyah	Ethanol	*Candida*	10.714±0.126	6.178±0.031	6.359±0.073	6.481±0.079	7.794±0.090
Erbil	Water	*Candida*	10.635±165	6	6	6	6.375±0.021
Mosul	Water	*Candida*	10.856±0.093	6	6	6	6
Duhok	Water	*Candida*	10.822±0.161	6	6	6	6
Sulaymaniyah	Water	*Candida*	10.401±0.257	6	6	6	6.346±0.058
Erbil	Ethanol	*Listeria*	10.340±0.115	6.092±0.059	6.282±0.035	6.562±0.084	6.916±0.112
Mosul	Ethanol	*Listeria*	10.439±0.149	6.038±0.039	6.232±0.053	6.554±0.097	6.745±0.127
Duhok	Ethanol	*Listeria*	10.508±0.096	6.205±0.073	6.254±0.052	6.319±0.040	6.459±0.099
Sulaymaniyah	Ethanol	*Listeria*	10.980±0.332	6.279±0.092	6.503±0.071	6.552±0.033	6.575±0.068
Erbil	Water	*Listeria*	11.077±0.140	6	6	6.257±0.051	6.348±0.047
Mosul	Water	*Listeria*	10.967±0.140	6	6	6	6
Duhok	Water	*Listeria*	11.089±0.356	6	6	6	6.385±0.018
Sulaymaniyah	Water	*Listeria*	11.085±0.236	6	6	6	6.320±0.025
Erbil	Ethanol	*E. coli*	8.971±0.164	6.367±0.086	6.542±0.062	6.614±0.111	6.781±0.126
Mosul	Ethanol	*E. coli*	8.948±0.155	6.347±0.082	6.422±0.045	6.458±0.046	6.514 ±0.024
Duhok	Ethanol	*E. coli*	8.731±0.148	6.037±0.012	6.227±0.055	6.326±0.045	6.339±0.034
Sulaymaniyah	Ethanol	*E. coli*	9.078±0.111	6.480±0.064	6.556 ±0.027	6.575±0.026	6.682±0.028
Erbil	Water	*E. coli*	8.862±0.132	6.047±0.034	6.100±0.051	6.269±0.051	6.603±0.054
Mosul	Water	*E. coli*	8.976±0.279	6.035±0.045	6.053±0.019	6.138±0.043	6.308±0.048
Duhok	Water	*E. coli*	9.154±0.428	6.097±0.030	6.187±0.032	6.213 ±0.028	6.406±0.063
Sulaymaniyah	Water	*E. coli*	9.407±0.221	6.062±0.042	6.150±0.045	6.199±0.043	6.495±0.056
Erbil	Ethanol	*Bacillus*	8.777±0.128	6.449±0.033	6.466±0.040	6.525±0.077	6.583±0.086
Mosul	Ethanol	*Bacillus*	8.637±0.144	6.222±0.068	6.335±0.061	6.380±0.052	6.425±0.061
Duhok	Ethanol	*Bacillus*	8.476±0.045	6.276±0.058	6.412±0.052	6.497±0.064	6.514±0.071
Sulaymaniyah	Ethanol	*Bacillus*	8.683±0.153	6.193 ±0.071	6.370±0.109	6.372±0.045	6.624±0.063
Erbil	Water	*Bacillus*	8.594±0.071	6.060±0.029	6.195±0.054	6.200 ±0.065	6.544±0.041
Mosul	Water	*Bacillus*	8.771±0.096	6.005±0.015	6.122±0.078	6.153 ±0.085	6.267±0.077
Duhok	Water	*Bacillus*	8.800±0.274	6.021±0.019	6.100±0.077	6.103±0.066	6.262±0.067
Sulaymaniyah	Water	*Bacillus*	8.367±0.100	6.019±0.019	6.050±0.030	6.067 ±0.065	6.204±0.033

Numerous studies have assessed effectiveness of propolis against bot Gram (+) and Gram (-) bacteria (Ghosh et al., 2022). A prevailing observation across these investigations is that Gram (-) bacteria typically exhibit a greater level of resistance to propolis than Gram (+) counterparts. For example, while propolis has shown notable inhibitory effects against *S. aureus* (Gram +), its activity against *E. coli* (Gram -) tends to be considerably lower (Pobiega et al., 2019). This disparity is often reflected in the higher MIC values observed for Gram (-) stains. One possible explanation lies in structural composition of Gram (-)

bacterial cell walls which are more complex and contain an outer membrane that serves as an effective barrier to many antimicrobial agents. On the other hand, Gram (+) bacteria are more consistently sensitive to propolis frequently show strong inhibition (Morawiec et al., 2015; Al-Ani et al., 2018; Grecka et al., 2019). The antibacterial potential of propolis is largely attributed to its rich chemical profile, particularly flavonoids (eg. pinocembrin, galangin) and phenolic acids (eg. caffeic acid) (Anjum et al., 2019; Gür et al., 2019). These compounds can interact with bacterial proteins altering their structural integrity and disrupting key cellular processes. Reported mode of action include interference with cell division, destabilization of cell membrane, suppression of enzymatic function, inhibition of protein biosynthesis and ultimately bacterial cell lysis. The studies using Italian propolis have identified galangin and pinocembrin as particularly active against *S. pyogenes* (Bosio et al., 2000). Other reports have demonstrated that propolis interferes with translation process in *S. aureus* (Grecka et al., 2019).

Propolis has demonstrated antifungal activity against a wide range of fungal genera including *Aspergillus* (Ding et al., 2024), *Fusarium* (Guerra-Fuentes et al., 2025), *Candida* (Al-Oebady, 2025), *Saccharomyces* (Oliveira et al., 2024) and *Cryptococcus* (Sabrina et al., 2022). Propolis extracts have been shown to interfere with Candida morphogenesis by preventing transition from yeat like to hyphal forms, a critical step in biofilm development (Tobaldini-Valerio et al., 2016). In some studies, propolis demonstrated stronger anti Candida effects than commonly used antifungal agents like amhphotericin B (Gucwa et al., 2018).

4.5. Sun Protection Activity

The sun protection factors (SPF) of both ethanolic and aqueous extracts of propolis samples collected from Erbil, Mosul, Duhok and Sulaymaniyah were quantitatively assessed via spectrophotometric analysis and the findings are presented in Table 4.8. The SPF values across all samples and concentrations ranged from 5.1 to 17.9 indicating moderate to high UV protective potential depending on extraction solvent and concentration. Among the tested samples, the highest SPF value (17.9±0.45) was recorded for the EEP of Mosul at concentration of 20 ppm while the lowest value (5.1±0.12) was observed in aqueous extract of Erbil propolis at 0.5 ppm. A general trend was evident wherein the SPF values of

ethanolic extracts consistently surpassed those of their corresponding water counterparts at all concentrations studied.

Specifically for Erbil propolis, the ethanolic extract at 20 ppm exhibited an SPF of 9.9±0.2 which was approximately 35% higher than that of aqueous extract at the same concentration (6.7±0.16). The lowest SPF value for this set was observed at 0.5 ppm in water extract (5.1±0.2). In case of Mosul propolis, the ethanolic extract not only demonstrated the highest overall SPF (17.9±0.45 at 20 ppm) but also significantly outperformed the water extract which displayed an SPF of 6.6±0.16 at the same concentration representing a nearly threefold reduction. The water extract at 0.5 ppm yielded a minimal SPF (5.4±0.15) at this concentration. Regarding the Sulaymaniyah propolis, the ethanolic extract again showed superior SPF values compared to water counterpart with the highest SPF recorded at 20 ppm (11.9±0.32). The water extract at this concentration exhibited a markedly lower SPF of 6.5±0.21 almost twofold less than the ethanolic sample. The lowest SPF for Sulaymaniyah sample was 5.5±0.11, observed in water extract at 0.5 ppm. The ethanolic extract of Duhok propolis at a concentration of 20 ppm displayed an SPF of 9.74±0.23 which was approximately 65% higher than that of aqueous extract at the same concentration (6.27±0.14). The lowest SPF value for this set was observed at 0.5 ppm in water extract (5.1±0.12).

The comparative analysis of SPF values across ethanolic and aqueous extracts from for different regional propolis samples revealed a consistent trend: ethanolic extracts demonstrated markedly superior photoprotective activity compared to their water counterparts. Among all samples, Mosul ethanolic extract exhibited the highest SPF highlighting its potential as a strong natural UV filters. In contract, water extracts across all regions showed minimal SPF variation ad consistently lower efficacy. These finfings suggest that ethanol is a more effective solvent for isolating the phootoprotective components of propolis with regional variation likely attributable to differences in botanical origin and local environmental factors.

Table 4.8. The SPF analysis of ethanolic and water extracts of Erbil, Mosul, Duhok and Sulayma niyah propolis. E denoted ethanol extract, W denotes water extract

Concentration (ppm)	**SPF (Erbil, E)**	**SPF (Erbil, W)**
20	9.9±0.2	6.7±0.16
10	6.6±0.12	6±0.14
5	5.9±0.14	5.7±0.14
1	5.5±0.12	5.6±0.1
0.5	5.4±0.1	5.1±0.12
Concentration (ppm)	**SPF (Mosul, E)**	**SPF (Mosul, W)**
20	17.9±0.45	6.6±0.16
10	10±0.24	5.9±0.1
5	7.5±0.22	5.7±0.15
1	5.7±0.12	5.4±0.12
0.5	5.5±0.15	5.4±0.1
Concentration (ppm)	**SPF (Duhok, E)**	**SPF (Duhok, W)**
20	9.74±0.23	6.27±0.14
10	7.05±0.14	5.48±0.11
5	6.3±0.11	5.3±0.1
1	5.47±0.12	5.11±0.1
0.5	5.64±0.13	5.1±0.12
Concentration (ppm)	**SPF (Sulaymaniyah, E)**	**SPF (Sulaymaniyah, W)**
20	11.9±0.32	6.5±0.21
10	8.7±0.23	5.9±0.22
5	6.9±0.20	5.7±0.14
1	5.9±0.14	5.5±0.16
0.5	5.5±0.11	5.5±0.11

Due to its recognized protective effects on skin, propolis has emerged as a promising candidate for incorporation into cosmeutical formulations including sunscreen products. Its pronounced anti-inflammatory properties make it particularly suitable for alleviating radiation induced skin conditions such as sunburn. Moreover, by contributing to the maintenance of collagen integrity in the skin, propolis may also serve as an effective anti-aging agent. The presence of diverse types off flavonoids within propolis further supports its potential in shielding the skin against the damaging effects of UV radiation.

Assessment of SPF of Lithuanian propolis ethanol extracts at a concentration of 10 µg/mL revealed SPF values ranging between 2.010 and 4.851 (Stanciauskaite et al., 2022). Notably, the SPF values recorded for pine bud extracts were comparatively lower and all were found to be lower than those reported for Northern Iraqi propolis. In another study, topical application of a hydroalcoholic extract derived from Brazilian red propolis exhibited photoprotective efficacy in an animal model (Batista et al., 2028). These

protective effects were attributed, at least in part, to the extract's anti-inflammatory and antioxidant activities. This suggests that red propolis extracts could represent viable natural ingredients in cosmetic formulations intended for UV protection. Furthermore, the inclusion of green propolis extracts in sun screen formulations prepared with Gel Permulen TR-1 was found to significantly enhance SPF values (Almeida et al., 2020). The HET-CAM assay demonstrated that these formulations were non-irritating and safe for dermal applications. Specifically, EEP at concentrations of 70% and 75%, maintained at both ambient and elevated temperatures, were observed to improve UV protection. These results support the utility of such formulations for potential use in photoprotective skin care products. Additionally, when incorporated into Polawax-based cream, red propolis etanolic extracts imparted measurable photoprotective properties (Valverde et al., 2023). While previous studies have often highlighted a direct association between UV protection and levels of total phenolics and flavonoid, this research suggested that antioxidant efficacy might also arise independently of high phenolic content, possibly due to other bioactive constituents retained during extraction. Importantly, the incorporation of EEP into these topical preparations not only augmented SPF values but also preserved the compound's multifunctional bioactivities including antimicrobial, antioxidant, anti-inflammatory and wound healing effects. Collectively, these findings underscore the potential of propolis as a multifunctional natural agent suitable for the development of innovative sunscreen and skin care formulations.

4.6. Purification of GST

SDS-PAGE analysis was performed to assess the purity of enzyme purified through affinity chromatography. The result revealed a single protein band on the gel indicating that the eluate contain a single protein band corresponding to the GST enzyme isolated from human erythrocyte (Figure 4.5).

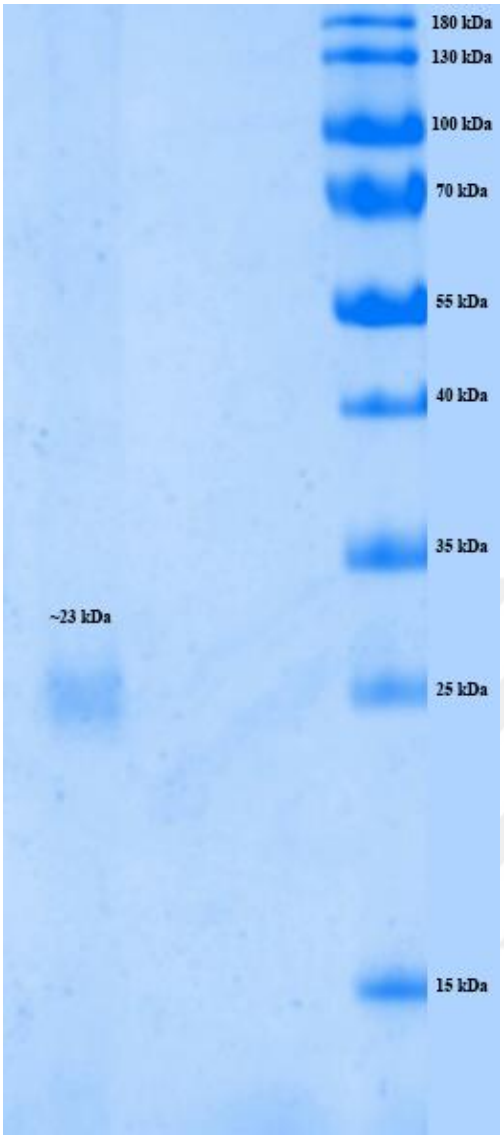

Figure 4.5. SDS-PAGE analysis of GST. The molecular weight of markers (lane 2) are depicted on the gel. The band corresponding to GST is shown in lane 1

4.7. GST Inhibitory Activity of Propolis

The GST inhibition activity of water and ethanol extracted propolis samples collected from four regions (Erbil, Mosul, Duhok and Sulaymaniyah) of Northern Iraq were evaluated (Figure 4.6, Figure 4.7, Figure 4.8, Figure 4.9 and Table 4.9). Among all samples, the ethanol extract of Sulaymaniyah exhibited the most potent GST inhibition with an IC_{50} value of 5.52±0.06 µg/mL, followed by water extract of Erbil at 6.27±0.09 µg/mL. Ethanol extract of Mosul, Duhok and Erbil also demonstrated significant inhibitory activities with

IC_{50} values of 7.04±0.12 µg/mL, 7.04±0.11 µg/mL and 16.83±0.21 µg/mL respectively. In contrast, the weakest GST inhibition was observed in water extract of Sulaymaniyah which had an IC_{50} of 98.57±1.21 µg/mL indicating minimal inhibitory activity.

