GC-MS Analysis of Propolis Samples from three Different Regions of Palestine

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Dedication

I dedicate this work to the people who always have inspired me starting with my father who always urged me for more work and to my mother whose prayers were with me all the way to success. To my friends who stood next to me and were always a source of motivation. To my grandmother, brothers, sister, cousins and aunts.

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List of Abbreviations

%	Percentage
˚C	Degree Celsius
AF-B1	Aflatoxin-B1
AG	Arabinogalactan
ALA	α-Linolenic acid
Area	Area under the peak
AXs	Arabinoxylans
BSTFA	Bis-(trimethyl-silyl) trifluoroacetamide
CAPE	Caffeic Acid Phenethyl Ester
CAPE	Caffeic Acid Phenethyl Ester
CFS	Chronic Fatigue Syndrome
DNA	Deoxyribonucleic acid
DOPC	1,2-dioleoyl-sn-glycero-3-phosphocholine
DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphocholine
e.g.	for example
EEP	Ethanol Extracts of Propolis
FKRP	Fukutin-related protein
FLU	Fluconazole
FLU	flurbiprofen
GC-MS	Gas Chromatograph - Mass Spectrometry
GRAS	Generally Regarded as Safe
kg	kilogram

LAM	Lipoarabinomannan
LS	Latency to seizures
m	meter
m/z	mass
ME	Myalgic Encephalomyelitis
MFC	Minimum Fungicidal Concentrations
Mg	milligram
Min	Minute
ml	milliliter
mm	millimeter
NBS	National Bureau of Standards
NF-kB	Nuclear Factor- protein Kinase-B
NK	Natural killer
OSA	Osteosarcoma
P. acnes	Propionibacterium acnes
PE	Propolis Extract
PKC	protein kinase C
PVC	Polyvinyl Chloride
RNA	Ribonucleic acid
RT	Retention Time
SD	Sprague-Dawley
TMCS	Trimethylchlorosilane
UMP	Uridine - Monophosphate
VOR	Voriconazole
VZV	Varicella zoster virus
WPP	Water-soluble Powder derivative of Propolis
γ-CD	γ-cyclodextrin
µg	microgram
µm	micro meter

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Abstract

Bees make propolis from the resin collected from trees buds. The resin chewed and mixed with saliva and the mixture (propolis) then used to close holes in the hive. It is also used as disinfectant that prevent viral and bacterial infections of the bee. Propolis organic compounds proved to have biological and pharmacological characteristics, such as antibacterial, anticancer, and anti-inflammatory activities, however, its popularity and value are just recently get noticed as one of the valuable sources for therapeutic compounds that is recently recognized by many researchers. The aim of the current study was to identify the various chemical compounds in propolis samples collected from three different regions of the Palestine and to shed the light on some of

these chemical compounds and their pharmaceutical potential. One sample of Propolis was collected from each of three different regions; Jericho, Hebron and Nablus districts during 2018-2019. Ethanol extracts of propolis (EEP) were prepared for chemical analysis, using gas chromatograph coupled with mass spectrometry (GC-MS).

Thirty-three, 27 and 17 different chemical compounds were found in tested samples from Hebron, Jericho and Nablus governorates, respectively. Samples from Nablus, Jericho and Hebron regions showed similar chemical composition for only four compounds including: ascorbic acid, glycerol, oleic acid and pimaric acid. GC-MS chromatograms showed that the total time of each sample spent on the column after injection was 141, 122, 104 minutes in Nablus, Jericho and Hebron, respectively.

The percentage of area under the peak (area) of the identified compounds in samples collected from Nablus region was measured where; 6 of these compounds were occupied an area more than 5%. The percentage of area for each of the compounds identified in Jericho samples was also calculated. Eight of these compounds were with a percentage area more than 5%. The percentage of area for each of the compounds identified in Hebron samples was calculated, seven of these compounds were with an area more than 5%.

Pimaric acid area in the chromatogram was the largest in samples collected from Nablus region, reflecting a significant amount of this compound, compared to the area size of propanoic, oleic and pimaric acid. Area size of this compound was also found to be the largest in all tested samples collected from the three regions.

To the best of our knowledge this is the first study of its kind that search for the propolis composition in Palestine with the hope to find and identify any promising components of pharmaceutical potential.

CHAPTER I

INTRODUCTION

1.1 Importance of the study

The popularity of herbal medicines is rapidly increasing in many countries where many individuals turn to natural products, because they promise a safe and natural remedy for a broad variety of health disorders**.**

In addition to their role in the pollination of different plant species, bees produce a diverse array of products including honey, bee pollen, propolis and royal jelly (Simone-Finstrom & Spivak, 2010).

Since ancient times, bee products have a long medicinal history due to its broad applications and their beneficial effect on human health. Honeybees make a sticky substance called propolis by mixing saliva with poplar tree resin and other botanical sources. Propolis, also called bee glue, is one of the most popular bee products, occurring usually as a sticky dark-colored material made by honeybees (*Apis mellifera)* from buds, leaves, exudates and bark of various plants, mixed with beeswax and salivary enzymes (Khan et al., 2018). According to several studies, propolis has large spectrum of biological properties. It can be used in food, cosmetic and pharmaceutical industries (El-Guendouz et al., 2016 a).

Although several studies reported the phenolic composition of propolis, much less is known on its volatile composition, despite its importance in propolis

chemical characterization (Alvarez-Suarez, 2017). The phenolic composition can be used as antioxidant, anti-inflammatory (Miguel et al., 2014; da Graça Miguel et al., 2014; Popova et al., 2015; El-Guendouz et al., 2017), anticancer (Mouse et al., 2012), antimicrobial (El-Guendouz et al., 2016 b) and enzymes inhibitory capacity (acetylcholinesterase, α-amylase, α-glucosidase, lipoxygenase, tyrosinase and xanthine oxidase) (da Graça Miguel et al., 2014; Popova et al., 2015; El- Guendouz et al., 2016 a).

In recent years, there has been renewed interest in the composition of propolis, a substance that can be considered as a potential natural source in folk medicine and in chemical industry. The composition of propolis depends on the place and time of collection.

1.2 Definitions of terms

Propolis, is a natural resinous substance collected by honeybees from buds and exudates of plants, has been used in the beehive as a protective barrier against their enemies. Recently propolis is extensively used in food and beverages to improve health and prevent diseases (Bankova et al., 2000). Much research and development has been implemented on propolis all over the world. Most of the studies are related to propolis because it has been reported to possess a characteristic biological activity (Marcucci 1999).

Propolis extracts were commonly obtained from continuous soaking in various solvents, such as ethanol, flavonoids and polyphenols, but there are other methods including ultrasonic and microwave (Ahangari et al., 2018).

1.3 Propolis uses

Propolis is used in medical and dental practices based on its chemical composition and its therapeutic properties (Bankova et al.,2000; Russo et al., 2002). Its chemical components, such as aromatic acid, chalcones and dihydrochalcones are very complex, and so far, more than 300 compounds have been identified (Almas et al., 2001). Many studies showed that observed effects of propolis might be the result of synergistic action of its complex constituents (Bueno-Silva et al., 2013), chemical properties were related to the geographic diversity of plant sources and bee species (Huang et al., 2015; Melliou and Chinou, 2004).

1.4 Propolis extract

Propolis is the bee product noted for multiple biological effects, and therefore it is widely used for the prevention and treatment of a variety of diseases. The active substances of propolis are easily soluble in ethanol. However, ethanolic extracts cannot be used in treatment of certain diseases encountered in ophthalmology, pediatrics, etc. As the main biologically active substances of

propolis are scarcely soluble in water, oil and other solvents usually used in pharmaceutical industry (Loreta Kubiliene, et al., 2015), investigated the chemical composition, radical scavenging and antimicrobial activity of propolis extracts differently made in non-ethanolic solvents.