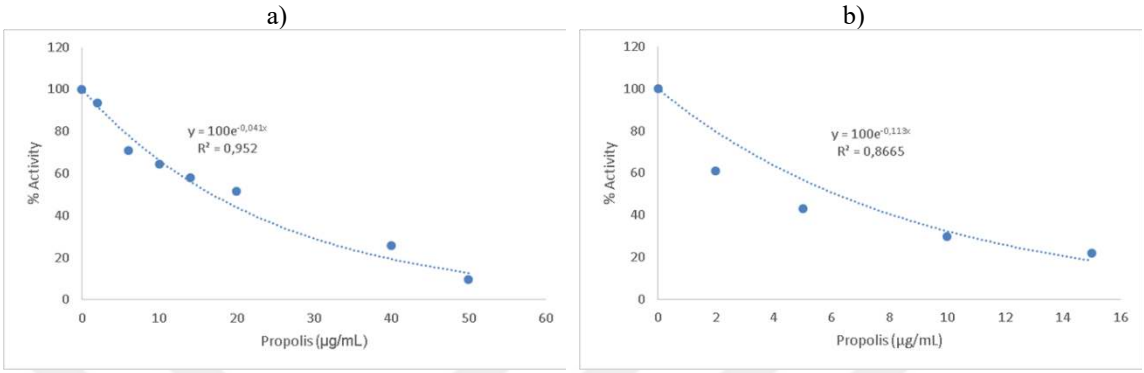

Figure 4.6. GST inhibitory effects of ethanol (a) and water (b) extract of Erbil propolis

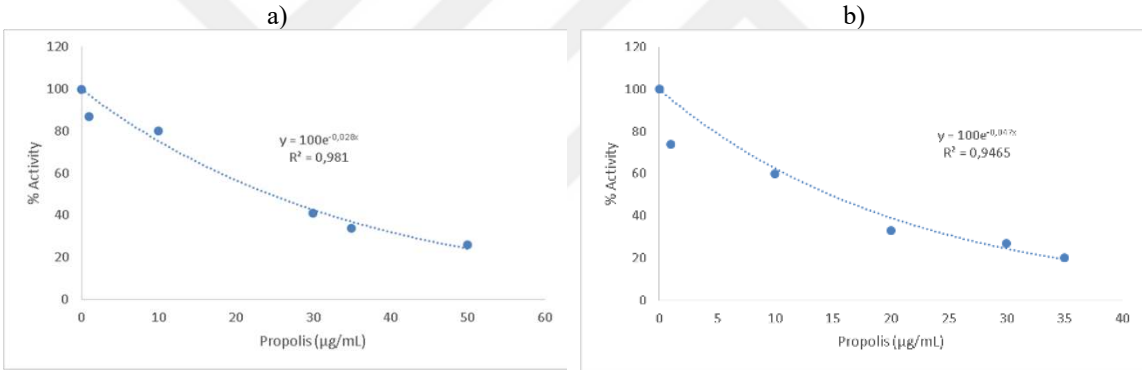

Figure 4.7. GST inhibitory effects of ethanol (a) and water (b) extract Mosul propolis

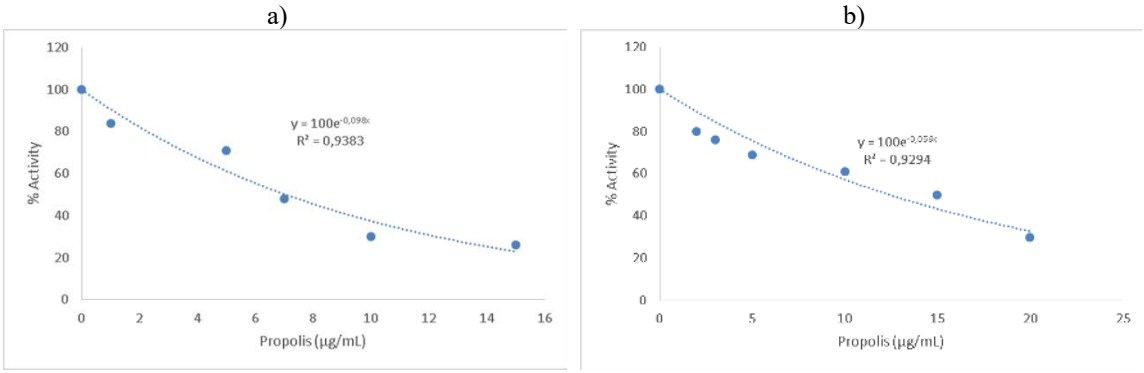

Figure 4.8. GST inhibitory effects of ethanol (a) and water (b) extract Duhok propolis

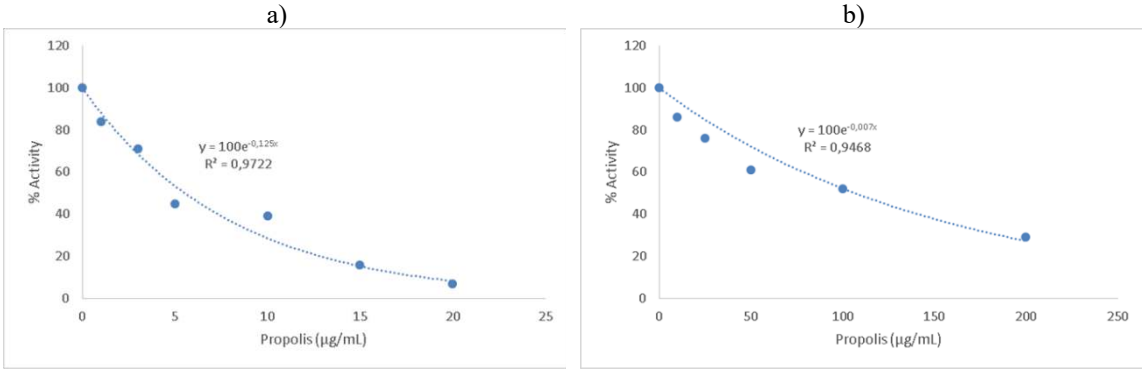

Figure 4.9. GST inhibitory effects of ethanol (a) and water (b) extract Sulaymaniyah propolis

The strong GST inhibition observed in ethanol extracts particularly Sulaymaniyah, correlates well with their rich flavonoid content. It contains high levels of galangin (4560.1 ppm), chrysin (4381.5 ppm) and diosmetin (2414.3 ppm). These flavonoids are known for their enzyme inhibitory and antioxidant properties suggesting a strong link between high flavonoid content and enzyme inhibition. Similarly, ethanol extract of propolis from Mosul and Duhok also displayed substantial GST inhibition aligning with their high concentrations of galangin (Mosul: 4981.6 ppm, Duhok: 1740.4 ppm) and chrysin (Mosul: 3767 ppm, Duhok: 3654.8 ppm). In contrast, the water extract of Sulaymaniyah which exhibited the weakest inhibition, also had the lowest phenolic compounds. Only trace amounts of ellagic acid (41.3 ppm), caffeic acid (41.2 ppm) and benzoic acid (7.5 ppm) were detected suggesting that the limited presence of bioactive compounds contributed to its poor inhibitory effect. Interestingly, despite being water based, some extracts like Erbil water extracts and Mosul water extracts displayed notable GST inhibition. This may be attributed to relatively higher levels of hydrophilic phenolic such as caffeic acid (Erbil: 1309.5 ppm, Mosul: 2280.5 ppm) which are still effective in enzyme inhibition.

Table 4.9. GST inhibitory effects of ethanol (E) and water (W) extract of propolis

Sample	**IC_{50} (µg/mL)**	**Concentration range (µg/mL)**
Erbil (E)	16.83±0.21	0-50
Erbil (W)	6.27±0.09	0-15
Mosul (E)	7.04±0.12	0-50
Mosul (W)	14.68±0.21	0-35
Duhok (E)	7.04±0.11	0-15
Duhok (W)	12,32±0.14	0-20
Sulaymaniyah (E)	5.52±0.06	0-20
Sulaymaniyah (W)	98.57±1.21	0-200

The inhibition of GST activity by propolis highlights the double edged sword nature of natural products. While propolis is renowned for its antioxidant, antimicrobial and anticancer properties, its ability to suppress key detoxification enzymes such as GST raises concerns especially in the context of healthy cells. GST plays a central role in maintaining redox balance and protecting cells from electrophilic and oxidative damage. Thus its inhibition could render normal cells more vulnerable to stress. On the other hand, in disease states such as cancer where GST overexpression contributes to drug resistance and tumor survival, this inhibitory effect may be advantageous (Townsend and Tew, 2023). This dualistic behavior underscores the importance of dose, duration and physiological context when evaluating the safety and efficacy of propolis. As such, GST inhibition by propolis may present a dose dependent effect emphasizing the need for further *in vivo* studies to delineate its biological consequences more precisely.

In recent years, various studies have highlighted the enzyme inhibitory potential of propolis which is largely attributed to its rich phenolic composition. For instance, Baltas et al. (2016) reported that ethanolic propolis extracts exhibited notable inhibitory activities against several enzymes including urease, xanthine oxidase and acetylcholine esterase (AchE) with IC_{50} values ranging from 0.074 to 1.560 mg/mL. The study demonstrated a clear correlation between enzyme inhibition and phenolic content suggesting that ethanolic extract of propolis may serve as a valuable source natural enzyme inhibitors for promoting human health. Similarly, Yıldız et al. (2014) investigated inhibitory effects of propolis, pollen and honey on monoamine oxidase. It was found that propolis showed strongest inhibitory effects. A more recent investigation by Izol and Turhan (2024) examined enzyme inhibitory effects of Bitlis propolis on various therapeutic enzymes. Their findings revealed potent inhibitory activities with IC_{50} of 7.19 µg/mL for human carbonic anhydrase I, 8.15 µg/mL for human carbonic anhydrase II, 5.17 µg/mL for AchE and 5.72 µg/mL for α-glucosidase. Importantly, the interaction of propolis with drug metabolizing enzymes has also been examined. Naramoto et al. (2014) conducted both *in vitro* and *in vivo* studies using Brazilian green propolis extract and demonstrated its concentration dependent inhibitory effects on major cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4). Zhang et al. (2015) further explored the inhibitory effects of propolis on glycosidase and sucrose. Their results indicated 75% ethanol extract of propolis exhibited the most effective inhibition. In alignment with these reports linking flavonoid

rich propolis to enzyme inhibition, the data of this thesis demonstrated that ethanol extracts of propolis particularly from Sulaymaniyah exhibited strong GST inhibition which correlated with their high concentration of galangin (4560.1 ppm), chrysin (4381.5 ppm) and diosmetin (2414.3 ppm).

5. CONCLUSIONS

This doctoral research presents a region-specific and comprehensive characterization of the phytochemical composition and biological promise of propolis samples collected from four geographically varied regions of Northern Iraq: Erbil, Mosul, Duhok, and Sulaymaniyah. The aim was to bridge the gap in recent scientific literature on the characterization of Iraqi propolis, which is less explored than its international analogues such as Brazilian, Turkish, and Chinese propolis.

LC-MS/MS chemical profiling revealed the phenolic and flavonoid composition of propolis to be solvent- and region-dependent. Ethanol extracts consistently contained a higher amount of total phenolic content (TPC), total flavonoid content (TFC), and a wider range of bioactive compounds than water extracts. Galangin, chrysin, and naringenin emerged as dominant flavonoids across the ethanol extracts, with Mosul and Sulaymaniyah propolis yielding the highest amount. This is in agreement with Populus origin propolis and reflects botanical similarity to some Turkish varieties of propolis. CAPE, a bioactive molecule of high activity, was extremely prevalent in the Duhok sample, which represents a characteristic regional variant of pharmacological significance.

GC-MS of hexane extracts contributed to this also, detecting a broad spectrum of fatty acids, hydrocarbons, esters, and alcohols, some of which are documented to be engaged in antioxidative and antimicrobial activities. The most prevalent fatty acid was palmitic acid (C16:0), and its prevalence in the Duhok samples statistically separated them in multivariate analysis. Exclusive substances like organoselenium compounds in Mosul and high concentrations of phenolic antioxidants in Sulaymaniyah indicate specific environmental and floristic impacts on each region's chemical profiles of propolis.

ICP-MS mineral profiling revealed widespread macro- and trace element variation with Ca, K, Mg, Fe, and Zn being dominant. While toxic metals like Pb were also found, their levels were within international accepted safety levels, again proving environmental safety and therapeutic value of these propolis samples. The elemental richness, particularly in the

Mosul and Sulaymaniyah samples, points to their potential for nutraceutical and pharmacological applications.

Bioassays confirmed the correlation between richness in chemicals and bioactivity. Ethanol extracts, especially Duhok and Sulaymaniyah, showed higher antioxidant activity in different parameters like scavenging of the DPPH radical and total antioxidant capacity (TAC). Water extracts showed lower TPC and TFC but exhibited unexpectedly high metal chelating activity, particularly in Sulaymaniyah samples indicating the presence of non-phenolic hydrophilic antioxidants.

Antimicrobial investigations revealed the increased activity of ethanol extracts against Gram-positive and Gram-negative bacterial cultures and *C. albicans*. The highest antimicrobial activity was demonstrated by Erbil and Sulaymaniyah propolis, with activity demonstrating increments dependent on concentration. Water extracts were much weaker, underlining the role played by polarity of the solvent in isolating bioactive compounds. These findings not only confirm propolis as a prospective antimicrobial agent but also show its selective use depending on the mode of extraction and microbial target.

Photoprotective screening revealed moderate to high SPF values for ethanol extracts, with maximum SPF value of 17.9 for Mosul propolis at 20 ppm. The consistency of ethanol extracts in all the regions assayed points towards elevated affinities of UV-absorbing compounds towards alcoholic solvents. These results confirm the feasibility of using Iraqi propolis in cosmeceutical applications such as sunscreens and anti-ageing creams.

Enzyme inhibition studies demonstrated that Sulaymaniyah ethanol extracts possessed the highest inhibitory activity against Glutathione S-Transferase (GST) with an IC_{50} value of 5.52 µg/mL. Inhibitory activity was most associated with high contents of flavonoids like galangin and chrysin. While such inhibition may be detrimental to detoxification in healthy cells, it has therapeutic value for modulation in diseases such as cancer, where GST is overexpressed and linked with chemoresistance. The findings emphasize the duality of propolis as both health promoter and metabolic modulator, for which strict dosage and context-dependent use must be practiced.

In total, this thesis establishes Northern Iraqi propolis to be a chemically variable and biologically active natural compound of high antioxidant, antimicrobial, photoprotective, and enzyme inhibitory activities. The regional variations observed attest to the impact of geographical, botanical, and climatic factors on the composition and bioactivity of propolis. The results identify ethanol extracts, particularly from Sulaymaniyah and Mosul, to be of high potential for pharmacological or cosmeceutical product development. This study forms the basis of continued research with:

1. *In vivo* bioactivity assays to determine therapeutic efficacy and safety.
2. Mechanistic studies to elucidate molecular targets of propolis-derived compounds.
3. Standardization efforts to develop quality standards for propolis products according to chemical and biological fingerprints.

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APPENDIX

Table S.1. The phenolic profiles of ethanol extract of Erbil propolis

N	Compound Name	RT	Quan Peak	Response	Conc	Units	Dev (min)
1	C vitamini (L-ascorbic acid)	N/F	175.02481 mz	N/F	N/F	ppb	N/F
2	Capsaicin	N/F	306.20637 mz	N/F	N/F		N/F
3	Gallic acid(3,4,5-trihydroxybenzoic acid)	N/F	169.01425 mz	N/F	N/F	ppb	N/F
4	Arbutin	N/F	271.08233 mz	N/F	N/F	ppb	N/F
5	Taxifolin M+3H	N/F	162.02118 mz	N/F	N/F	ppb	N/F
6	Quinic acid	1,10	191.05611 mz	36558024	47,926	ppb	-0,13
7	Protocatechuic acid (3,4-Dihydroxybenzoic acid)	4,34	153.01933 mz	4572433	8,699	ppb	0,10
8	3,4-Dihydroxyphenylacetic acid(DOPAC, Homoprotocatechuic acid)	N/F	167.03498 mz	N/F	N/F	ppb	N/F
9	3,4-dihydroxybenzaldehyde (Protocatechuic aldehyde)	5,65	137.02442 mz	82977537	19,909	ppb	-0,06
10	Esculin hydrate	N/F	339.07216 mz	N/F	N/F	ppb	N/F
11	4-Hydroxybenzoic acid	6,19	137.02442 mz	8989030	8,259	ppb	-0,08
12	Epigallocatechin	N/F	305.06668 mz	N/F	N/F	ppb	N/F
13	Catechin (Cianidanol)-p	6,43	289.07176 mz	324623	7,731	ppb	0,08
14	Gentisic acid	6,46	153.01933 mz	335751	2,018	ppb	-0,06
15	Taxifolin	N/F	305.06558 mz	N/F	N/F	ppb	N/F
16	Procyanidin B2	N/F	577.13515 mz	N/F	N/F	ppb	N/F
17	Chlorogenic acid	7,07	353.08781 mz	2125250	3,968	ppb	0,10
18	3-hydroxyphenylacetic acid (3-HPA)	N/F	107.05053 mz	N/F	N/F	ppb	N/F
19	Benzoic acid	9,09	121.02940 mz	7419688	22,712	ppb	0,12
20	Epigallocatechin gallate	N/F	457.07763 mz	N/F	N/F	ppb	N/F
21	3-hydroxybenzoic acid (3-HBA)	7,03	137.02442 mz	3662256	2,994	ppb	-0,13
22	Vanillic acid	N/F	167.03498 mz	N/F	N/F	ppb	N/F
23	Homovanillic acid((4-Hydroxy-3-methoxyphenylacetic acid)	N/F	181.05063 mz	N/F	N/F	ppb	N/F
24	Caffeic acid	7,22	179.03498 mz	2224384938	444,277	ppb	-0,01
25	2,4-dihydroxybenzoic acid (beta-Resorcylic acid)	7,29	153.01933 mz	480991	2,884	ppb	0,04

26	Quercetin 3-rutinoside 7-glucoside	N/F	771.20374 mz	N/F	N/F	ppb	N/F
27	Syringic acid	N/F	197.04555 mz	N/F	N/F	ppb	N/F
28	3-(4-Hydroxyphenyl) propionic acid	7,57	165.05572 mz	1860247	139,481	ppb	-0,16
29	Vanillin	7,83	151.04007 mz	92323	-1,088	ppb	0,07
30	Vicenin 2	N/F	593.15119 mz	N/F	N/F	ppb	N/F
31	Ethylgallate	N/F	197.04555 mz	N/F	N/F	ppb	N/F
32	Daidzin	N/F	255.06433 mz	N/F	N/F	ppb	N/F
33	Luteolin 7-rutinoside	N/F	595.16376 mz	N/F	N/F	ppb	N/F
34	Rutin trihydrate M-3H2O	N/F	609.14611 mz	N/F	N/F	ppb	N/F
35	Schaftoside	N/F	563.14063 mz	N/F	N/F	ppb	N/F
36	Eriocitrin	N/F	595.16684 mz	N/F	N/F	ppb	N/F
37	Coumaric acid (trans-3-Hydroxycinnamic acid)	8,26	163.04007 mz	1038629200	276,803	ppb	-0,09
38	Luteoloside (Luteolin 7-glucoside)	N/F	449.10784 mz	N/F	N/F	ppb	N/F
39	Orientin	N/F	447.09328 mz	N/F	N/F	ppb	N/F
40	Isoorientin	N/F	447.09328 mz	N/F	N/F	ppb	N/F
41	Liquiritin (4′,7-Dihydroxyflavanone 4′-glucoside)	8,60	417.11911 mz	72271989	69,745	ppb	0,04
42	Sinapic acid	N/F	223.06120 mz	N/F	N/F	ppb	N/F
43	Ferulic acid	8,52	193.05063 mz	102825242	55,956	ppb	-0,10
44	Vitexin (Apigenin 8-C-glucoside)	8,67	431.09837 mz	491358	4,24	ppb	-0,04
45	Narirutin (Narirutinsa, Naringenin rutinoside)	N/F	579.17193 mz	N/F	N/F	ppb	N/F
46	Doxorubicin Hydrchloride	N/F	544.18134 mz	N/F	N/F	ppb	N/F
47	Naringin	N/F	609.18390 mz	N/F	N/F	ppb	N/F
48	Hesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
49	α-Cyano-4-hydroxycinnamic acid	N/F	188.03532 mz	N/F	N/F	ppb	N/F
50	Rutin hydrate M-OH2	9,23	609.14611 mz	585507	6,596	ppb	0,02
51	İsoquercitrin (Quercetin 3-glucoside)	9,23	463.08820 mz	5371354	1,582	ppb	0,00
52	Hyperoside (Quercetin 3-D-galactoside)	9,23	463.08820 mz	5371354	1,582	ppb	0,00
53	Eriodictyol (3,4,5,7-Tetrahydroxyflavanone)	9,10	287.05501 mz	2150089	1,606	ppb	-0,13
54	Neohesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
55	Kaempferitrin	N/F	579.17083 mz	N/F	N/F	ppb	N/F
56	Protocatechuic acid ethyl ester (Ethyl 3,4-Dihydroxybenzoate)	9,14	181.05063 mz	884878	-3,381	ppb	-0,21
57	Ellagic acid	9,64	300.99899 mz	224262	37,059	ppb	0,12
58	Apiin (Apigenin-7-(2-O-apiosylglucoside)	N/F	565.15179 mz	N/F	N/F	ppb	N/F
59	Phloridzin	N/F	435.12967 mz	N/F	N/F	ppb	N/F
60	Myricetin	N/F	319.04291 mz	N/F	N/F	ppb	N/F

61	Luteolin-7-O-glucuronide (Luteolin-7-O-β-D-glucuronide)	N/F	461.07255 mz	N/F	N/F	ppb	N/F
62	Nicotiflorin (Kaempferol 3-rutinoside, Kaempferol 3-O-β –rutinoside)	9,74	593.15106 mz	118624	0,219	ppb	0,24
63	Rhoifolin (Apigenin 7-O- neohesperidoside)	N/F	577.15692 mz	N/F	N/F	ppb	N/F
64	Rosmarinic acid	9,49	359.07724 mz	137144	-0,008	ppb	-0,05
65	Apigenin 7-glucoside	9,55	431.09837 mz	433338	-1,859	ppb	-0,01
66	Astragalin (Kaempferol 3-glucoside)	9,62	447.09328 mz	4274687	-0,866	ppb	0,06
67	Kuromanine (Cyanidin 3-glucoside chloride)	9,62	447.09328 mz	4274687	-0,866	ppb	0,06
68	Salicylic acid	9,88	137.02442 mz	14620153	-2,124	ppb	0,12
69	Narcissin (Narcissoside, Isorhamnetin 3-rutinoside)	9,78	623.16176 mz	178366	1,844	ppb	-0,03
70	Leucoside (Kaempferol 3-sambubioside)	9,77	289.06924 mz	136785571	56,771	ppb	-0,14
71	Liquiritigenin	10,16	255.06628 mz	1378000	-3,128	ppb	0,22
72	Fisetin hydrate	9,76	285.04046 mz	3526454	3,154	ppb	-0,23
73	Etoposide	N/F	383.11053 mz	N/F	N/F	ppb	N/F
74	Afzelin (Kaempferol 3-rhamnoside)	N/F	431.09837 mz	N/F	N/F	ppb	N/F
75	Apigenin 7-glucuronide	N/F	445.07763 mz	N/F	N/F	ppb	N/F
76	Trans Cinnamic acid	N/F	147.04515 mz	N/F	N/F	ppb	N/F
77	Kaempferol	10,27	287.05350 mz	3435768	-0,325	ppb	-0,04
78	Quercetin	10,44	301.03538 mz	678546431	324,79	ppb	0,10
79	Naringenin	10,49	271.06120 mz	6502892899	1911,019	ppb	0,14
80	Tiliroside	N/F	595.14264 mz	N/F	N/F	ppb	N/F
81	Luteolin	10,71	285.04046 mz	378209403	163,127	ppb	-0,07
82	Genistein (5,7-Dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one)	10,68	269.04555 mz	611267	-4,81	ppb	-0,14
83	Apigenin (5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one)	11,25	269.04555 mz	2927401897	1027,597	ppb	0,20
84	Isorhamnetin (Quercetin 3′-methyl ether)	11,16	315.05103 mz	645142630	797,5	ppb	-0,14
85	Diosmetin (Luteolin 4′-methyl ether)	11,39	299.05611 mz	1925222593	1695,503	ppb	0,04
86	Formononetin (Neochanin)	11,37	267.06628 mz	4819905	0,378	ppb	-0,15
87	Sakuranetin (Naringenin 7-O-methyl ether)	11,68	285.07685 mz	1319677201	729,824	ppb	0,00
88	Caffeic acid phenhyl ester (CAPE)	11,97	283.09758 mz	10316931718	1605,413	ppb	-0,17
89	Chrysin (5,7-Dihydroxy-2-phenyl-4H-chromen-4-one)	12,15	253.05063 mz	9562990916	3339,983	ppb	-0,13
90	Galangin (3,5,7-Trihydroxy-2-phenyl-4H-chromen-4-one)	12,29	269.04555 mz	9651302710	4639,736	ppb	-0,13
91	Acacetin (5,7-Dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one)	12,37	283.06120 mz	3905037466	329,17	ppb	-0,15
92	Genkwanin(4’,5-Dihydroxy-7-metthoxyflavone, Apigenin 7-O-methyl ether)	12,37	283.06120 mz	3905037466	331,252	ppb	-0,15
93	Kaempferide	N/F	299.05611 mz	N/F	N/F	ppb	N/F
94	Glabridin	12,84	323.12888 mz	15528393	5,159	ppb	-0,02
95	Emodin	13,44	269.04555 mz	95095996	3,405	ppb	-0,17

Table S.2. The phenolic profiles of water extract of Erbil propolis

N	Compound Name	RT	Quan Peak	Response	Conc	Units	Dev (min)
1	C vitamini (L-ascorbic acid)	N/F	175.02481 mz	N/F	N/F	ppb	N/F
2	Capsaicin	N/F	306.20637 mz	N/F	N/F		N/F
3	Gallic acid(3,4,5-trihydroxybenzoic acid)	N/F	169.01425 mz	N/F	N/F	ppb	N/F
4	Arbutin	N/F	271.08233 mz	N/F	N/F	ppb	N/F
5	Taxifolin M+3H	N/F	162.02118 mz	N/F	N/F	ppb	N/F
6	Quinic acid	1,21	191.05611 mz	99057338	130,064	ppb	-0,03
7	Protocatechuic acid (3,4-Dihydroxybenzoic acid)	4,33	153.01933 mz	170435176	148,837	ppb	0,09
8	3,4-Dihydroxyphenylacetic acid(DOPAC, Homoprotocatechuic acid)	7,14	167.03498 mz	1889307	24,97	ppb	-0,07
9	3,4-dihydroxybenzaldehyde (Protocatechuic aldehyde)	5,77	137.02442 mz	155215069	37,973	ppb	0,06
10	Esculin hydrate	N/F	339.07216 mz	N/F	N/F	ppb	N/F
11	4-Hydroxybenzoic acid	6,26	137.02442 mz	124320026	126,637	ppb	-0,01
12	Epigallocatechin	N/F	305.06668 mz	N/F	N/F	ppb	N/F
13	Catechin (Cianidanol)-p	N/F	289.07176 mz	N/F	N/F	ppb	N/F
14	Gentisic acid	6,53	153.01933 mz	137874	1,914	ppb	0,01
15	Taxifolin	N/F	305.06558 mz	N/F	N/F	ppb	N/F
16	Procyanidin B2	N/F	577.13515 mz	N/F	N/F	ppb	N/F
17	Chlorogenic acid	6,83	353.08781 mz	796583	1,903	ppb	-0,14
18	3-hydroxyphenylacetic acid (3-HPA)	N/F	107.05053 mz	N/F	N/F	ppb	N/F
19	Benzoic acid	8,98	121.02940 mz	14293720	58,84	ppb	0,01
20	Epigallocatechin gallate	N/F	457.07763 mz	N/F	N/F	ppb	N/F
21	3-hydroxybenzoic acid (3-HBA)	7,24	137.02442 mz	4735203	3,985	ppb	0,08
22	Vanillic acid	7,14	167.03498 mz	1889307	24,211	ppb	-0,07
23	Homovanillic acid((4-Hydroxy-3-methoxyphenylacetic acid)	N/F	181.05063 mz	N/F	N/F	ppb	N/F
24	Caffeic acid	7,26	179.03498 mz	6998307523	1309,495	ppb	0,02
25	2,4-dihydroxybenzoic acid (beta-Resorcylic acid)	7,40	153.01933 mz	3270321	3,934	ppb	0,15
26	Quercetin 3-rutinoside 7-glucoside	N/F	771.20374 mz	N/F	N/F	ppb	N/F
27	Syringic acid	N/F	197.04555 mz	N/F	N/F	ppb	N/F
28	3-(4-Hydroxyphenyl) propionic acid	7,64	165.05572 mz	5553067	266,539	ppb	-0,09
29	Vanillin	7,65	151.04007 mz	3665104	2,293	ppb	-0,11
30	Vicenin 2	N/F	593.15119 mz	N/F	N/F	ppb	N/F
31	Ethylgallate	N/F	197.04555 mz	N/F	N/F	ppb	N/F
32	Daidzin	N/F	255.06433 mz	N/F	N/F	ppb	N/F