Another study by (Galeotti et al., 2018) managed to obtain different products from a unique propolis extract using various solvents such as hydro alcoholic, glycolic (98% propylene glycol), and glycerol solutions, and oil, as well as in powder form, named ESIT12, were prepared. The chemical composition of the different preparations was evaluated, and their antioxidant activity was determined. All the preparations showed a quite similar polyphenol composition and comparable percentage even if ESIT12 was found to be richer in phenolic acids (caffeic, coumaric, ferulic, and isoferulic). Overall, flavones and flavanols ranged from ~20% up to ~36% in the glyceric extract, while flavanones and diidroflavonols were between ~28% and ~41%. Besides their quite similar composition, glycolic and hydro alcoholic extracts were found to be richer in the total polyphenols content. When the antioxidant properties were determined for the preparations, the activity was similar among them, thus revealing that it is strictly related to the polyphenols content for propolis products whose composition is quite comparable. Nowadays, very few data are available on propolis composition in glyceric and glycolic

extracts. A study shows that propolis preparations useful for active finished products can be produced by providing suitable solvents and conditions (Galeotti et al., 2018).

1.5 Chemical composition of propolis

Propolis chemical composition is highly variable mainly due to the variability of plant species growing around the hive, from which the bees collect the exudates (Castaldo and Capasso, 2002; Markham et al., 1996; Kumazawa et al., 2004). In review study, propolis was reported as a complex resinous mixture which contains approximately 50% resin and balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% impurities (Thomson, 1990). Variations in chemical composition of propolis also seems to vary depending on the seasonality, illumination, altitude, collector type, and food availability and activity developed during propolis exploitation (Mercan 2006; Castro et al., 2007).

Much work has been conducted on the chemical composition and biological activities. More than 300 chemical constituents have been identified in propolis samples collected from different regions worldwide (Bankova et al., 2000). The main chemical classes present in propolis were flavonoids, phenolics, and aromatic compounds (Xu et al., 2009; Toreti et al., 2013).

Propolis also was reported to contains some volatile oils, terpenes, and bee wax, however, these compounds do not contribute to the chemical properties and effects of propolis (Toreti et al., 2013)

1.6 Pharmaceutical importance

At present, several studies showed that propolis have large number of organic compounds that could be used to overcome many types of bacterial, viral and fungal diseases.

1.7 Aims of this study

The current study aimed at identifying the derivative chemical composition of propolis samples collected from several representative regions of the Palestine. Within the scope of this study the medical compounds will be evaluated for their future use as pharmaceutical products.

CHAPTER II

LITRETURE REVIEW

2. Literature Review

2.1 Biological and Pharmacological Properties of Propolis

2.1.1 Antibacterial activity

The propolis extract synergistically enhance the efficacy of antibiotics, especially those acting on cell wall synthesis (vancomycin and oxacillin) against drug-resistant microorganisms (AL-Ani et al., 2018). Dental caries is a multi-factorial disease and an important health problem worldwide. Various *Streptococcus* mutants were considered as cariogenic agents in oral cavity. This bacterium synthesizes soluble and insoluble glucans from sucrose by glycosyltransferases enzymes and generate stable biofilms on the tooth surface. Biological properties of Chilean propolis have been described to have antimicrobial, antifungal, and anti-biofilm activities (Veloz et al., 2019).

A study showed that the concentrations of main flavonoids presents in Chilean propolis were qualified and some biological properties such as antimicrobial and antibiofilm activity of individual compounds, were compared against *S. mutans* cultures (Veloz et al., 2019). Chilean propolis was studied by some researchers and some polyphenols present in this extract were quantified by

HPLC-DAD using commercial standards of apigenin, pinocembrin, quercetin, and caffeic acid phenethyl ester (CAPE). Pinocembrin, apigenin, quercetin, and (CAPE) were the most abundant compounds in Chilean propolis. These polyphenols have strong antimicrobial and antibiofilm potential at low concentrations. However, pinocembrin and apigenin have a greater contribution to this action. The effect of polyphenols on *S. mutans* is produced by a combination of mechanisms to decrease bacterial growth and affect bio-film proliferation due to changes in their architecture (Veloz et al., 2019).

Propolis hydro-ethanolic extracts (PHEE), prepared using three different methods and two solvent mixtures contained high amounts of flavonoids (20.95–28.11 % TIC), aromatic acids (8.17–15.91 % TIC) and their esters (9.27-11.91 % TIC) (Mašek et al., 2018). A study that obtained the PHEE shows that high antioxidant activity (DPPH IC50 values from 9.96–19.95 µg/ml and FRAP 38.0–41.9 mM Fe2+/mg PHEE) (Mašek et al., 2018). Despite differences in composition, the PHEE samples exhibited significant antibacterial activities, affecting tested strains of *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Moraxella catarrhalis* (Mašek et al., 2018).

2.1.2 Antiviral activity

The antiviral activity and antiherpetic effect of propolis was reported in a study against Varicella zoster virus (VZV) in cell culture. The study revealed a moderate cytotoxicity on lung fibroblasts with a CC50 of 380 μg/ml, the results of study found that, 50 % inhibitory concentration (IC50) of propolis extract for VZV plaque formation at 64 μg/ml. In viral suspension tests the propolis extract exhibited high levels of antiviral activity against VZV, and infectivity was significantly reduced by 93.9 % and a concentration-dependent antiviral activity could be demonstrated. To determine the mode of virus suppression by propolis, the extract was added at different times during viral infection cycle (Labská et al., 2018). Propolis was added to uninfected cells (pretreatment cells) prior to infection or to infected cells (replication) during intracellular replication had no effect on virus multiplication. However, propolis exhibited high anti-VZV activity when viruses were pre-treated with propolis prior to infection, thus indicating an unspecific interaction between the virus and propolis (Labská et al., 2018).

In an invitro study, propolis showed immunomodulatory effects on macrophages and it increased the ratio of CD4+/CD8+ T-cells in vivo in mice (Al-Hariri, 2019). This could explain why it is used in acute and chronic

inflammations in the lower and upper airway diseases, cutaneous ulcers, laryngotracheitis, periodontist, and sinusitis (Al-Hariri, 2019).

Some researchers showed that propolis could relieve allergic disorders through the inhibition of histamine release and reported that caffeic acid phenethyl ester induced caspase-3 expression and inhibited nuclear factor-kB (NF-kB) and protein Kinase-B signaling pathways in primary human CD4 and T cells. The anti-allergy effect of propolis was due to the suppression of immunoglobulin E levels that cause inhibition of platelet-activating factor release and NF-κB activation, the use of oral propolis 200 mg/kg in an ova albumin-induced rat model of allergic rhinitis inflammation, and allergic symptom was lower (Al-Hariri, 2019). Propolis could act directly on the T-cells inhibiting their differentiation and consequently the improvement of acquired immune response (Al-Hariri, 2019).

2.1.3 Antifungal activity

The antifungal agents in the treatment of candidemia and the toxic activities of these drugs was failure. Several research works were conducted to develop new nontoxic and effective antifungal agents for optimal control of fungal pathogens. Antifungal activity of propolis against yeasts clinical isolates from intensive care unit patients showed that it had a significant antifungal activity

against all *Candida* strains and the MIC was determined as 0185 to 3 μg/ml (Sariguzel et al., 2016).