33	Luteolin 7-rutinoside	N/F	595.16376 mz	N/F	N/F	ppb	N/F
34	Rutin trihydrate M-3H2O	N/F	609.14611 mz	N/F	N/F	ppb	N/F
35	Schaftoside	8,36	563.14063 mz	202903	5,175	ppb	0,04
36	Eriocitrin	N/F	595.16684 mz	N/F	N/F	ppb	N/F
37	Coumaric acid (trans-3-Hydroxycinnamic acid)	8,31	163.04007 mz	910510266	231,047	ppb	-0,04
38	Luteoloside (Luteolin 7-glucoside)	N/F	449.10784 mz	N/F	N/F	ppb	N/F
39	Orientin	8,41	447.09328 mz	2328379	1,437	ppb	0,00
40	Isoorientin	8,41	447.09328 mz	2328379	1,437	ppb	0,00
41	Liquiritin (4′,7-Dihydroxyflavanone 4′-glucoside)	8,62	417.11911 mz	5532401	5,21	ppb	0,06
42	Sinapic acid	8,43	223.06120 mz	143594	-2,483	ppb	-0,16
43	Ferulic acid	8,55	193.05063 mz	82223640	44,191	ppb	-0,07
44	Vitexin (Apigenin 8-C-glucoside)	N/F	431.09837 mz	N/F	N/F	ppb	N/F
45	Narirutin (Narirutinsa, Naringenin rutinoside)	N/F	579.17193 mz	N/F	N/F	ppb	N/F
46	Doxorubicin Hydrchloride	N/F	544.18134 mz	N/F	N/F	ppb	N/F
47	Naringin	9,04	609.18390 mz	343895	3,919	ppb	-0,09
48	Hesperidin	9,04	609.18249 mz	343895	4,61	ppb	-0,09
49	α-Cyano-4-hydroxycinnamic acid	N/F	188.03532 mz	N/F	N/F	ppb	N/F
50	Rutin hydrate M-OH2	9,23	609.14611 mz	236776	5,37	ppb	0,02
51	Isoquercitrin (Quercetin 3-glucoside)	9,26	463.08820 mz	210395	-1,041	ppb	0,03
52	Hyperoside (Quercetin 3-D-galactoside)	9,26	463.08820 mz	210395	-1,041	ppb	0,03
53	Eriodictyol (3,4,5,7-Tetrahydroxyflavanone)	9,13	287.05501 mz	11659114	4,343	ppb	-0,10
54	Neohesperidin	9,04	609.18249 mz	343895	5,474	ppb	-0,25
55	Kaempferitrin	N/F	579.17083 mz	N/F	N/F	ppb	N/F
56	Protocatechuic acid ethyl ester (Ethyl 3,4-Dihydroxybenzoate)	9,16	181.05063 mz	12721820	-1,143	ppb	-0,19
57	Ellagic acid	N/F	300.99899 mz	N/F	N/F	ppb	N/F
58	Apiin (Apigenin-7-(2-O-apiosylglucoside)	N/F	565.15179 mz	N/F	N/F	ppb	N/F
59	Phloridzin	N/F	435.12967 mz	N/F	N/F	ppb	N/F
60	Myricetin	N/F	319.04291 mz	N/F	N/F	ppb	N/F
61	Luteolin-7-O-glucuronide (Luteolin-7-O-β-D-glucuronide)	N/F	461.07255 mz	N/F	N/F	ppb	N/F
62	Nicotiflorin (Kaempferol 3-rutinoside, Kaempferol 3-O-β –rutinoside)	9,62	593.15106 mz	110772	0,204	ppb	0,11
63	Rhoifolin (Apigenin 7-O- neohesperidoside)	N/F	577.15692 mz	N/F	N/F	ppb	N/F
64	Rosmarinic acid	9,40	359.07724 mz	605828	0,583	ppb	-0,14
65	Apigenin 7-glucoside	9,52	431.09837 mz	3785734	0,925	ppb	-0,04
66	Astragalin (Kaempferol 3-glucoside)	N/F	447.09328 mz	N/F	N/F	ppb	N/F
67	Kuromanine (Cyanidin 3-glucoside chloride)	N/F	447.09328 mz	N/F	N/F	ppb	N/F

68	Salicylic acid	9,92	137.02442 mz	42709742	3,419	ppb	0,16
69	Narcissin (Narcissoside, Isorhamnetin 3-rutinoside)	N/F	623.16176 mz	N/F	N/F	ppb	N/F
70	Leucoside (Kaempferol 3-sambubioside)	9,80	289.06924 mz	13133054	-5,073	ppb	-0,11
71	Liquiritigenin	N/F	255.06628 mz	N/F	N/F	ppb	N/F
72	Fisetin hydrate	9,81	285.04046 mz	119758	1,391	ppb	-0,17
73	Etoposide	10,15	383.11053 mz	215022	1,343	ppb	0,15
74	Afzelin (Kaempferol 3-rhamnoside)	10,12	431.09837 mz	4262159	3,496	ppb	0,06
75	Apigenin 7-glucuronide	10,38	445.07763 mz	161146	0,724	ppb	0,21
76	Trans Cinnamic acid	N/F	147.04515 mz	N/F	N/F	ppb	N/F
77	Kaempferol	N/F	287.05350 mz	N/F	N/F	ppb	N/F
78	Quercetin	10,38	301.03538 mz	1806470	1,403	ppb	0,04
79	Naringenin	10,50	271.06120 mz	3188878850	936,082	ppb	0,15
80	Tiliroside	N/F	595.14264 mz	N/F	N/F	ppb	N/F
81	Luteolin	10,75	285.04046 mz	4689023	1,08	ppb	-0,03
82	Genistein (5,7-Dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one)	10,76	269.04555 mz	806754	-4,759	ppb	-0,05
83	Apigenin (5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one)	11,28	269.04555 mz	50302437	-60,215	ppb	0,24
84	Isorhamnetin (Quercetin 3′-methyl ether)	11,20	315.05103 mz	4951122	8,992	ppb	-0,11
85	Diosmetin (Luteolin 4′-methyl ether)	11,40	299.05611 mz	44744651	36,636	ppb	0,05
86	Formononetin (Neochanin)	11,42	267.06628 mz	411483	-0,558	ppb	-0,10
87	Sakuranetin (Naringenin 7-O-methyl ether)	11,70	285.07685 mz	166500893	91,57	ppb	0,02
88	Caffeic acid phenhyl ester (CAPE)	11,99	283.09758 mz	132474762	-125,309	ppb	-0,15
89	Chrysin (5,7-Dihydroxy-2-phenyl-4H-chromen-4-one)	12,16	253.05063 mz	315230381	-23,127	ppb	-0,12
90	Galangin (3,5,7-Trihydroxy-2-phenyl-4H-chromen-4-one)	12,30	269.04555 mz	150898749	73,538	ppb	-0,12
91	Acacetin (5,7-Dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one)	12,39	283.06120 mz	140179885	8,676	ppb	-0,13
92	Genkwanin(4’,5-Dihydroxy-7-metthoxyflavone, Apigenin 7-O-methyl ether)	12,39	283.06120 mz	140179885	9,158	ppb	-0,13
93	Kaempferide	N/F	299.05611 mz	N/F	N/F	ppb	N/F
94	Glabridin	N/F	323.12888 mz	N/F	N/F	ppb	N/F
95	Emodin	13,55	269.04555 mz	261943	-7,623	ppb	-0,06

Table S.3. The phenolic profiles of ethanol extract of Mosul propolis

N	Compound Name	RT	Quan Peak	Response	Conc	Units	Dev (min)
1	C vitamini (L-ascorbic acid)	N/F	175.02481 mz	N/F	N/F	ppb	N/F
2	Capsaicin	N/F	306.20637 mz	N/F	N/F		N/F
3	Gallic acid(3,4,5-trihydroxybenzoic acid)	0,79	169.01425 mz	1214043	14,924	ppb	0,10
4	Arbutin	N/F	271.08233 mz	N/F	N/F	ppb	N/F
5	Taxifolin M+3H	N/F	162.02118 mz	N/F	N/F	ppb	N/F
6	Quinic acid	1,08	191.05611 mz	97216852	127,645	ppb	-0,15
7	Protocatechuic acid (3,4-Dihydroxybenzoic acid)	4,33	153.01933 mz	16571484	18,837	ppb	0,09
8	3,4-Dihydroxyphenylacetic acid(DOPAC, Homoprotocatechuic acid)	7,12	167.03498 mz	1145144	18,904	ppb	-0,09
9	3,4-dihydroxybenzaldehyde (Protocatechuic aldehyde)	5,66	137.02442 mz	248088866	61,198	ppb	-0,05
10	Esculin hydrate	N/F	339.07216 mz	N/F	N/F	ppb	N/F
11	4-Hydroxybenzoic acid	6,19	137.02442 mz	46800502	47,069	ppb	-0,08
12	Epigallocatechin	N/F	305.06668 mz	N/F	N/F	ppb	N/F
13	Catechin (Cianidanol)-p	N/F	289.07176 mz	N/F	N/F	ppb	N/F
14	Gentisic acid	6,45	153.01933 mz	1655347	2,71	ppb	-0,07
15	Taxifolin	N/F	305.06558 mz	N/F	N/F	ppb	N/F
16	Procyanidin B2	N/F	577.13515 mz	N/F	N/F	ppb	N/F
17	Chlorogenic acid	7,04	353.08781 mz	6504231	10,775	ppb	0,07
18	3-hydroxyphenylacetic acid (3-HPA)	N/F	107.05053 mz	N/F	N/F	ppb	N/F
19	Benzoic acid	8,92	121.02940 mz	14657169	60,75	ppb	-0,05
20	Epigallocatechin gallate	N/F	457.07763 mz	N/F	N/F	ppb	N/F
21	3-hydroxybenzoic acid (3-HBA)	7,21	137.02442 mz	2379951	1,811	ppb	0,05
22	Vanillic acid	7,12	167.03498 mz	1145144	18,153	ppb	-0,09
23	Homovanillic acid((4-Hydroxy-3-methoxyphenylacetic acid)	N/F	181.05063 mz	N/F	N/F	ppb	N/F
24	Caffeic acid	7,21	179.03498 mz	3665560824	705,473	ppb	-0,02
25	2,4-dihydroxybenzoic acid (beta-Resorcylic acid)	7,30	153.01933 mz	1775475	3,371	ppb	0,05
26	Quercetin 3-rutinoside 7-glucoside	N/F	771.20374 mz	N/F	N/F	ppb	N/F
27	Syringic acid	N/F	197.04555 mz	N/F	N/F	ppb	N/F
28	3-(4-Hydroxyphenyl) propionic acid	7,83	165.05572 mz	105951	79,121	ppb	0,10
29	Vanillin	7,59	151.04007 mz	13156617	11,276	ppb	-0,17
30	Vicenin 2	N/F	593.15119 mz	N/F	N/F	ppb	N/F
31	Ethylgallate	N/F	197.04555 mz	N/F	N/F	ppb	N/F
32	Daidzin	N/F	255.06433 mz	N/F	N/F	ppb	N/F

33	Luteolin 7-rutinoside	9,12	595.16376 mz	605426	2,711	ppb	0,13
34	Rutin trihydrate M-3H2O	N/F	609.14611 mz	N/F	N/F	ppb	N/F
35	Schaftoside	8,35	563.14063 mz	5576159	18,421	ppb	0,03
36	Eriocitrin	N/F	595.16684 mz	N/F	N/F	ppb	N/F
37	Coumaric acid (trans-3-Hydroxycinnamic acid)	8,26	163.04007 mz	1353929694	389,408	ppb	-0,09
38	Luteoloside (Luteolin 7-glucoside)	N/F	449.10784 mz	N/F	N/F	ppb	N/F
39	Orientin	N/F	447.09328 mz	N/F	N/F	ppb	N/F
40	Isoorientin	N/F	447.09328 mz	N/F	N/F	ppb	N/F
41	Liquiritin (4′,7-Dihydroxyflavanone 4′-glucoside)	8,61	417.11911 mz	22929284	22,033	ppb	0,05
42	Sinapic acid	N/F	223.06120 mz	N/F	N/F	ppb	N/F
43	Ferulic acid	8,52	193.05063 mz	211828180	118,21	ppb	-0,10
44	Vitexin (Apigenin 8-C-glucoside)	N/F	431.09837 mz	N/F	N/F	ppb	N/F
45	Narirutin (Narirutinsa, Naringenin rutinoside)	N/F	579.17193 mz	N/F	N/F	ppb	N/F
46	Doxorubicin Hydrchloride	N/F	544.18134 mz	N/F	N/F	ppb	N/F
47	Naringin	N/F	609.18390 mz	N/F	N/F	ppb	N/F
48	Hesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
49	α-Cyano-4-hydroxycinnamic acid	N/F	188.03532 mz	N/F	N/F	ppb	N/F
50	Rutin hydrate M-OH2	9,21	609.14611 mz	2651640	13,86	ppb	0,00
51	İsoquercitrin (Quercetin 3-glucoside)	9,23	463.08820 mz	5307395	1,55	ppb	0,00
52	Hyperoside (Quercetin 3-D-galactoside)	9,23	463.08820 mz	5307395	1,55	ppb	0,00
53	Eriodictyol (3,4,5,7-Tetrahydroxyflavanone)	9,29	287.05501 mz	1716730	1,481	ppb	0,06
54	Neohesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
55	Kaempferitrin	N/F	579.17083 mz	N/F	N/F	ppb	N/F
56	Protocatechuic acid ethyl ester (Ethyl 3,4-Dihydroxybenzoate)	9,13	181.05063 mz	1277370	-3,307	ppb	-0,22
57	Ellagic acid	9,44	300.99899 mz	5873043	49,829	ppb	-0,09
58	Apiin (Apigenin-7-(2-O-apiosylglucoside)	N/F	565.15179 mz	N/F	N/F	ppb	N/F
59	Phloridzin	N/F	435.12967 mz	N/F	N/F	ppb	N/F
60	Myricetin	9,38	319.04291 mz	306915	-0,163	ppb	-0,12
61	Luteolin-7-O-glucuronide (Luteolin-7-O-β-D-glucuronide)	N/F	461.07255 mz	N/F	N/F	ppb	N/F
62	Nicotiflorin (Kaempferol 3-rutinoside, Kaempferol 3-O-β –rutinoside)	9,71	593.15106 mz	5745477	11,437	ppb	0,20
63	Rhoifolin (Apigenin 7-O- neohesperidoside)	N/F	577.15692 mz	N/F	N/F	ppb	N/F
64	Rosmarinic acid	N/F	359.07724 mz	N/F	N/F	ppb	N/F
65	Apigenin 7-glucoside	9,54	431.09837 mz	6262956	2,982	ppb	-0,02
66	Astragalin (Kaempferol 3-glucoside)	9,66	447.09328 mz	8020682	1,068	ppb	0,10
67	Kuromanine (Cyanidin 3-glucoside chloride)	9,66	447.09328 mz	8020682	1,068	ppb	0,10