Other study listed the activity of 50 ethanolic extracts of propolis (EEPs), harvested in Polish apiaries, on a group of 69 clinical isolates of *C. albicans*, most of the EEPs showed satisfactory activity, with minimum fungicidal concentrations (MFC) mainly in the range of 0.08–1.25% (v/v). High activity was also observed in eradication of biofilm formed by *C. glabrata* and *C. krusei* on the surfaces of PVC (Polyvinyl Chloride) and silicone catheters, at subinhibitory concentrations EEPs inhibited yeast-to-mycelia morphological transformation of *C. albicans* in liquid medium and mycelial growth on solid medium, a synergistic effect was observed for the action of EEP in combination with fluconazole (FLU) and voriconazole (VOR) against *C. albicans*. In the presence of EEP at concentrations as low as 0.02%, the MICs of FLU and VOR were 256 to 32 times lower in comparison to those of the drug alone. Evidence for the fungal cell membrane as the most probable target of EEPs were presented (Gucwa et al., 2018).

The in vitro activity of propolis extract against 67 yeasts isolated from onychomycosis in patients attending at the Teaching and Research Laboratory of Clinical Analysis. Propolis extract showed excellent performance regarding its antifungal activity: the concentration capable of inhibiting all of the yeasts

was 10^{-2} mg/ml of flavonoids and *Trichosporon* sp. were the most sensitive species, showing MIC50 and MIC90 of 10^{-2} mg/ml of flavonoids, *and C. tropicalis* was the most resistant, with CFM50 of 10^{-2} mg/ml of flavonoids and MFC90 of 10^{-2} mg/ml. Since propolis is a natural, low cost, non-toxic product with proven antifungal activity, it should be considered as another option in the onychomycosis treatment (Oliveira et al., 2006).

The in vitro antifungal activity of propolis extract was also tested against yeasts *C. albicans* and C. non-albicans isolated from vaginal exudates, in comparison with nystatin (Dalben-Dota, et al., 2010). All tested yeasts isolates were inhibited by low concentrations of PE (maximum of 393.19 μg/mL of the total flavonoid content), including an isolate of the women and the species of yeast resistant to nystatin, regardless of the clinical conditions (Dalben-Dota, et al., 2010). The PE showed an outstanding performance against the tested vaginal yeast strains and could be included among the novel therapeutic options for the treatment of Volvo-vaginal candidiasis (Dalben-Dota, et al., 2010).

2.1.4 Cytotoxic activity

Natural products (propolis) are invaluable resource of anticancer drug discovery. The cytotoxic activity of different propolis extracts were evaluated

in many types of cancer cell lines. Solid nanoparticles from the organic solvent extracts were prepared and their cytotoxicity was evaluated. The compounds 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) liposomes were prepared from the most cytotoxic organic solvent extracts and their cytotoxicity was also evaluated. The results showed that hexane extract and its solid nanoparticles as well as its liposomal form exhibited high cytotoxic activity, DPPC/DOPC-hexane extract cytotoxicity selectively depends on the cell line and DOPC liposomal form, the anti-proliferative activity of propolis was associated to multiple modes of actions including apoptosis and nitric oxide production and as indicated by the HPLC and FTIR results, it is functioning in many propolis ingredients rather than a single component and influenced by the presence of more lipophilic components within the extract (Sherif, et al., 2018).

The effects of the oral ingestion of propolis on natural killer (NK) cell activity, which is important in immune surveillance against cancer and viral infections, was assessed. The effects of the major components of the water-soluble powder derivative of propolis (WPP), suggest that oral ingestion of WPP enhances NK cell cytotoxic activity, but not proliferation, in a manner dependent on IFN-γ and without the contribution of acquired immune

responses. Further, artepillin C or p-coumaric acid, but not drupanin, may be the components responsible for this augmentation of NK cell cytotoxicity. These findings suggest the possible utility of WPP as a therapeutic for prevention of cancer development and against viral infection through NK cell activation (Takeda et al., 2018).

The potential use of propolis for the development of new antitumor drugs has been evaluated (Mora, et al., 2018). He studies the chemical composition of Colombian propolis samples, and the mechanisms involved in their cytotoxic effects on Osteosarcoma (OSA). The samples were grouped: Cluster 1 contained diterpenes and benzophenones and showed the highest antiradical activity; Cluster 2 was characterized by triterpenes, fatty acid, and diterpenes. Apoptosis, mitochondrial membrane alteration, and suppression of cell invasion were the main mechanisms involved in the inhibition of OSA cells in vitro, suggesting the potential of Colombian propolis to discover new antitumor drugs (Mora, et al., 2018).

2.1.5 Therapeutic activity

Propolis is a anti toxic natural product; however, some cases of allergy and contact dermatitis to this compound have been described, the important factor in impaired wound healing is biofilm formation; propolis as an antimicrobial

agent can reduce biofilm generation and result in accelerated healing processes. Most of the in vivo studies suggested the beneficial roles of propolis on wound healing and this has also been approved in the clinical trial studies, the effectiveness of propolis between different products is variable, more characterizations should be done and future investigations comparing different propolis based products and characterization of their specific roles on different models of wounds were highly appreciated (Oryan et al., 2018).

The molecular mechanism of caffeic acid phenethyl ester (CAPE) as anticancer activity, isolated from New Zealand propolis, showed that CAPE activates DNA damage signaling in cancer cells. It found that CAPE-induced growth arrest cells by mediating the downregulation of mortalin and activation of p53 tumor suppressor protein. Also they reported an enhanced activity of CAPE when complexed with γ-cyclodextrin (γCD) and the CAPE-γCD complex showed higher cytotoxicity activity to a wide range of cancer cells in stable acidic milieu and therefore recommended as an anticancer amalgam. Thus, propolis was suggested for therapeutic benefits as treating agent for cancer (Ishida, et al., 2018).

Various pharmaceutical properties of the propolis, including antibacterial action against microorganisms involved in the beginning and progression of dental caries. Analysis of propolis chemical composition and its antimicrobial

activity against microorganisms within dental biofilm was evaluated by Machado et al (2017) to elucidate the possibility of employment of propolis compounds as an adjunct to prevention and ultra conservative treatment of dental caries. The results confirmed the great potential of the propolis activities as a natural medicine with solid therapeutic properties and proven anti-cariogenic action (Machado et al., 2017).

Immune-related diseases such as allergic diseases, Type 1 diabetes mellitus, rheumatoid arthritis, multiple sclerosis, psoriasis, inflammatory bowel disease and other such as immunodeficiency, various infectious diseases, vaccines, and malignancies were among the targeted projects for scientific research were propolis was suggested as one of the most promising immunomodulation agents (Al-Hariri, 2019).

2.1.6 In dermatology

Propolis is well known for its antimicrobial, antioxidant, antiviral, and antifungal properties. There was a highly significant clinical efficacy of topical solution of ethanolic extract of propolis in the treatment of acne vulgaris. The antibacterial effect of topical solution of ethanolic extract of propolis on gram-positive aerobic (*Staphylococcus epidermidis*) and gram-positive anaerobic bacteria (*Propionibacterium acnes*), is well demonstrated

and it showed a promising, effective, well-tolerated, safe, and alternative medication for acne vulgaris. It was also showed that it has anti-inflammatory properties (Ali et al., 2005).