68	Salicylic acid	9,85	137.02442 mz	14062285	-2,235	ppb	0,09
69	Narcissin (Narcissoside, Isorhamnetin 3-rutinoside)	9,78	623.16176 mz	411252	2,496	ppb	-0,03
70	Leucoside (Kaempferol 3-sambubioside)	9,77	289.06924 mz	136245621	56,501	ppb	-0,14
71	Liquiritigenin	10,13	255.06628 mz	14681182	1,423	ppb	0,19
72	Fisetin hydrate	9,75	285.04046 mz	4337264	3,573	ppb	-0,24
73	Etoposide	N/F	383.11053 mz	N/F	N/F	ppb	N/F
74	Afzelin (Kaempferol 3-rhamnoside)	N/F	431.09837 mz	N/F	N/F	ppb	N/F
75	Apigenin 7-glucuronide	N/F	445.07763 mz	N/F	N/F	ppb	N/F
76	Trans Cinnamic acid	10,25	147.04515 mz	527383	47,176	ppb	0,01
77	Kaempferol	N/F	287.05350 mz	N/F	N/F	ppb	N/F
78	Quercetin	10,44	301.03538 mz	756347494	361,968	ppb	0,10
79	Naringenin	10,49	271.06120 mz	8958722559	2633,49	ppb	0,14
80	Tiliroside	10,90	595.14264 mz	602996	-3,131	ppb	0,12
81	Luteolin	10,71	285.04046 mz	446978114	192,961	ppb	-0,07
82	Genistein (5,7-Dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one)	10,70	269.04555 mz	11651262	-1,923	ppb	-0,12
83	Apigenin (5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one)	11,25	269.04555 mz	2685875550	936,278	ppb	0,20
84	Isorhamnetin (Quercetin 3′-methyl ether)	11,20	315.05103 mz	914919474	1129,778	ppb	-0,11
85	Diosmetin (Luteolin 4′-methyl ether)	11,39	299.05611 mz	2167911717	1909,592	ppb	0,04
86	Formononetin (Neochanin)	11,39	267.06628 mz	96249172	19,783	ppb	-0,14
87	Sakuranetin (Naringenin 7-O-methyl ether)	11,85	285.07685 mz	704514130	389,347	ppb	0,18
88	Caffeic acid phenhyl ester (CAPE)	11,97	283.09758 mz	7256704513	1085,365	ppb	-0,17
89	Chrysin (5,7-Dihydroxy-2-phenyl-4H-chromen-4-one)	12,15	253.05063 mz	10737250354	3767,023	ppb	-0,13
90	Galangin (3,5,7-Trihydroxy-2-phenyl-4H-chromen-4-one)	12,29	269.04555 mz	10362608041	4981,612	ppb	-0,13
91	Acacetin (5,7-Dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one)	12,39	283.06120 mz	3501268684	294,798	ppb	-0,13
92	Genkwanin(4’,5-Dihydroxy-7-metthoxyflavone, Apigenin 7-O-methyl ether)	12,39	283.06120 mz	3501268684	296,709	ppb	-0,13
93	Kaempferide	N/F	299.05611 mz	N/F	N/F	ppb	N/F
94	Glabridin	12,84	323.12888 mz	9126035	3,712	ppb	-0,02
95	Emodin	N/F	269.04555 mz	N/F	N/F	ppb	N/F

Table S.4. The phenolic profiles of water extract of Mosul propolis

N	Compound Name	RT	Quan Peak	Response	Conc	Units	Dev (min)
1	C vitamini (L-ascorbic acid)	N/F	175.02481 mz	N/F	N/F	ppb	N/F
2	Capsaicin	N/F	306.20637 mz	N/F	N/F		N/F
3	Gallic acid(3,4,5-trihydroxybenzoic acid)	0,79	169.01425 mz	381051	8,67	ppb	0,10
4	Arbutin	N/F	271.08233 mz	N/F	N/F	ppb	N/F
5	Taxifolin M+3H	N/F	162.02118 mz	N/F	N/F	ppb	N/F
6	Quinic acid	1,21	191.05611 mz	105911676	139,072	ppb	-0,03
7	Protocatechuic acid (3,4-Dihydroxybenzoic acid)	4,31	153.01933 mz	69805499	63,815	ppb	0,07
8	3,4-Dihydroxyphenylacetic acid(DOPAC, Homoprotocatechuic acid)	7,07	167.03498 mz	5217876	52,102	ppb	-0,14
9	3,4-dihydroxybenzaldehyde (Protocatechuic aldehyde)	5,62	137.02442 mz	227242373	55,985	ppb	-0,09
10	Esculin hydrate	N/F	339.07216 mz	N/F	N/F	ppb	N/F
11	4-Hydroxybenzoic acid	6,15	137.02442 mz	177780654	181,51	ppb	-0,12
12	Epigallocatechin	N/F	305.06668 mz	N/F	N/F	ppb	N/F
13	Catechin (Cianidanol)-p	N/F	289.07176 mz	N/F	N/F	ppb	N/F
14	Gentisic acid	6,46	153.01933 mz	4797651	4,358	ppb	-0,06
15	Taxifolin	N/F	305.06558 mz	N/F	N/F	ppb	N/F
16	Procyanidin B2	N/F	577.13515 mz	N/F	N/F	ppb	N/F
17	Chlorogenic acid	7,10	353.08781 mz	31440115	49,537	ppb	0,13
18	3-hydroxyphenylacetic acid (3-HPA)	7,10	107.05053 mz	111182	10,974	ppb	0,11
19	Benzoic acid	8,95	121.02940 mz	21331521	95,828	ppb	-0,02
20	Epigallocatechin gallate	N/F	457.07763 mz	N/F	N/F	ppb	N/F
21	3-hydroxybenzoic acid (3-HBA)	7,38	137.02442 mz	20034715	18,106	ppb	0,22
22	Vanillic acid	7,07	167.03498 mz	5217876	51,31	ppb	-0,14
23	Homovanillic acid((4-Hydroxy-3-methoxyphenylacetic acid)	N/F	181.05063 mz	N/F	N/F	ppb	N/F
24	Caffeic acid	7,19	179.03498 mz	12355947626	2280,506	ppb	-0,04
25	2,4-dihydroxybenzoic acid (beta-Resorcylic acid)	7,31	153.01933 mz	6168259	5,025	ppb	0,06
26	Quercetin 3-rutinoside 7-glucoside	N/F	771.20374 mz	N/F	N/F	ppb	N/F
27	Syringic acid	7,48	197.04555 mz	116242	0,583	ppb	-0,11
28	3-(4-Hydroxyphenyl) propionic acid	7,57	165.05572 mz	5983329	281,343	ppb	-0,16
29	Vanillin	7,59	151.04007 mz	8014121	6,409	ppb	-0,17
30	Vicenin 2	7,81	593.15119 mz	347369	8,446	ppb	-0,01
31	Ethylgallate	7,83	197.04555 mz	34353048	8,524	ppb	-0,18
32	Daidzin	7,93	255.06433 mz	812803	2,871	ppb	-0,10

33	Luteolin 7-rutinoside	N/F	595.16376 mz	N/F	N/F	ppb	N/F
34	Rutin trihydrate M-3H2O	N/F	609.14611 mz	N/F	N/F	ppb	N/F
35	Schaftoside	8,35	563.14063 mz	16226120	44,675	ppb	0,03
36	Eriocitrin	N/F	595.16684 mz	N/F	N/F	ppb	N/F
37	Coumaric acid (trans-3-Hydroxycinnamic acid)	8,26	163.04007 mz	1344030595	385,873	ppb	-0,09
38	Luteoloside (Luteolin 7-glucoside)	N/F	449.10784 mz	N/F	N/F	ppb	N/F
39	Orientin	8,33	447.09328 mz	635381	0,452	ppb	-0,08
40	Isoorientin	8,33	447.09328 mz	635381	0,452	ppb	-0,08
41	Liquiritin (4′,7-Dihydroxyflavanone 4′-glucoside)	8,61	417.11911 mz	702507	0,54	ppb	0,05
42	Sinapic acid	8,54	223.06120 mz	169467	-2,455	ppb	-0,05
43	Ferulic acid	8,54	193.05063 mz	255039344	142,888	ppb	-0,08
44	Vitexin (Apigenin 8-C-glucoside)	8,83	431.09837 mz	166994	3,855	ppb	0,12
45	Narirutin (Narirutinsa, Naringenin rutinoside)	N/F	579.17193 mz	N/F	N/F	ppb	N/F
46	Doxorubicin Hydrchloride	8,89	544.18134 mz	414433	10,661	ppb	-0,14
47	Naringin	N/F	609.18390 mz	N/F	N/F	ppb	N/F
48	Hesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
49	α-Cyano-4-hydroxycinnamic acid	N/F	188.03532 mz	N/F	N/F	ppb	N/F
50	Rutin hydrate M-OH2	N/F	609.14611 mz	N/F	N/F	ppb	N/F
51	İsoquercitrin (Quercetin 3-glucoside)	9,02	463.08820 mz	141465	-1,076	ppb	-0,21
52	Hyperoside (Quercetin 3-D-galactoside)	9,02	463.08820 mz	141465	-1,076	ppb	-0,21
53	Eriodictyol (3,4,5,7-Tetrahydroxyflavanone)	9,26	287.05501 mz	4229931	2,205	ppb	0,03
54	Neohesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
55	Kaempferitrin	N/F	579.17083 mz	N/F	N/F	ppb	N/F
56	Protocatechuic acid ethyl ester (Ethyl 3,4-Dihydroxybenzoate)	9,14	181.05063 mz	15666207	-0,586	ppb	-0,21
57	Ellagic acid	9,43	300.99899 mz	1404226	39,727	ppb	-0,09
58	Apiin (Apigenin-7-(2-O-apiosylglucoside)	N/F	565.15179 mz	N/F	N/F	ppb	N/F
59	Phloridzin	N/F	435.12967 mz	N/F	N/F	ppb	N/F
60	Myricetin	N/F	319.04291 mz	N/F	N/F	ppb	N/F
61	Luteolin-7-O-glucuronide (Luteolin-7-O-β-D-glucuronide)	N/F	461.07255 mz	N/F	N/F	ppb	N/F
62	Nicotiflorin (Kaempferol 3-rutinoside, Kaempferol 3-O-β –rutinoside)	9,28	593.15106 mz	765501	1,509	ppb	-0,23
63	Rhoifolin (Apigenin 7-O- neohesperidoside)	N/F	577.15692 mz	N/F	N/F	ppb	N/F
64	Rosmarinic acid	9,78	359.07724 mz	4830223	5,903	ppb	0,24
65	Apigenin 7-glucoside	9,61	431.09837 mz	308913	-1,962	ppb	0,05
66	Astragalin (Kaempferol 3-glucoside)	9,73	447.09328 mz	2393864	-1,837	ppb	0,17
67	Kuromanine (Cyanidin 3-glucoside chloride)	9,73	447.09328 mz	2393864	-1,837	ppb	0,17

68	Salicylic acid	9,88	137.02442 mz	22320563	-0,605	ppb	0,12
69	Narcissin (Narcissoside, Isorhamnetin 3-rutinoside)	N/F	623.16176 mz	N/F	N/F	ppb	N/F
70	Leucoside (Kaempferol 3-sambubioside)	9,79	289.06924 mz	21863523	-0,707	ppb	-0,12
71	Liquiritigenin	10,14	255.06628 mz	742430	-3,345	ppb	0,20
72	Fisetin hydrate	9,80	285.04046 mz	307541	1,489	ppb	-0,19
73	Etoposide	N/F	383.11053 mz	N/F	N/F	ppb	N/F
74	Afzelin (Kaempferol 3-rhamnoside)	10,11	431.09837 mz	216469	0,448	ppb	0,05
75	Apigenin 7-glucuronide	N/F	445.07763 mz	N/F	N/F	ppb	N/F
76	Trans Cinnamic acid	10,26	147.04515 mz	250031	42,426	ppb	0,03
77	Kaempferol	N/F	287.05350 mz	N/F	N/F	ppb	N/F
78	Quercetin	10,45	301.03538 mz	1675174	1,34	ppb	0,11
79	Naringenin	10,51	271.06120 mz	6464317148	1899,67	ppb	0,16
80	Tiliroside	N/F	595.14264 mz	N/F	N/F	ppb	N/F
81	Luteolin	10,75	285.04046 mz	4444028	0,974	ppb	-0,03
82	Genistein (5,7-Dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one)	10,71	269.04555 mz	113570	-4,94	ppb	-0,10
83	Apigenin (5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one)	11,26	269.04555 mz	47569894	-61,248	ppb	0,22
84	Isorhamnetin (Quercetin 3′-methyl ether)	11,20	315.05103 mz	6443576	10,83	ppb	-0,11
85	Diosmetin (Luteolin 4′-methyl ether)	11,39	299.05611 mz	64426486	53,999	ppb	0,04
86	Formononetin (Neochanin)	11,40	267.06628 mz	19780595	3,553	ppb	-0,12
87	Sakuranetin (Naringenin 7-O-methyl ether)	11,84	285.07685 mz	249533660	137,526	ppb	0,16
88	Caffeic acid phenhyl ester (CAPE)	11,97	283.09758 mz	39236796	-141,154	ppb	-0,17
89	Chrysin (5,7-Dihydroxy-2-phenyl-4H-chromen-4-one)	12,16	253.05063 mz	579249895	72,888	ppb	-0,12
90	Galangin (3,5,7-Trihydroxy-2-phenyl-4H-chromen-4-one)	12,30	269.04555 mz	182633443	88,791	ppb	-0,12
91	Acacetin (5,7-Dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one)	12,39	283.06120 mz	191402562	13,037	ppb	-0,13
92	Genkwanin(4’,5-Dihydroxy-7-metthoxyflavone, Apigenin 7-O-methyl ether)	12,39	283.06120 mz	191402562	13,541	ppb	-0,13
93	Kaempferide	N/F	299.05611 mz	N/F	N/F	ppb	N/F
94	Glabridin	N/F	323.12888 mz	N/F	N/F	ppb	N/F
95	Emodin	13,63	269.04555 mz	1079964	-7,527	ppb	0,02