2.1.7 In Cosmetics

Propolis contains almost all necessary elements such as, magnesium, potassium, sodium, iron, zinc, manganese, cobalt, phosphorus, sulfur, aluminum, chromium, selenium, silicon, strontium, titanium, vanadium, zinc, tin, and copper fluoride. Various vitamins were also reported in propolis, among these vitamins B (B1, B2, B6), vitamin A, C, E, H and R. Large number of applications of propolis in dermatology are well known, their action is effective for burns, frostbite, skin non-healing ulcers. Successfully used ointments and gels of propolis in the vast deep wounds, chronic eczema, itchy dermatitis, furunculosis, fungal diseases on their feet (Teslenko et al., 2014). Propolis extract (PE) was reported to have antifungal activities and was recommended as a potential alternative for conventional antifungal agents because of its low cost, accessibility, and low toxicity (Veiga et al., 2018). These properties recommend propolis as good candidate for cosmetics use as an antibacterial, antifungal and regenerating agent. Nowadays one can find very popular lip balms and lipsticks hygiene based on propolis and several

products for hair care and toothpastes. Lipsticks based on such compound seems to prevent drying, cracking and peeling of skin. Mascara based on propolis was also reported to have anti-inflammatory and disinfectant properties, which is very important for especially sensitive eyes. Propolis tincture is perfect for those who want to get rid of blackheads. Propolis cream makes the skin smooth and silky, and massage oil on its basis is an excellent way to combat premature aging of the skin. Its application allows to enrich the skin with oxygen and contributes to the process of cell regeneration (Teslenko et al., 2014).

2.2 Propolis chemical compounds found in the study and their reported Properties and pharmacological use

As we will see in the following result section, table 1 shows the major chemical compounds found in the study regions as determined by GC-MS analysis. The table shows the majority of found compounds and their reported properties and pharmacological use. The selection of these compounds was based on importance and use of these compounds as reported by many researchers.

Table 1. General compounds found in Propolis

COMPOUND	Properties and pharmaceutical use of propolis chemical compounds found in the study regions
GLYCEROL	Glycerol was used in pharmaceutical, medical and personal care preparations, often as a means of improving smoothness, providing lubrication, and as a humectant. It is found in allergen immuno-therapies, cough syrups, elixirs and expectorants, toothpaste, mouthwashes, skin care products, shaving cream, hair care products, soaps, and water-based personal lubricants (Mark et al., 2017).
CARBODIIMIDE	The functionality of the Carbodiimides are dehydration agents and are often used to activate carboxylic acids towards amide or ester formation. Carbodiimides can also react with amines to form guanidine's. Polycarbodiimides can also be used as crosslinkers for aqueous resins, such a polyurethane dispersions or acrylic dispersion, the Polycarbodiimides reacts with carboxylic acids, which functional groups are often present in such aqueous resins, to form N-acyl urea (Hesselmans et al., 2006).
ETHYL ALCOHOL	Ethyl alcohol responsible for increases the secretion of acids in the stomach, the metabolite acetaldehyde is responsible for much of the short term, and long-term effects of ethyl alcohol toxicity. Polysaccharides precipitate from aqueous solution in the presence of alcohol, and ethanol precipitation is used for this reason in the purification of DNA and RNA (Wallner & Olsen, 2008).

1,4-DICHLOROBENZENE	p-DCB is used as a disinfectant, pesticide, and deodorant, most familiarly in mothballs in which it is a replacement for the more traditional naphthalene because of naphthalene's greater flammability. It is also used as a precursor in the production of the chemically and thermally resistant polymer (Rossberg et al., 2006).
VITAMIN E	vitamin E is fat-soluble, it is incorporated into cell membranes, and protect cells from oxidative damage. It also acts as enzyme activity regulator, such as for protein kinase C (PKC) – which plays a role in smooth muscle growth – vitamin E participates in deactivation of PKC to inhibit smooth muscle growth (Schneider, 2005)
PROPANOIC ACID	Phenyl propanoic acid is widely used for flavoring, food additives, spices, fragrance, and medicines, it is used frequently in cosmetic. It has a role as an antifungal agent, human and plant metabolite. Used for the treatment and prevention of diseases and to their use for preparing medicaments for the treatment and/or prevention of diseases, for the treatment and/or prevention of cardiovascular diseases (Hahn, et al., 2018).
DECANOL	Decanol is used in the manufacture of plasticizers, lubricants, solvents and surfactants. Its ability to permeate the skin has led to it being investigated as a penetration enhancer for transdermal drug delivery (Kanikkannan & Singh, 2002).
PROPIONIC ACID	The human skin is host of several species of bacteria known as Propionic bacteria, which are named after their ability to produce propionic acid. The most notable one is the Propionibacterium acnes, which lives mainly in the sebaceous glands

	of the skin and is one of the principal causes of acne, some researchers demonstrate that fermentation of glycerol with Propionibacterium acnes (P. acnes), a skin commensal bacterium, can function as a skin probiotic, and therefore it can eliminate the skin diseases (Shu, et al, 2013).
CAFFEIC ACID	Caffeic acid has a variety of potential pharmacological effects in in vitro studies and in animal models, and the inhibitory effect of caffeic acid on cancer cell proliferation by an oxidative mechanism in the human HT-1080 fibrosarcoma cell line has recently been established. It is also having immunomodulatory and anti-inflammatory activity (Rajendra Prasad, et al, 2011).
BENZALDEHYDE	Benzaldehyde is commonly employed to confer almond flavor to foods and scented products. It is sometimes used in cosmetics products (Andersen, 2006). Benzaldehyde is an aromatic aldehyde used in cosmetics as a denaturant, a flavoring agent, and as a fragrance. Benzaldehyde is a generally regarded as safe (GRAS) food additive in the United States and is accepted as a flavoring substance in the European Union (Andersen, 2006).
CINNAMIC ACID	Cinnamic acid is used in certain pharmaceuticals, flavorings and synthetic indigo. A major use is in the manufacturing of the ethyl, methyl, and benzyl esters for the perfume industry. Cinnamic acid is a precursor to the sweetener aspartame via enzyme-catalyzed animation to phenylalanine (Alqahtani, 2017). Cinnamic acid can dimerize in non-polar solvents resulting in different linear free energy relationships (Bradley, et al, 2015).
ARABINOSE	Arabinose could be used in foods to attenuate the peak of glycemic response after the consumption of

	sucrose. The long-term effects of arabinose consumption on blood glucose parameters such as fasting blood glucose and HbA1c. Foods that contain arabinose were designed for prediabetic and diabetic patients. (Seri, et al., 1996). Arabinose is a potential prebiotic, because it cannot be absorbed by human intestine and could be utilized by probiotics such as bifidobacterial (Degnan & Macfarlane, 1993).
D-GALACTOSE	Galactose may have a role in treatment of focal segmental glomerulosclerosis (a kidney disease resulting in kidney failure and proteinuria) (De Smet, et al., 2009).
MYRISTIC ACID	Myristic acid's reported to have positive effects on HDL cholesterol and hence improving HDL (good cholesterol) to total cholesterol ratio (Kromhout, et al., 1995).
LINOLEIC ACID	Linoleic acid has become increasingly use in the beauty products industry because of its beneficial properties on the skin, also has anti-inflammatory, acne reductive, skin-lightening and moisture retentive properties when applied topically on the skin (Darmstadt, et al., 2002).
OLEIC ACID	Oleic acid is used commercially in the preparation of foliates and lotions, and as a pharmaceutical solvent. The principal use of oleic acid is as a component in many foods, in the form of its triglycerides. It is a component of the normal human diet as a part of animal fats and vegetable oils. Oleic acid as its sodium salt is a major component of soap as an emulsifying agent. It is also used as an emollient (Carrasco, 2009). Small amounts of oleic acid were used as an excipient in pharmaceuticals, and it is used as an emulsifying or