Table S.5. The phenolic profiles of ethanol extract of Duhok propolis

N	Compound Name	RT	Quan Peak	Response	Conc	Units	Dev (min)
1	C vitamini (L-ascorbic acid)	N/F	175.02481 mz	N/F	N/F	ppb	N/F
2	Capsaicin	N/F	306.20637 mz	N/F	N/F		N/F
3	Gallic acid(3,4,5-trihydroxybenzoic acid)	0,79	169.01425 mz	22598818	175,462	ppb	0,10
4	Arbutin	0,81	271.08233 mz	6227254	96,983	ppb	0,00
5	Taxifolin M+3H	N/F	162.02118 mz	N/F	N/F	ppb	N/F
6	Quinic acid	1,07	191.05611 mz	41708810	54,695	ppb	-0,17
7	Protocatechuic acid (3,4-Dihydroxybenzoic acid)	4,33	153.01933 mz	13949905	16,622	ppb	0,09
8	3,4-Dihydroxyphenylacetic acid(DOPAC, Homoprotocatechuic acid)	7,29	167.03498 mz	576836	14,271	ppb	0,08
9	3,4-dihydroxybenzaldehyde (Protocatechuic aldehyde)	5,64	137.02442 mz	85287445	20,487	ppb	-0,07
10	Esculin hydrate	N/F	339.07216 mz	N/F	N/F	ppb	N/F
11	4-Hydroxybenzoic acid	6,17	137.02442 mz	27929627	27,7	ppb	-0,10
12	Epigallocatechin	N/F	305.06668 mz	N/F	N/F	ppb	N/F
13	Catechin (Cianidanol)-p	N/F	289.07176 mz	N/F	N/F	ppb	N/F
14	Gentisic acid	6,41	153.01933 mz	939823	2,335	ppb	-0,11
15	Taxifolin	N/F	305.06558 mz	N/F	N/F	ppb	N/F
16	Procyanidin B2	N/F	577.13515 mz	N/F	N/F	ppb	N/F
17	Chlorogenic acid	6,97	353.08781 mz	21928835	34,752	ppb	0,00
18	3-hydroxyphenylacetic acid (3-HPA)	N/F	107.05053 mz	N/F	N/F	ppb	N/F
19	Benzoic acid	8,94	121.02940 mz	9786070	35,149	ppb	-0,03
20	Epigallocatechin gallate	N/F	457.07763 mz	N/F	N/F	ppb	N/F
21	3-hydroxybenzoic acid (3-HBA)	7,24	137.02442 mz	1844778	1,317	ppb	0,08
22	Vanillic acid	7,29	167.03498 mz	576836	13,526	ppb	0,08
23	Homovanillic acid((4-Hydroxy-3-methoxyphenylacetic acid)	N/F	181.05063 mz	N/F	N/F	ppb	N/F
24	Caffeic acid	7,21	179.03498 mz	1736459058	355,846	ppb	-0,02
25	2,4-dihydroxybenzoic acid (beta-Resorcylic acid)	7,28	153.01933 mz	1356822	3,213	ppb	0,03
26	Quercetin 3-rutinoside 7-glucoside	N/F	771.20374 mz	N/F	N/F	ppb	N/F
27	Syringic acid	N/F	197.04555 mz	N/F	N/F	ppb	N/F
28	3-(4-Hydroxyphenyl) propionic acid	7,54	165.05572 mz	3206616	185,805	ppb	-0,19
29	Vanillin	N/F	151.04007 mz	N/F	N/F	ppb	N/F
30	Vicenin 2	N/F	593.15119 mz	N/F	N/F	ppb	N/F
31	Ethylgallate	7,85	197.04555 mz	39092594	9,647	ppb	-0,16
32	Daidzin	8,07	255.06433 mz	325850	2,694	ppb	0,04

33	Luteolin 7-rutinoside	N/F	595.16376 mz	N/F	N/F	ppb	N/F
34	Rutin trihydrate M-3H2O	N/F	609.14611 mz	N/F	N/F	ppb	N/F
35	Schaftoside	N/F	563.14063 mz	N/F	N/F	ppb	N/F
36	Eriocitrin	N/F	595.16684 mz	N/F	N/F	ppb	N/F
37	Coumaric acid (trans-3-Hydroxycinnamic acid)	8,26	163.04007 mz	601135103	120,558	ppb	-0,09
38	Luteoloside (Luteolin 7-glucoside)	N/F	449.10784 mz	N/F	N/F	ppb	N/F
39	Orientin	N/F	447.09328 mz	N/F	N/F	ppb	N/F
40	Isoorientin	N/F	447.09328 mz	N/F	N/F	ppb	N/F
41	Liquiritin (4′,7-Dihydroxyflavanone 4′-glucoside)	8,61	417.11911 mz	196677	0,051	ppb	0,05
42	Sinapic acid	N/F	223.06120 mz	N/F	N/F	ppb	N/F
43	Ferulic acid	8,52	193.05063 mz	104958254	57,175	ppb	-0,10
44	Vitexin (Apigenin 8-C-glucoside)	N/F	431.09837 mz	N/F	N/F	ppb	N/F
45	Narirutin (Narirutinsa, Naringenin rutinoside)	N/F	579.17193 mz	N/F	N/F	ppb	N/F
46	Doxorubicin Hydrchloride	N/F	544.18134 mz	N/F	N/F	ppb	N/F
47	Naringin	N/F	609.18390 mz	N/F	N/F	ppb	N/F
48	Hesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
49	α-Cyano-4-hydroxycinnamic acid	N/F	188.03532 mz	N/F	N/F	ppb	N/F
50	Rutin hydrate M-OH2	9,21	609.14611 mz	2932922	14,849	ppb	0,00
51	İsoquercitrin (Quercetin 3-glucoside)	9,23	463.08820 mz	17234534	7,612	ppb	0,00
52	Hyperoside (Quercetin 3-D-galactoside)	9,23	463.08820 mz	17234534	7,612	ppb	0,00
53	Eriodictyol (3,4,5,7-Tetrahydroxyflavanone)	9,24	287.05501 mz	4029879	2,147	ppb	0,01
54	Neohesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
55	Kaempferitrin	N/F	579.17083 mz	N/F	N/F	ppb	N/F
56	Protocatechuic acid ethyl ester (Ethyl 3,4-Dihydroxybenzoate)	9,13	181.05063 mz	558966	-3,443	ppb	-0,22
57	Ellagic acid	9,42	300.99899 mz	9035907	56,979	ppb	-0,10
58	Apiin (Apigenin-7-(2-O-apiosylglucoside)	N/F	565.15179 mz	N/F	N/F	ppb	N/F
59	Phloridzin	N/F	435.12967 mz	N/F	N/F	ppb	N/F
60	Myricetin	N/F	319.04291 mz	N/F	N/F	ppb	N/F
61	Luteolin-7-O-glucuronide (Luteolin-7-O-β-D-glucuronide)	N/F	461.07255 mz	N/F	N/F	ppb	N/F
62	Nicotiflorin (Kaempferol 3-rutinoside, Kaempferol 3-O-β –rutinoside)	9,71	593.15106 mz	1311619	2,598	ppb	0,20
63	Rhoifolin (Apigenin 7-O- neohesperidoside)	N/F	577.15692 mz	N/F	N/F	ppb	N/F
64	Rosmarinic acid	N/F	359.07724 mz	N/F	N/F	ppb	N/F
65	Apigenin 7-glucoside	9,56	431.09837 mz	658690	-1,672	ppb	0,00
66	Astragalin (Kaempferol 3-glucoside)	9,71	447.09328 mz	25953088	10,325	ppb	0,15
67	Kuromanine (Cyanidin 3-glucoside chloride)	9,71	447.09328 mz	25953088	10,325	ppb	0,15

68	Salicylic acid	9,85	137.02442 mz	22418742	-0,585	ppb	0,09
69	Narcissin (Narcissoside, Isorhamnetin 3-rutinoside)	9,80	623.16176 mz	579073	2,965	ppb	-0,01
70	Leucoside (Kaempferol 3-sambubioside)	9,77	289.06924 mz	74156164	25,447	ppb	-0,14
71	Liquiritigenin	10,13	255.06628 mz	26848059	5,585	ppb	0,19
72	Fisetin hydrate	9,76	285.04046 mz	2063256	2,397	ppb	-0,22
73	Etoposide	N/F	383.11053 mz	N/F	N/F	ppb	N/F
74	Afzelin (Kaempferol 3-rhamnoside)	N/F	431.09837 mz	N/F	N/F	ppb	N/F
75	Apigenin 7-glucuronide	N/F	445.07763 mz	N/F	N/F	ppb	N/F
76	Trans Cinnamic acid	N/F	147.04515 mz	N/F	N/F	ppb	N/F
77	Kaempferol	10,29	287.05350 mz	8124155	1,069	ppb	-0,02
78	Quercetin	10,44	301.03538 mz	255117684	122,45	ppb	0,10
79	Naringenin	10,49	271.06120 mz	8078419072	2374,517	ppb	0,14
80	Tiliroside	10,57	595.14264 mz	271261	-3,611	ppb	-0,21
81	Luteolin	10,71	285.04046 mz	171972349	73,654	ppb	-0,07
82	Genistein (5,7-Dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one)	N/F	269.04555 mz	N/F	N/F	ppb	N/F
83	Apigenin (5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one)	11,25	269.04555 mz	2614913546	909,448	ppb	0,20
84	Isorhamnetin (Quercetin 3′-methyl ether)	11,18	315.05103 mz	278642581	346,091	ppb	-0,12
85	Diosmetin (Luteolin 4′-methyl ether)	11,37	299.05611 mz	1844756043	1624,519	ppb	0,02
86	Formononetin (Neochanin)	11,39	267.06628 mz	109525460	22,6	ppb	-0,14
87	Sakuranetin (Naringenin 7-O-methyl ether)	11,87	285.07685 mz	411077380	226,937	ppb	0,19
88	Caffeic acid phenhyl ester (CAPE)	11,98	283.09758 mz	4790954072	666,342	ppb	-0,17
89	Chrysin (5,7-Dihydroxy-2-phenyl-4H-chromen-4-one)	12,15	253.05063 mz	10428746620	3654,83	ppb	-0,13
90	Galangin (3,5,7-Trihydroxy-2-phenyl-4H-chromen-4-one)	12,29	269.04555 mz	7780200530	3740,424	ppb	-0,13
91	Acacetin (5,7-Dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one)	12,39	283.06120 mz	3296341426	277,353	ppb	-0,13
92	Genkwanin(4’,5-Dihydroxy-7-metthoxyflavone, Apigenin 7-O-methyl ether)	12,39	283.06120 mz	3296341426	279,176	ppb	-0,13
93	Kaempferide	N/F	299.05611 mz	N/F	N/F	ppb	N/F
94	Glabridin	12,84	323.12888 mz	6238494	3,06	ppb	-0,02
95	Emodin	13,62	269.04555 mz	46763617	-2,215	ppb	0,01

Table S.6. The phenolic profiles of water extract of Duhok propolis

N	Compound Name	RT	Quan Peak	Response	Conc	Units	Dev (min)
1	C vitamini (L-ascorbic acid)	N/F	175.02481 mz	N/F	N/F	ppb	N/F
2	Capsaicin	N/F	306.20637 mz	N/F	N/F		N/F
3	Gallic acid(3,4,5-trihydroxybenzoic acid)	N/F	169.01425 mz	N/F	N/F	ppb	N/F
4	Arbutin	N/F	271.08233 mz	N/F	N/F	ppb	N/F
5	Taxifolin M+3H	N/F	162.02118 mz	N/F	N/F	ppb	N/F
6	Quinic acid	1,21	191.05611 mz	95487886	125,373	ppb	-0,03
7	Protocatechuic acid (3,4-Dihydroxybenzoic acid)	4,29	153.01933 mz	26137466	26,92	ppb	0,05
8	3,4-Dihydroxyphenylacetic acid(DOPAC, Homoprotocatechuic acid)	7,07	167.03498 mz	1487095	21,691	ppb	-0,14
9	3,4-dihydroxybenzaldehyde (Protocatechuic aldehyde)	5,62	137.02442 mz	71463557	17,03	ppb	-0,09
10	Esculin hydrate	N/F	339.07216 mz	N/F	N/F	ppb	N/F
11	4-Hydroxybenzoic acid	6,15	137.02442 mz	68010537	68,84	ppb	-0,12
12	Epigallocatechin	N/F	305.06668 mz	N/F	N/F	ppb	N/F
13	Catechin (Cianidanol)-p	6,39	289.07176 mz	443325	7,854	ppb	0,04
14	Gentisic acid	6,45	153.01933 mz	3377661	3,614	ppb	-0,07
15	Taxifolin	N/F	305.06558 mz	N/F	N/F	ppb	N/F
16	Procyanidin B2	N/F	577.13515 mz	N/F	N/F	ppb	N/F
17	Chlorogenic acid	7,07	353.08781 mz	22986822	36,397	ppb	0,10
18	3-hydroxyphenylacetic acid (3-HPA)	N/F	107.05053 mz	N/F	N/F	ppb	N/F
19	Benzoic acid	8,95	121.02940 mz	7619156	23,761	ppb	-0,02
20	Epigallocatechin gallate	N/F	457.07763 mz	N/F	N/F	ppb	N/F
21	3-hydroxybenzoic acid (3-HBA)	7,24	137.02442 mz	3676099	3,007	ppb	0,08
22	Vanillic acid	7,07	167.03498 mz	1487095	20,937	ppb	-0,14
23	Homovanillic acid((4-Hydroxy-3-methoxyphenylacetic acid)	7,36	181.05063 mz	118100	49,372	ppb	0,14
24	Caffeic acid	7,19	179.03498 mz	3099391116	602,862	ppb	-0,04
25	2,4-dihydroxybenzoic acid (beta-Resorcylic acid)	7,29	153.01933 mz	2444218	3,623	ppb	0,04
26	Quercetin 3-rutinoside 7-glucoside	N/F	771.20374 mz	N/F	N/F	ppb	N/F
27	Syringic acid	7,46	197.04555 mz	125180	0,585	ppb	-0,13
28	3-(4-Hydroxyphenyl) propionic acid	7,57	165.05572 mz	1511999	127,499	ppb	-0,16
29	Vanillin	7,60	151.04007 mz	2970702	1,636	ppb	-0,16
30	Vicenin 2	N/F	593.15119 mz	N/F	N/F	ppb	N/F
31	Ethylgallate	7,84	197.04555 mz	57482691	14,003	ppb	-0,17
32	Daidzin	N/F	255.06433 mz	N/F	N/F	ppb	N/F