	solubilizing agent in aerosol products (Smolinske, 1992).
ISOMERIC ACID	Isomeric acid originates from many sorts of trees, especially conifers (da Rosa Chagas, et al., 2019).
D-RIBOSE	D-ribose has been suggested for use in management of congestive heart failure (as well as other forms of heart disease) and for chronic fatigue syndrome (CFS), also called myalgic encephalomyelitis (ME) (Teitelbaum, et al., 2006).
OCTADECYL GLYCEROL	Octadecyl glycerol is used as a detergent or as an inhibitor of Phosphoinositide-specific phospholipase C. It is also used as cleaning and washing agent (Barreleiro et al., 2018). It is also used in dermal cleansing because it is effective against antimicrobial activity. Phospholipid which provides a reduction in potential irritancy of other ingredients. To further improve the mildness of the formulations, skin adjuvants can be included which do not inhibit the antimicrobial effectiveness of the formulations. The adjuvants include, for example, glycerol and its derivatives (octadecyl glycerol) (Grundhofer, 2017).
D-LYXOSE	D-Lyxose is an aldopentose — a monosaccharide containing five carbon atoms and including an aldehyde functional group. Lyxose occurs only rarely in nature, for example, as a component of bacterial glycolipids (Khoo, et al., 1996).
9.12.15-OCTADECATRIENOIC ACID	9.12.15-octadecatrienoic acid: a fatty acid is mainly used in the production of soap, both for cosmetic purposes. Fatty acids are also converted, via their

	methyl esters, to fatty alcohols and fatty amines, which are precursors to surfactants, detergents, and lubricants. Other applications include their use as emulsifiers, texturizing agents, wetting agents, anti-foam agents, or stabilizing agents (Anneken et al., 2000).
GERANYLGERANIOL	Geranylgeraniol is a diterpene alcohol which plays a role in several important biological processes. It is an intermediate in the biosynthesis of other diterpenes and of vitamins E and K (Farrell and Merkler, 2008). It also used in the post-translational modification known as geranylgeranylation. Geranylgeraniol is a pheromone for bumblebees and a variety of other insects. Geranylgeraniol is a potent inhibitor of Mycobacterium tuberculosis in vitro (Vik et al., 2007).
FARNESYL ALCOHOL	Farnesyl has been suggested to function as a chemo preventative and anti-tumor agent (Joo and Jetten, 2010). It is also used as a deodorant in cosmetic products because of its anti-bacterial activity. Farnesol is subject to restrictions on its use in perfumery as some people may become sensitized to it, however the evidence that farnesol can cause an allergic reaction in humans is disputed (Younan, 2013).
GLUCOPYRANOSE (GLUCOSE)	Glucopyranose play a role in glucose metabolism, antioxidant function, and inflammatory cytokines in patients with type 2 diabetes mellitus (Gao et al., 2018). TGPE delayed the development and progression of T2DM and reduced the severity of β-cell failure. TGPE also attenuated inflammation and reactive oxygen species ROS in the rats. Moreover, there

	were higher levels of oxidant cytokines, leptin, and adiponectin in the serum of the TGPE-treated group. TGPE may thus delay the progression of T2DM through anti-inflammation effects, anti-oxidation effects, and balancing lipid metabolism. It is suggested that TGPE can be a potential alternative medicine for T2DM (Chen et al., 2018)
XYLOPYRANOSE	Xylopyranose is a polysaccharide GCP composed of arabinose, galactose, glucose, xylose, mannose and glucuronic acid. Antibacterial characteristics of GCP against many types of bacteria, such as *Escherichia coli* and *Staphlococcus aureus*, GCP (xylose) could be used as antibacterial agent in food and pharmaceutical industries (Wang, et al., 2019). Some researchers discovered that, the Consumption of food contaminated with Aflatoxin-B1(AF-B1), caused deleterious effects on different body's systems. Results revealed that intestinal absorption function of D-Xylose sugar was reduced in AFB-1 exposed rats, meanwhile Propolis improve D-xylose absorption in rats exposed to the AFB-1. Analysis of light microscopic photograph revealed that administration of aflatoxin-B1 cause deleterious changes in intestinal tissues and stomach, while Propolis was efficient in improving these changes to normal condition (Alqayim and Shehab, 2017).
OCTADECYL GLYCEROL	Octadecyl glycerol can be used as a detergent or as an inhibitor of Phosphoinositide-specific phospholipase C, uses as cleaning agents and washing agents (Barreleiro et al., 2018).
MONOLINOLEOYLGLYCEROL (GLYCEROL)	Glycerol is used in medical, pharmaceutical and personal care preparations, often as a means of improving smoothness, providing lubrication, and

	as a humectant. Ichthyosis and xerosis have been relieved by the topical use glycerin. It is also found in allergen immunotherapies, cough syrups, elixirs and expectorants, toothpaste, mouthwashes, skin care products, shaving cream, hair care products, soaps, and water-based personal lubricants (Lebwohl et al., 2013).
FLURBIPROFEN	Flurbiprofen, the model drug used to the class of non-steroidal anti-inflammatory drugs. flurbiprofen (FLU) used for treatment of bacterial conjunctivitis which aims to increase the residence time in ocular tissue thus enhancing patient compliance and improved efficacy (Shinde et al., 2019).
EUDESMOL	Beta- eudesmol synthase is an enzyme with systematic name (2E,6E)-farnesyl-diphosphate diphosphate-lyase (beta-eudesmol-forming). This enzyme catalysis chemical reactions (Yu et al., 2008).
9,12,15-OCTADECATRIENOIC ACID (ALA)	α-Linolenic acid (ALA) is an n−3 fatty acid. It is one of two essential fatty acids (the other being linoleic acid), so called because they are necessary for health and cannot be produced within the human body. They must be acquired through diet. ALA is an omega-3 fatty acid found in seeds (chia, flaxseed, hemp, see also table below), nuts (notably walnuts), and many common vegetable oils. In terms of its structure, it is named all-cis-9,12,15-octadecatrienoic acid (Beare-Rogers et al., 2001). Flax is a rich source of α-linolenic acid. Although the best source of ALA is seeds, most seeds and seed oils are much richer in an n−6 fatty acid, linoleic acid. Exceptions include flaxseed (must be ground for proper nutrient absorption) and chia seeds. Linoleic acid is the other essential fatty acid, but it, and the other n−6 fatty acids, compete with

	n−3s for positions in cell membranes and have very different effects on human health. There is a complex set of essential fatty acid interactions (Beare-Rogers et al., 2001).
METHYL KETON	Researcher showed that, ketone ester (1,3-butanediol acetoacetate diester, KE) administration delayed latency to seizures (LS) in 3-month-old Sprague-Dawley (SD) rats, so the effect of exogenous ketone supplements in additional dosages and formulations on CNS-OT seizures in 18 months old SD rats, an age group correlating to human middle age. In these groups, the severity of seizures appeared to be reduced, although these changes were significant only in KE-treated animals (Ari et al., 2019).
DIETHYLENE GLYCOL	Diethylene glycol was synthesized as a monomer unit and further utilized for polymerization with FeCl2 in order to form water soluble coordination polymers. Viscosity measurements and film-forming properties indicate the formation of linear coordination polymers or larger ring structures (Schmatloch et al., 2002).
ERYTHRITOL	Erythritol is tooth-friendly; it cannot be metabolized by oral bacteria, so it does not contribute to tooth decay (Kawanabe et al., 1992).
2-FURANACETALDEHYDE	Furfural participates in the same kinds of reactions as other aldehydes and other aromatic compounds. Furfural is also a specialized chemical solvent (Dalvand et al., 2018).
ARABINO FURANOSE	Arabinoxylans (AXs) are major dietary fibers. Recently, attracted a great deal of attention because of their biological activities such as their immunomodulatory potential. Extraction of AXs has some difficulties; therefore, various methods