33	Luteolin 7-rutinoside	N/F	595.16376 mz	N/F	N/F	ppb	N/F
34	Rutin trihydrate M-3H2O	N/F	609.14611 mz	N/F	N/F	ppb	N/F
35	Schaftoside	N/F	563.14063 mz	N/F	N/F	ppb	N/F
36	Eriocitrin	N/F	595.16684 mz	N/F	N/F	ppb	N/F
37	Coumaric acid (trans-3-Hydroxycinnamic acid)	8,28	163.04007 mz	337193193	26,295	ppb	-0,07
38	Luteoloside (Luteolin 7-glucoside)	N/F	449.10784 mz	N/F	N/F	ppb	N/F
39	Orientin	N/F	447.09328 mz	N/F	N/F	ppb	N/F
40	Isoorientin	N/F	447.09328 mz	N/F	N/F	ppb	N/F
41	Liquiritin (4′,7-Dihydroxyflavanone 4′-glucoside)	N/F	417.11911 mz	N/F	N/F	ppb	N/F
42	Sinapic acid	N/F	223.06120 mz	N/F	N/F	ppb	N/F
43	Ferulic acid	8,53	193.05063 mz	52064566	26,966	ppb	-0,09
44	Vitexin (Apigenin 8-C-glucoside)	N/F	431.09837 mz	N/F	N/F	ppb	N/F
45	Narirutin (Narirutinsa, Naringenin rutinoside)	N/F	579.17193 mz	N/F	N/F	ppb	N/F
46	Doxorubicin Hydrchloride	N/F	544.18134 mz	N/F	N/F	ppb	N/F
47	Naringin	N/F	609.18390 mz	N/F	N/F	ppb	N/F
48	Hesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
49	α-Cyano-4-hydroxycinnamic acid	N/F	188.03532 mz	N/F	N/F	ppb	N/F
50	Rutin hydrate M-OH2	N/F	609.14611 mz	N/F	N/F	ppb	N/F
51	İsoquercitrin (Quercetin 3-glucoside)	N/F	463.08820 mz	N/F	N/F	ppb	N/F
52	Hyperoside (Quercetin 3-D-galactoside)	N/F	463.08820 mz	N/F	N/F	ppb	N/F
53	Eriodictyol (3,4,5,7-Tetrahydroxyflavanone)	9,05	287.05501 mz	5045552	2,44	ppb	-0,18
54	Neohesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
55	Kaempferitrin	N/F	579.17083 mz	N/F	N/F	ppb	N/F
56	Protocatechuic acid ethyl ester (Ethyl 3,4-Dihydroxybenzoate)	9,14	181.05063 mz	8121638	-2,013	ppb	-0,21
57	Ellagic acid	9,43	300.99899 mz	2351814	41,869	ppb	-0,09
58	Apiin (Apigenin-7-(2-O-apiosylglucoside)	N/F	565.15179 mz	N/F	N/F	ppb	N/F
59	Phloridzin	N/F	435.12967 mz	N/F	N/F	ppb	N/F
60	Myricetin	N/F	319.04291 mz	N/F	N/F	ppb	N/F
61	Luteolin-7-O-glucuronide (Luteolin-7-O-β-D-glucuronide)	N/F	461.07255 mz	N/F	N/F	ppb	N/F
62	Nicotiflorin (Kaempferol 3-rutinoside, Kaempferol 3-O-β –rutinoside)	N/F	593.15106 mz	N/F	N/F	ppb	N/F
63	Rhoifolin (Apigenin 7-O- neohesperidoside)	9,64	577.15692 mz	143322	-2,041	ppb	0,10
64	Rosmarinic acid	9,67	359.07724 mz	137652	-0,007	ppb	0,13
65	Apigenin 7-glucoside	N/F	431.09837 mz	N/F	N/F	ppb	N/F
66	Astragalin (Kaempferol 3-glucoside)	9,76	447.09328 mz	121611	-3,01	ppb	0,20
67	Kuromanine (Cyanidin 3-glucoside chloride)	9,76	447.09328 mz	121611	-3,01	ppb	0,20

68	Salicylic acid	9,88	137.02442 mz	16056201	-1,841	ppb	0,12
69	Narcissin (Narcissoside, Isorhamnetin 3-rutinoside)	N/F	623.16176 mz	N/F	N/F	ppb	N/F
70	Leucoside (Kaempferol 3-sambubioside)	9,79	289.06924 mz	1948223	-10,668	ppb	-0,13
71	Liquiritigenin	10,14	255.06628 mz	1195027	-3,19	ppb	0,20
72	Fisetin hydrate	N/F	285.04046 mz	N/F	N/F	ppb	N/F
73	Etoposide	N/F	383.11053 mz	N/F	N/F	ppb	N/F
74	Afzelin (Kaempferol 3-rhamnoside)	N/F	431.09837 mz	N/F	N/F	ppb	N/F
75	Apigenin 7-glucuronide	N/F	445.07763 mz	N/F	N/F	ppb	N/F
76	Trans Cinnamic acid	N/F	147.04515 mz	N/F	N/F	ppb	N/F
77	Kaempferol	10,32	287.05350 mz	492199	-1,201	ppb	0,01
78	Quercetin	N/F	301.03538 mz	N/F	N/F	ppb	N/F
79	Naringenin	10,50	271.06120 mz	2243376792	657,929	ppb	0,15
80	Tiliroside	N/F	595.14264 mz	N/F	N/F	ppb	N/F
81	Luteolin	N/F	285.04046 mz	N/F	N/F	ppb	N/F
82	Genistein (5,7-Dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one)	10,73	269.04555 mz	141084	-4,933	ppb	-0,09
83	Apigenin (5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one)	11,26	269.04555 mz	13277099	-74,214	ppb	0,22
84	Isorhamnetin (Quercetin 3′-methyl ether)	N/F	315.05103 mz	N/F	N/F	ppb	N/F
85	Diosmetin (Luteolin 4′-methyl ether)	11,40	299.05611 mz	16949983	12,117	ppb	0,05
86	Formononetin (Neochanin)	11,40	267.06628 mz	8004734	1,054	ppb	-0,12
87	Sakuranetin (Naringenin 7-O-methyl ether)	11,83	285.07685 mz	39121715	21,069	ppb	0,16
88	Caffeic acid phenhyl ester (CAPE)	11,97	283.09758 mz	3019700	-147,308	ppb	-0,17
89	Chrysin (5,7-Dihydroxy-2-phenyl-4H-chromen-4-one)	12,16	253.05063 mz	287292351	-33,288	ppb	-0,12
90	Galangin (3,5,7-Trihydroxy-2-phenyl-4H-chromen-4-one)	12,30	269.04555 mz	52819668	26,398	ppb	-0,12
91	Acacetin (5,7-Dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one)	12,39	283.06120 mz	82861436	3,797	ppb	-0,13
92	Genkwanin(4’,5-Dihydroxy-7-metthoxyflavone, Apigenin 7-O-methyl ether)	12,39	283.06120 mz	82861436	4,255	ppb	-0,13
93	Kaempferide	N/F	299.05611 mz	N/F	N/F	ppb	N/F
94	Glabridin	N/F	323.12888 mz	N/F	N/F	ppb	N/F
95	Emodin	13,61	269.04555 mz	99589	-7,641	ppb	0,00

Table S.7. The phenolic profiles of ethanol extract of Sulaymaniyah propolis

N	Compound Name	RT	Quan Peak	Response	Conc	Units	Dev (min)
1	C vitamini (L-ascorbic acid)	N/F	175.02481 mz	N/F	N/F	ppb	N/F
2	Capsaicin	N/F	306.20637 mz	N/F	N/F		N/F
3	Gallic acid(3,4,5-trihydroxybenzoic acid)	0,79	169.01425 mz	4577885	40,176	ppb	0,10
4	Arbutin	0,84	271.08233 mz	7845197	120,421	ppb	0,04
5	Taxifolin M+3H	N/F	162.02118 mz	N/F	N/F	ppb	N/F
6	Quinic acid	1,08	191.05611 mz	66658457	87,485	ppb	-0,15
7	Protocatechuic acid (3,4-Dihydroxybenzoic acid)	4,29	153.01933 mz	16930531	19,141	ppb	0,05
8	3,4-Dihydroxyphenylacetic acid(DOPAC, Homoprotocatechuic acid)	7,12	167.03498 mz	1940749	25,389	ppb	-0,09
9	3,4-dihydroxybenzaldehyde (Protocatechuic aldehyde)	5,62	137.02442 mz	91873885	22,134	ppb	-0,09
10	Esculin hydrate	N/F	339.07216 mz	N/F	N/F	ppb	N/F
11	4-Hydroxybenzoic acid	6,16	137.02442 mz	20771681	20,353	ppb	-0,11
12	Epigallocatechin	N/F	305.06668 mz	N/F	N/F	ppb	N/F
13	Catechin (Cianidanol)-p	N/F	289.07176 mz	N/F	N/F	ppb	N/F
14	Gentisic acid	6,38	153.01933 mz	2113765	2,95	ppb	-0,14
15	Taxifolin	N/F	305.06558 mz	N/F	N/F	ppb	N/F
16	Procyanidin B2	N/F	577.13515 mz	N/F	N/F	ppb	N/F
17	Chlorogenic acid	6,97	353.08781 mz	71103323	111,192	ppb	0,00
18	3-hydroxyphenylacetic acid (3-HPA)	N/F	107.05053 mz	N/F	N/F	ppb	N/F
19	Benzoic acid	8,94	121.02940 mz	9473203	33,505	ppb	-0,03
20	Epigallocatechin gallate	N/F	457.07763 mz	N/F	N/F	ppb	N/F
21	3-hydroxybenzoic acid (3-HBA)	7,33	137.02442 mz	4568703	3,831	ppb	0,17
22	Vanillic acid	7,12	167.03498 mz	1940749	24,63	ppb	-0,09
23	Homovanillic acid((4-Hydroxy-3-methoxyphenylacetic acid)	N/F	181.05063 mz	N/F	N/F	ppb	N/F
24	Caffeic acid	7,19	179.03498 mz	2800510028	548,693	ppb	-0,04
25	2,4-dihydroxybenzoic acid (beta-Resorcylic acid)	7,26	153.01933 mz	5534758	4,786	ppb	0,01
26	Quercetin 3-rutinoside 7-glucoside	N/F	771.20374 mz	N/F	N/F	ppb	N/F
27	Syringic acid	7,52	197.04555 mz	122541	0,584	ppb	-0,07
28	3-(4-Hydroxyphenyl) propionic acid	7,57	165.05572 mz	6043569	283,415	ppb	-0,16
29	Vanillin	7,61	151.04007 mz	6831732	5,29	ppb	-0,15
30	Vicenin 2	N/F	593.15119 mz	N/F	N/F	ppb	N/F
31	Ethylgallate	7,85	197.04555 mz	126042	0,415	ppb	-0,16
32	Daidzin	8,07	255.06433 mz	118738	2,618	ppb	0,04

33	Luteolin 7-rutinoside	N/F	595.16376 mz	N/F	N/F	ppb	N/F
34	Rutin trihydrate M-3H2O	N/F	609.14611 mz	N/F	N/F	ppb	N/F
35	Schaftoside	8,54	563.14063 mz	34230603	89,059	ppb	0,22
36	Eriocitrin	N/F	595.16684 mz	N/F	N/F	ppb	N/F
37	Coumaric acid (trans-3-Hydroxycinnamic acid)	8,26	163.04007 mz	757775324	176,5	ppb	-0,09
38	Luteoloside (Luteolin 7-glucoside)	N/F	449.10784 mz	N/F	N/F	ppb	N/F
39	Orientin	N/F	447.09328 mz	N/F	N/F	ppb	N/F
40	Isoorientin	N/F	447.09328 mz	N/F	N/F	ppb	N/F
41	Liquiritin (4′,7-Dihydroxyflavanone 4′-glucoside)	N/F	417.11911 mz	N/F	N/F	ppb	N/F
42	Sinapic acid	8,35	223.06120 mz	132438	-2,495	ppb	-0,24
43	Ferulic acid	8,52	193.05063 mz	201409091	112,259	ppb	-0,10
44	Vitexin (Apigenin 8-C-glucoside)	N/F	431.09837 mz	N/F	N/F	ppb	N/F
45	Narirutin (Narirutinsa, Naringenin rutinoside)	N/F	579.17193 mz	N/F	N/F	ppb	N/F
46	Doxorubicin Hydrchloride	N/F	544.18134 mz	N/F	N/F	ppb	N/F
47	Naringin	N/F	609.18390 mz	N/F	N/F	ppb	N/F
48	Hesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
49	α-Cyano-4-hydroxycinnamic acid	N/F	188.03532 mz	N/F	N/F	ppb	N/F
50	Rutin hydrate M-OH2	9,19	609.14611 mz	4961989	21,983	ppb	-0,02
51	İsoquercitrin (Quercetin 3-glucoside)	9,25	463.08820 mz	20758758	9,403	ppb	0,02
52	Hyperoside (Quercetin 3-D-galactoside)	9,25	463.08820 mz	20758758	9,403	ppb	0,02
53	Eriodictyol (3,4,5,7-Tetrahydroxyflavanone)	9,08	287.05501 mz	18247141	6,239	ppb	-0,15
54	Neohesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
55	Kaempferitrin	N/F	579.17083 mz	N/F	N/F	ppb	N/F
56	Protocatechuic acid ethyl ester (Ethyl 3,4-Dihydroxybenzoate)	9,11	181.05063 mz	362152	-3,48	ppb	-0,24
57	Ellagic acid	9,44	300.99899 mz	3423754	44,292	ppb	-0,09
58	Apiin (Apigenin-7-(2-O-apiosylglucoside)	N/F	565.15179 mz	N/F	N/F	ppb	N/F
59	Phloridzin	N/F	435.12967 mz	N/F	N/F	ppb	N/F
60	Myricetin	9,65	319.04291 mz	14224357	11,352	ppb	0,15
61	Luteolin-7-O-glucuronide (Luteolin-7-O-β-D-glucuronide)	N/F	461.07255 mz	N/F	N/F	ppb	N/F
62	Nicotiflorin (Kaempferol 3-rutinoside, Kaempferol 3-O-β –rutinoside)	9,73	593.15106 mz	1354688	2,684	ppb	0,22
63	Rhoifolin (Apigenin 7-O- neohesperidoside)	N/F	577.15692 mz	N/F	N/F	ppb	N/F
64	Rosmarinic acid	N/F	359.07724 mz	N/F	N/F	ppb	N/F
65	Apigenin 7-glucoside	9,56	431.09837 mz	1959825	-0,591	ppb	0,00
66	Astragalin (Kaempferol 3-glucoside)	9,71	447.09328 mz	24890912	9,777	ppb	0,15
67	Kuromanine (Cyanidin 3-glucoside chloride)	9,71	447.09328 mz	24890912	9,777	ppb	0,15

68	Salicylic acid	9,85	137.02442 mz	33614776	1,624	ppb	0,09
69	Narcissin (Narcissoside, Isorhamnetin 3-rutinoside)	9,80	623.16176 mz	246910	2,036	ppb	-0,01
70	Leucoside (Kaempferol 3-sambubioside)	9,77	289.06924 mz	121089385	48,92	ppb	-0,14
71	Liquiritigenin	10,13	255.06628 mz	31381442	7,135	ppb	0,19
72	Fisetin hydrate	9,76	285.04046 mz	6854881	4,876	ppb	-0,22
73	Etoposide	10,10	383.11053 mz	253915	1,406	ppb	0,10
74	Afzelin (Kaempferol 3-rhamnoside)	N/F	431.09837 mz	N/F	N/F	ppb	N/F
75	Apigenin 7-glucuronide	N/F	445.07763 mz	N/F	N/F	ppb	N/F
76	Trans Cinnamic acid	N/F	147.04515 mz	N/F	N/F	ppb	N/F
77	Kaempferol	10,31	287.05350 mz	1797643	-0,812	ppb	0,00
78	Quercetin	10,44	301.03538 mz	455734847	218,317	ppb	0,10
79	Naringenin	10,49	271.06120 mz	7407940866	2177,271	ppb	0,14
80	Tiliroside	N/F	595.14264 mz	N/F	N/F	ppb	N/F
81	Luteolin	10,73	285.04046 mz	419086852	180,861	ppb	-0,05
82	Genistein (5,7-Dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one)	10,70	269.04555 mz	8732898	-2,686	ppb	-0,12
83	Apigenin (5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one)	11,27	269.04555 mz	2681720401	934,707	ppb	0,22
84	Isorhamnetin (Quercetin 3′-methyl ether)	11,22	315.05103 mz	835942247	1032,504	ppb	-0,09
85	Diosmetin (Luteolin 4′-methyl ether)	11,39	299.05611 mz	2740058551	2414,312	ppb	0,04
86	Formononetin (Neochanin)	11,41	267.06628 mz	237142114	49,685	ppb	-0,12
87	Sakuranetin (Naringenin 7-O-methyl ether)	11,87	285.07685 mz	658269498	363,751	ppb	0,19
88	Caffeic acid phenhyl ester (CAPE)	11,97	283.09758 mz	2076062315	204,979	ppb	-0,17
89	Chrysin (5,7-Dihydroxy-2-phenyl-4H-chromen-4-one)	12,16	253.05063 mz	12426982792	4381,523	ppb	-0,12
90	Galangin (3,5,7-Trihydroxy-2-phenyl-4H-chromen-4-one)	12,28	269.04555 mz	9485712286	4560,148	ppb	-0,14
91	Acacetin (5,7-Dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one)	12,39	283.06120 mz	4440426186	374,746	ppb	-0,13
92	Genkwanin(4’,5-Dihydroxy-7-metthoxyflavone, Apigenin 7-O-methyl ether)	12,39	283.06120 mz	4440426186	377,056	ppb	-0,13
93	Kaempferide	N/F	299.05611 mz	N/F	N/F	ppb	N/F
94	Glabridin	12,86	323.12888 mz	6230076	3,058	ppb	0,00
95	Emodin	13,64	269.04555 mz	58842257	-0,811	ppb	0,03

Table S.8. The phenolic profiles of water extract of Sulaymaniyah propolis

N	Compound Name	RT	Quan Peak	Response	Conc	Units	Dev (min)
1	C vitamini (L-ascorbic acid)	N/F	175.02481 mz	N/F	N/F	ppb	N/F
2	Capsaicin	N/F	306.20637 mz	N/F	N/F		N/F
3	Gallic acid(3,4,5-trihydroxybenzoic acid)	N/F	169.01425 mz	N/F	N/F	ppb	N/F
4	Arbutin	N/F	271.08233 mz	N/F	N/F	ppb	N/F
5	Taxifolin M+3H	N/F	162.02118 mz	N/F	N/F	ppb	N/F
6	Quinic acid	N/F	191.05611 mz	N/F	N/F	ppb	N/F
7	Protocatechuic acid (3,4-Dihydroxybenzoic acid)	N/F	153.01933 mz	N/F	N/F	ppb	N/F
8	3,4-Dihydroxyphenylacetic acid(DOPAC, Homoprotocatechuic acid)	N/F	167.03498 mz	N/F	N/F	ppb	N/F
9	3,4-dihydroxybenzaldehyde (Protocatechuic aldehyde)	5,65	137.02442 mz	2514735	-0,212	ppb	-0,06
10	Esculin hydrate	N/F	339.07216 mz	N/F	N/F	ppb	N/F
11	4-Hydroxybenzoic acid	6,46	137.02442 mz	438393	-0,518	ppb	0,19
12	Epigallocatechin	N/F	305.06668 mz	N/F	N/F	ppb	N/F
13	Catechin (Cianidanol)-p	N/F	289.07176 mz	N/F	N/F	ppb	N/F
14	Gentisic acid	N/F	153.01933 mz	N/F	N/F	ppb	N/F
15	Taxifolin	N/F	305.06558 mz	N/F	N/F	ppb	N/F
16	Procyanidin B2	N/F	577.13515 mz	N/F	N/F	ppb	N/F
17	Chlorogenic acid	N/F	353.08781 mz	N/F	N/F	ppb	N/F
18	3-hydroxyphenylacetic acid (3-HPA)	N/F	107.05053 mz	N/F	N/F	ppb	N/F
19	Benzoic acid	8,86	121.02940 mz	4520698	7,476	ppb	-0,11
20	Epigallocatechin gallate	N/F	457.07763 mz	N/F	N/F	ppb	N/F
21	3-hydroxybenzoic acid (3-HBA)	6,95	137.02442 mz	3186882	2,556	ppb	-0,21
22	Vanillic acid	N/F	167.03498 mz	N/F	N/F	ppb	N/F
23	Homovanillic acid((4-Hydroxy-3-methoxyphenylacetic acid)	N/F	181.05063 mz	N/F	N/F	ppb	N/F
24	Caffeic acid	7,24	179.03498 mz	242415	41,176	ppb	0,01
25	2,4-dihydroxybenzoic acid (beta-Resorcylic acid)	N/F	153.01933 mz	N/F	N/F	ppb	N/F
26	Quercetin 3-rutinoside 7-glucoside	N/F	771.20374 mz	N/F	N/F	ppb	N/F
27	Syringic acid	N/F	197.04555 mz	N/F	N/F	ppb	N/F
28	3-(4-Hydroxyphenyl) propionic acid	N/F	165.05572 mz	N/F	N/F	ppb	N/F
29	Vanillin	7,88	151.04007 mz	222218	-0,965	ppb	0,12
30	Vicenin 2	N/F	593.15119 mz	N/F	N/F	ppb	N/F
31	Ethylgallate	N/F	197.04555 mz	N/F	N/F	ppb	N/F
32	Daidzin	N/F	255.06433 mz	N/F	N/F	ppb	N/F

33	Luteolin 7-rutinoside	N/F	595.16376 mz	N/F	N/F	ppb	N/F
34	Rutin trihydrate M-3H2O	N/F	609.14611 mz	N/F	N/F	ppb	N/F
35	Schaftoside	N/F	563.14063 mz	N/F	N/F	ppb	N/F
36	Eriocitrin	N/F	595.16684 mz	N/F	N/F	ppb	N/F
37	Coumaric acid (trans-3-Hydroxycinnamic acid)	8,36	163.04007 mz	126042	-94,084	ppb	0,01
38	Luteoloside (Luteolin 7-glucoside)	N/F	449.10784 mz	N/F	N/F	ppb	N/F
39	Orientin	N/F	447.09328 mz	N/F	N/F	ppb	N/F
40	Isoorientin	N/F	447.09328 mz	N/F	N/F	ppb	N/F
41	Liquiritin (4′,7-Dihydroxyflavanone 4′-glucoside)	N/F	417.11911 mz	N/F	N/F	ppb	N/F
42	Sinapic acid	N/F	223.06120 mz	N/F	N/F	ppb	N/F
43	Ferulic acid	8,85	193.05063 mz	111312	-2,705	ppb	0,23
44	Vitexin (Apigenin 8-C-glucoside)	N/F	431.09837 mz	N/F	N/F	ppb	N/F
45	Narirutin (Narirutinsa, Naringenin rutinoside)	N/F	579.17193 mz	N/F	N/F	ppb	N/F
46	Doxorubicin Hydrchloride	N/F	544.18134 mz	N/F	N/F	ppb	N/F
47	Naringin	N/F	609.18390 mz	N/F	N/F	ppb	N/F
48	Hesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
49	α-Cyano-4-hydroxycinnamic acid	N/F	188.03532 mz	N/F	N/F	ppb	N/F
50	Rutin hydrate M-OH2	N/F	609.14611 mz	N/F	N/F	ppb	N/F
51	İsoquercitrin (Quercetin 3-glucoside)	N/F	463.08820 mz	N/F	N/F	ppb	N/F
52	Hyperoside (Quercetin 3-D-galactoside)	N/F	463.08820 mz	N/F	N/F	ppb	N/F
53	Eriodictyol (3,4,5,7-Tetrahydroxyflavanone)	N/F	287.05501 mz	N/F	N/F	ppb	N/F
54	Neohesperidin	N/F	609.18249 mz	N/F	N/F	ppb	N/F
55	Kaempferitrin	N/F	579.17083 mz	N/F	N/F	ppb	N/F
56	Protocatechuic acid ethyl ester (Ethyl 3,4-Dihydroxybenzoate)	N/F	181.05063 mz	N/F	N/F	ppb	N/F
57	Ellagic acid	9,43	300.99899 mz	2116632	41,337	ppb	-0,09
58	Apiin (Apigenin-7-(2-O-apiosylglucoside)	N/F	565.15179 mz	N/F	N/F	ppb	N/F
59	Phloridzin	N/F	435.12967 mz	N/F	N/F	ppb	N/F
60	Myricetin	N/F	319.04291 mz	N/F	N/F	ppb	N/F
61	Luteolin-7-O-glucuronide (Luteolin-7-O-β-D-glucuronide)	N/F	461.07255 mz	N/F	N/F	ppb	N/F
62	Nicotiflorin (Kaempferol 3-rutinoside, Kaempferol 3-O-β –rutinoside)	N/F	593.15106 mz	N/F	N/F	ppb	N/F
63	Rhoifolin (Apigenin 7-O- neohesperidoside)	N/F	577.15692 mz	N/F	N/F	ppb	N/F
64	Rosmarinic acid	N/F	359.07724 mz	N/F	N/F	ppb	N/F
65	Apigenin 7-glucoside	N/F	431.09837 mz	N/F	N/F	ppb	N/F
66	Astragalin (Kaempferol 3-glucoside)	N/F	447.09328 mz	N/F	N/F	ppb	N/F
67	Kuromanine (Cyanidin 3-glucoside chloride)	N/F	447.09328 mz	N/F	N/F	ppb	N/F

68	Salicylic acid	9,83	137.02442 mz	5546664	-3,915	ppb	0,07
69	Narcissin (Narcissoside, Isorhamnetin 3-rutinoside)	N/F	623.16176 mz	N/F	N/F	ppb	N/F
70	Leucoside (Kaempferol 3-sambubioside)	9,79	289.06924 mz	273450	-11,505	ppb	-0,13
71	Liquiritigenin	N/F	255.06628 mz	N/F	N/F	ppb	N/F
72	Fisetin hydrate	N/F	285.04046 mz	N/F	N/F	ppb	N/F
73	Etoposide	N/F	383.11053 mz	N/F	N/F	ppb	N/F
74	Afzelin (Kaempferol 3-rhamnoside)	N/F	431.09837 mz	N/F	N/F	ppb	N/F
75	Apigenin 7-glucuronide	N/F	445.07763 mz	N/F	N/F	ppb	N/F
76	Trans Cinnamic acid	N/F	147.04515 mz	N/F	N/F	ppb	N/F
77	Kaempferol	10,22	287.05350 mz	272449	-1,266	ppb	-0,09
78	Quercetin	N/F	301.03538 mz	N/F	N/F	ppb	N/F
79	Naringenin	10,49	271.06120 mz	167551	-1,992	ppb	0,14
80	Tiliroside	N/F	595.14264 mz	N/F	N/F	ppb	N/F
81	Luteolin	N/F	285.04046 mz	N/F	N/F	ppb	N/F
82	Genistein (5,7-Dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one)	10,71	269.04555 mz	140214	-4,933	ppb	-0,11
83	Apigenin (5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one)	N/F	269.04555 mz	N/F	N/F	ppb	N/F
84	Isorhamnetin (Quercetin 3′-methyl ether)	N/F	315.05103 mz	N/F	N/F	ppb	N/F
85	Diosmetin (Luteolin 4′-methyl ether)	N/F	299.05611 mz	N/F	N/F	ppb	N/F
86	Formononetin (Neochanin)	11,38	267.06628 mz	149563	-0,613	ppb	-0,14
87	Sakuranetin (Naringenin 7-O-methyl ether)	N/F	285.07685 mz	N/F	N/F	ppb	N/F
88	Caffeic acid phenhyl ester (CAPE)	11,97	283.09758 mz	350976	-147,762	ppb	-0,17
89	Chrysin (5,7-Dihydroxy-2-phenyl-4H-chromen-4-one)	12,16	253.05063 mz	3338213	-136,553	ppb	-0,12
90	Galangin (3,5,7-Trihydroxy-2-phenyl-4H-chromen-4-one)	12,30	269.04555 mz	2947760	2,428	ppb	-0,12
91	Acacetin (5,7-Dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one)	12,37	283.06120 mz	885354	-3,182	ppb	-0,15
92	Genkwanin(4’,5-Dihydroxy-7-metthoxyflavone, Apigenin 7-O-methyl ether)	12,37	283.06120 mz	885354	-2,759	ppb	-0,15
93	Kaempferide	N/F	299.05611 mz	N/F	N/F	ppb	N/F
94	Glabridin	N/F	323.12888 mz	N/F	N/F	ppb	N/F
95	Emodin	N/F	269.04555 mz	N/F	N/F	ppb	N/F