	have been used to increase the extractability of AXs with varying degrees of success, such as alkaline, enzymatic, mechanical extraction (Fadel et al., 2018).Lipoarabinomannan (LAM) and arabinogalactan (AG) are the two major mycobacterial cell wall (lipo) polysaccharides, in addition to playing an essential role in mycobacterial physiology, LAM and its biochemical precursor lipomannan possess potent immunomodulatory activities that affect the host immune response (Jankute et al., 2016).
RIBITOL	Ribitol, a pentose alcohol with previously unknown function in mammalian cells, partially restores functional O-mannosylation of α-DG (F-α-DG) in the dystroglycanopathy model containing a P448L mutation in fukutin-related protein (FKRP) gene, which is clinically associated with severe congenital muscular dystrophy. Oral administration of ribitol increases levels of ribitol-5-phosphate and CDP-ribitol and restores therapeutic levels of F-α-DG in skeletal and cardiac muscles. Furthermore, ribitol, given before and after the onset of disease phenotype, reduces skeletal muscle pathology, significantly decreases cardiac fibrosis and improves skeletal and respiratory functions in the FKRP mutant mice. Ribitol treatment presents a new class, low risk, and easy to administer experimental therapy to restore F-α-DG in FKRP-related muscular dystrophy (Cataldi et al., 2018).
5,8,11-EICOSATRIYNOIC ACID	5,8,11-Eicosatriynoic acid is a fatty acid mainly used in the production of soap, both for cosmetic purposes and, in the case of metallic soaps, as lubricants. Fatty acids are also converted, via their methyl esters, to fatty alcohols and fatty amines, which are precursors to surfactants, detergents, and

	lubricants. Other applications include their use as emulsifiers, texturizing agents, wetting agents, anti-foam agents, or stabilizing agents (Anneken et al., 2000).
URIDINE	In infants consuming mother's milk or commercial infant formulas, uridine is present as its monophosphate, UMP (Wurtman, 2014), and this source of uridine is indeed bioavailable and enters the blood (Carver, 2003).
SPINACENE (SQUALENE)	As a common lipid produced by sebaceous glands, squalene has a role in topical skin lubrication and protection (Pappas, 2009). Toxicology studies indicate that in the concentrations used in cosmetics, squalene has low acute toxicity, and is not a significant contact allergen or irritant (Huang et al., 2009).
OCTANOL	Octanol is used commercially as a component in perfumes and in flavor production for the food industry. It is usually produced by hydroformylation of heptane and the dehydrogenation of 1-octanol Octanal can also be referred to as caprylic aldehyde or aldehyde C-8 (Kohlpaintner et al., 2000).
D-FUCOSE	Fucose was used in cosmetics, pharmaceuticals, and dietary supplements. Fucosylation of antibodies has been established to reduce binding to the Fc receptor of Natural Killer cells and thereby reduce antigen-dependent cellular cytotoxicity. Therefore, a fucosylated monoclonal antibodies have been designed to recruit the immune system to cancers cells have been manufactured in cell lines deficient in the enzyme for core fucosylation (FUT8) and thereby enhancing the in vivo cell killing (Dalziel et al., 2014; Yu et al., 2017)

LYXOSE	D-Lyxose is an aldopentoseamonosaccharide containing five carbon atoms and including an aldehyde functional group. Lyxose occurs only rarely in nature, for example, as a component of bacterial glycolipids (Khoo et al., 1996).

2.3 Environmental conditions and plant diversity of the studied regions

As previously mentioned, the selected sampling regions were representative regions of good yield of propolis. These is an expected result due to various climatic conditions which is reflected on plant biodiversity, thus, influencing the chemical composition of the produced propolis in the different regions.

2.3.1 Hebron region

Hebron has a unique biodiversity and climate in the world as a mountainous highlands, which affected by the climate of the Mediterranean basin; the eastern region, which is affected by the climate of the Dead Sea and the Jordan Valley; the western region, which is affected by the climate of the Palestinian coast and the Mediterranean coast and the southern region, which is affected by the climate of the African Sahara, as Sinai and the Red Sea (Ighbareyeh and Carmona 2018).

Variations in climatic conditions resulted in the generation of great biodiversity as reflected by the finding of large number of plant species as well as large number of animal species (Ighbareyehetal., 2015). The following paragraph represent a study that clearly show the effect of these climatic changes on plant diversity and growth requirement.

Using bioclimatic classification of the Earth of Salvador Rivas-Martinez to study the relationship between the almond yield and climate and bioclimate factors (variables). The climatic and bioclimatic variables of greatest importance to almond were used to develop regressions analysis relating yield to climatic conditions. Hebron was positively affected by annual homoeothermic index, simple continentality index, precipitation, water soil reserve, and mean annual temperature (Ighbareyeh et al.,2018).

The previous study also showed that climate is one of the roles controlling factors in grape and grapevine production in Hebron and all over the world. Grapes are sensitive climate and the surrounding environment factors, and in order to get a high production, high-quality grapes and achieve food security, grapes were adapted in places where the mean monthly temperature is between 15˚C - 20˚C. Furthermore, Hebron grapes are the finest grapes in the world, because it has all the best nutritional standards, and the climates of Hebron.

2.3.2 Nablus region

Nablus located within the Mediterranean region, enjoys a moderate climate with average maximum temperature reaches 13.1 centigrade during the coldest month (January). As for the month of August, the average maximum temperature reaches 29,4 centigrade, while the minimum reaches 19,5 centigrade. North west is the dominant wind with average speed of 10 km/hr, while humidity average reaches 61% (Nablus Municipal, 2019).

The major fruit tree plantations are olive orchards, representing almost 77% of the total area of fruit trees under rain fed cultivation (942,000 dunums). In terms of production value, olives represent 20% of the average agricultural production. Almond and fig trees cover more area in the chromatogram than grapes and plums and are scattered in the hilly mountains of the West Bank. Grapes and plums are concentrated in the southern districts. Grape and plum trees are planted in intensive areas and require more care and attention from farmers than fig and almond trees. Figs' high per unit production value indicates the demand for this fruit although production itself is considerably low (Isaac & Gasteyer 1995).

2.3.3 Jericho region

Jericho is located at an altitude of 273m below sea level with a mean annual rainfall of 133mm. The average annual temperature is 24˚C, and the average annual humidity is approximately 49.3%. Furthermore, there are 4 public harvesting reservoirs in the city; the combined capacity of which reached 4,500 cubic meters (Jericho Municipal Council, 2011).

The bioclimatic of Jericho is located within the zones of the thermal model under the infra Mediterranean basin, the dry and arid regions. Jericho is belonging to Mediterranean desertic-oceanic (Ighbareyeh, 2019).

Adjusting to different habitats (Mediterranean, semi-arid, coastal plain) the flora & fauna are represented in all their diversity. Over 2,800 species of plants have been identified here on a comparatively small area. Today, fruit trees olive, almond, orange, apricot etc. Dominate the countryside while wild species such as pine, cypress, carob, acacia and turpentine trees are limited to certain regions (in the Galilee and on Mt. Carmel), on the edges of villages and in wadis (Visit Palestine Flora and Fauna,2019).

CHAPTER III

MATERIALS AND METHODS

3.1 Propolis source

Propolis samples were collected from three regions representative of the most important propolis producing regions in Palestine (Nablus – northern), (Jericho- eastern) and (Hebron – southern). In all these regions the collected honeybee type was the same species of local origin known as Harathy.

The samples were collected from these regions having in mind seasonal changes. The collection time for Jericho was the 8th of April 2018, for Hebron was the 14th of April 2018 and for Nablus was the 10th of September 2018. Hand-collected propolis samples were stored at -80˚C in dark until use. Voucher specimens were deposited at the Analysis Poison Control and Calibration Center at An - Najah National University, Palestine.

3.2 Chemicals

Bis-(trimethyl-silyl) trifluoroacetamide (BSTFA)(Merck-10255) and trimethylchlorosilane (TMCS) (Merck-2333), were used as sialylation reagents with spectrophotometric grade pyridine, were purchased from (Merck-7460).

3.3 Instruments and measurements

The GC-MS (Gas chromatography (Clarus 500) Perkin Elmer) Mass Spectrometer (Clarus 560D) Perkin Elmer) (Singapore). SLBTM-5ms fused silica capillary column 30m X 0.25mm X 0.25µm film thickness (SIGMA-ALDRICH- SUPELCO). Helium was used as a carrier gas at a standard flow rate of 1 ml/min.

The area size for generated peaks usually calculated by the system and it is usually = 1/2 X Hight X Width of the base. The area under the peak is considered as a function of that compound's amount in the sample.

The temperature of the injector was set at 220℃. Initial temperature 60℃, Initial Hold 2.00 min, Ramp (1) 3.0 min to 170℃, Hold 3.00 min to 250℃, Hold 120.00 min total run time 188.33 min. Source Temperature 250℃, GC Line Temperature 250℃, Electron Energy 70 eV.

Solvent delay 0 to 5.0 min, MS Scan Time 5.00 to 188.33 min, Mass 50.00 to 550.00 m/z,El+ Type MS Scan, Lon Mode El+, Data Format Centroid, Start Mass 50.00, End Mass 550.00 m/z

3.4 Sample preparation

Five grams of propolis samples were extracted for 30 min in an ultrasonic bath (Sonicor; SC-50Ð 22) with 100 ml of 70% ethanol, at room temperature. After filtration; extracts were evaporated to dryness under vacuum (nitrogen) at 50°C. After that, 1 mg of the dry extracted material was added to 50µl pyridine + 100µl bis-(trimethylsilyl) trifluoroacetamide (BSTFA) including 1% trimethylchlorosilane (TMCS) in a sealed glass tube and left to react for 30 min at 100°C. The reactant was applied into gas chromatography for analysis. The used sample size was1µl that directly injected to the column for GC-MS analysis (Kartal et al.,2002).

Derivatives of above used solvents were eliminated from the found chemicals in the tested samples.

3.5 Identification of compounds

Peaks were identified by computer searches using commercial reference libraries. The currently used program was Wiley and National Bureau of Standards (NBS) mass spectral library.

CHAPTER IV

RESULTS

4 Result

4.1 GC-MS analysis of different propolis samples collected from different regions of Palestine

Identification of chemical composition of propolis extracts was assessed by mass spectral analysis and the retention time RT of each compound are shown in figures (Figure 1, 2 and 3). The figures show the RT of each compound in each region, the X-axis of the gas chromatogram shows the amount of time taken for the analytes to pass through the column and reach the mass spectrometer detector. The peaks correspond to the time in minutes at which each of the components reached the detector. Y-axis area of each peak, the area in the chromatogram will be based on the number of counts taken by the mass spectrometer detector at the point of retention.

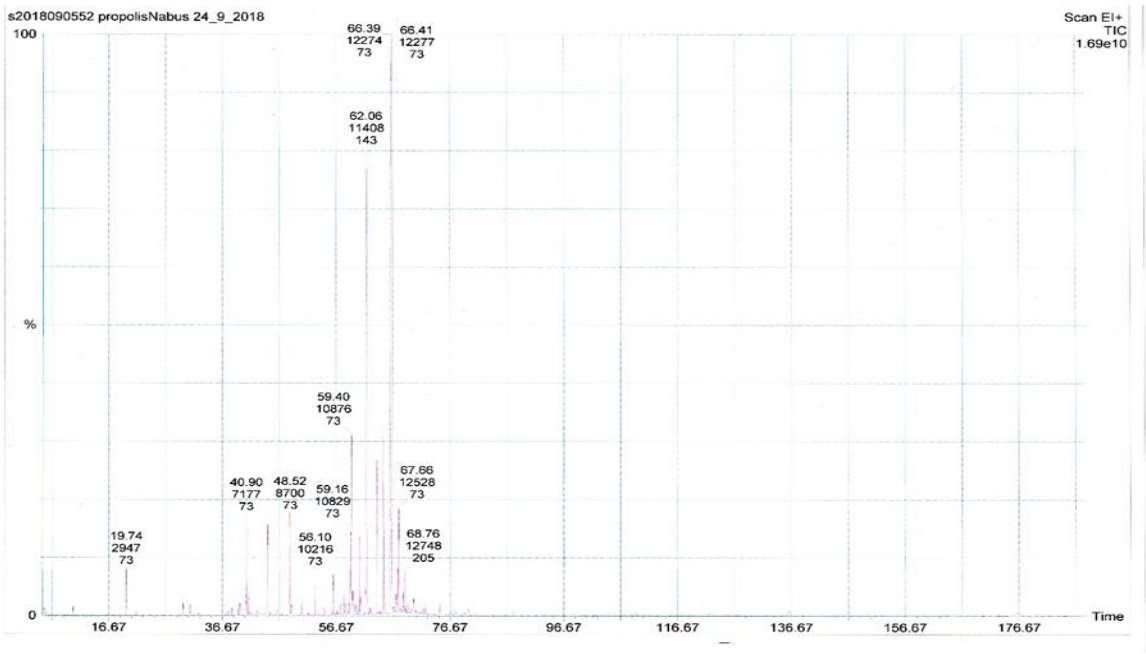

Figure 1. Mass spectrum analysis of the propolis sample collected from Nablus region

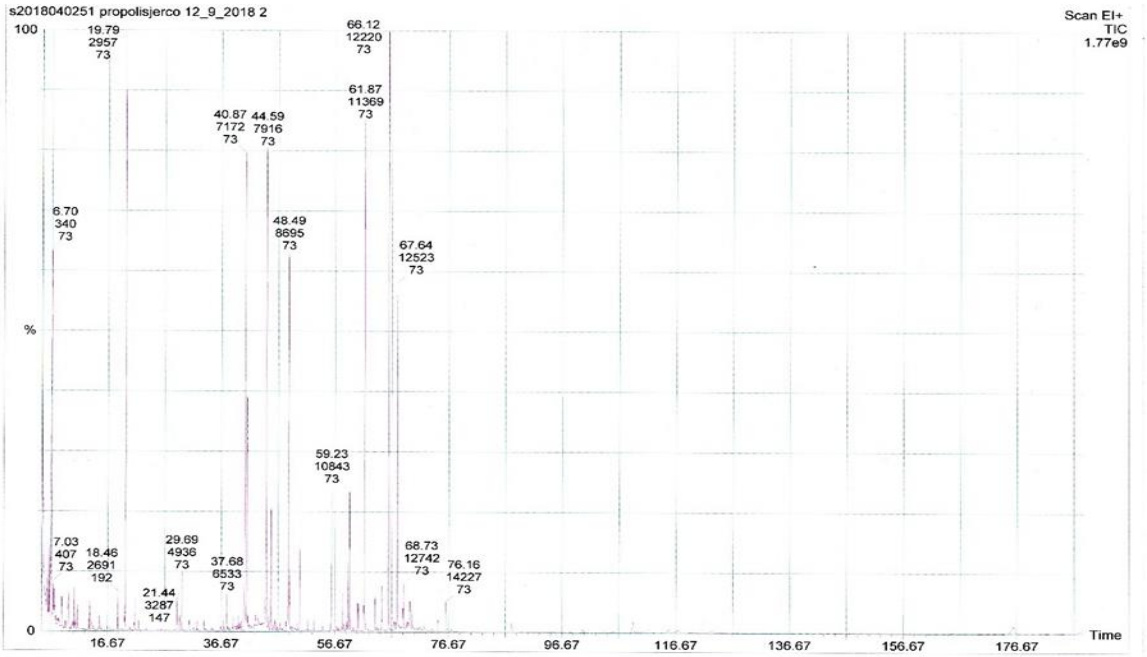

Figure 2. Mass spectrum of the propolis sample collected from Jericho region

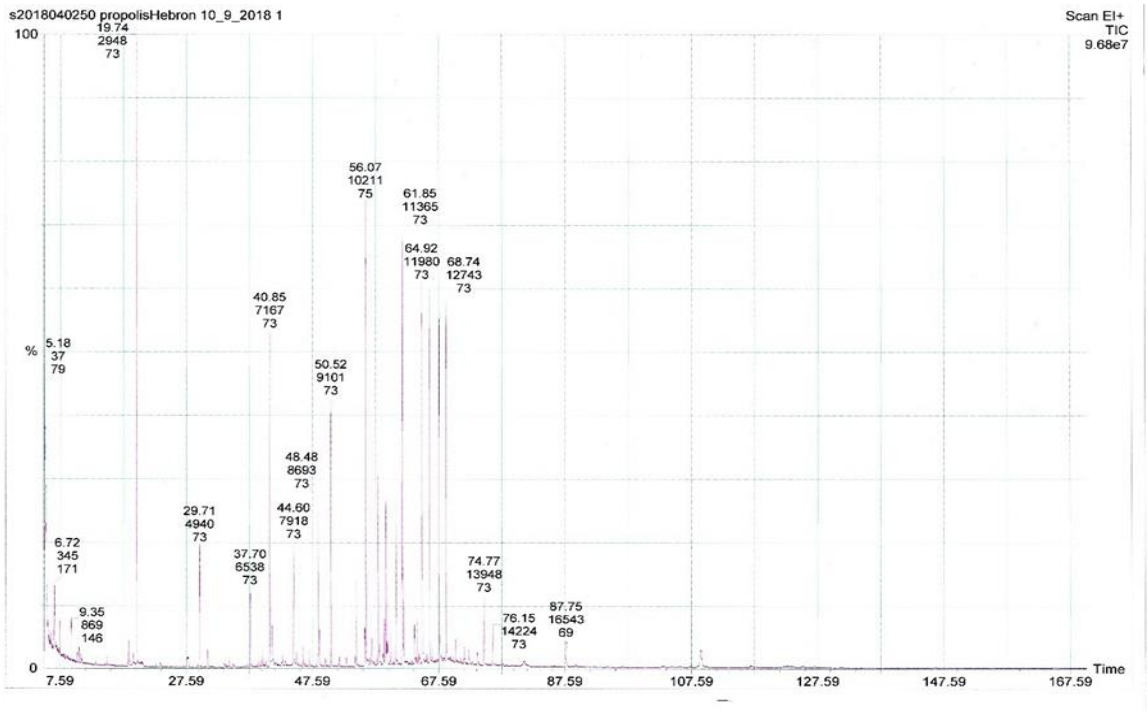

Figure 3. Mass spectrum of the propolis sample collected from Hebron region

Mass spectrum analysis showing magnified chromatogram showing some of these compounds

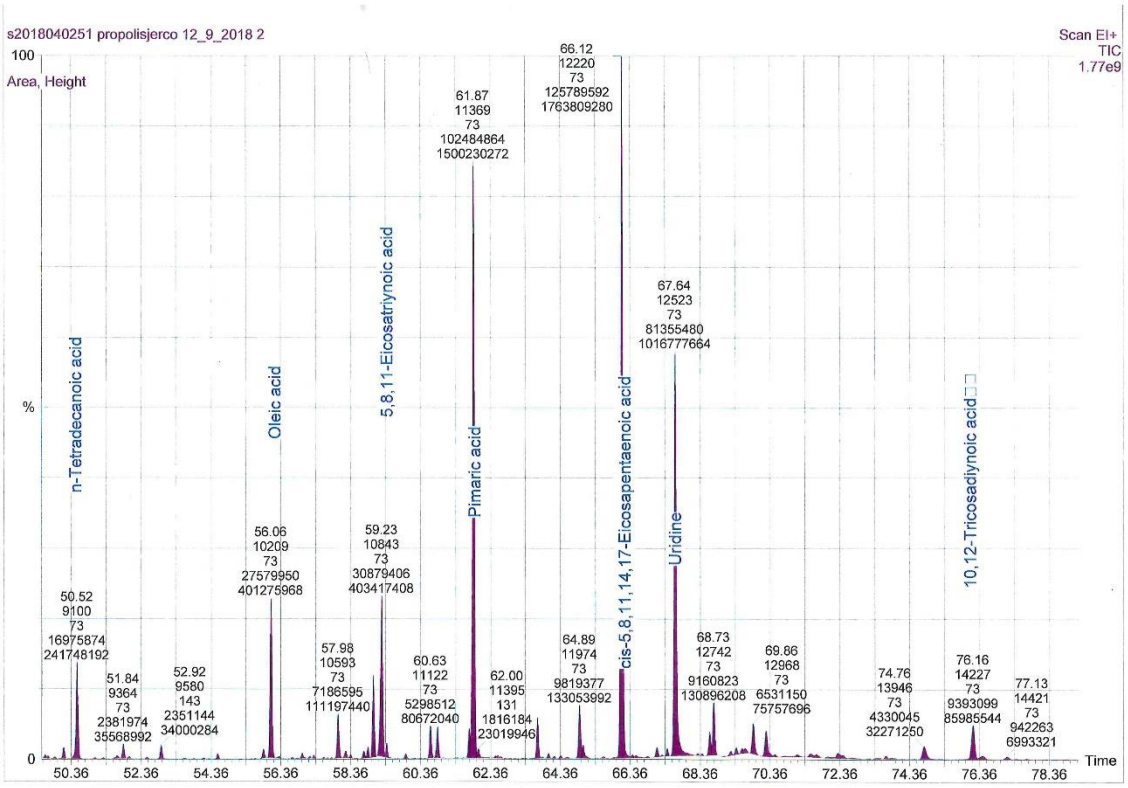

Figure 4. Mass spectrum analysis showing some of these compounds

In the above, Packs 1-7 represent: N-tetra decanoic acid, oleic acid, 5,8,11 eicosatriynoic acid, pimaric acid, cis 5,8,11,14,17-eicosapentaenoic acid, uridine and 10,12-tricosadiyynoic acid, respectively. This is a representative example of how compounds were identified.

The various components of propolis collected from different regions were identified and listed in Table 2. The total number of various compounds from different examined regions was 65. Some of these compounds were present in

one area and absent in the other area such as methyl ketone which was detected in samples collected only from Jericho and D-lyxose which was also found in samples collected from Hebron region, and the compound glucopyranose was present in samples collected from both Nablus and Jericho regions and absent in those samples collected from Hebron region. Three of the identified compounds were commune in the three tested regions (Table 6). Three compounds shared by Jericho and Nablus regions (Table 7). Only one compound shared by Jericho and Hebron regions (Table 8). None of the found compounds shared by Nablus and Hebron regions.

Table 2. Chemical composition of propolis samples collected from the different study regions of Palestine

Number	Chemical composition	Nablus	Jericho	Hebron
1	Propanoic acid	*	*	*
2	Glycerol	*	*	*
3	Oleic acid	*	*	*
4	Pimaric acid	*	*	*
5	D-Ribofuranose			*
6	D-robose	*	*	
7	Glucopyranose	*	*	
8	D-Xylopyranose	*	*	
9	10,12-Docosadiynedioic acid	*		
10	5,8,11 Eicosatriynoic acid		*	
11	Methyl Keton		*	
12	Ribitol		*	
13	1-Heptatriacotanol	*		

14	Androast-2, 16-diene	*		
16	1-O-Octadecylglycerol	*		
17	Monolinoleoylglycerol	*		
18	1-Naphthalenepropanol	*		
19	Flurbiprofen	*		
20	Eudemon	*		
21	9,12,15-Octadecatrienoic acid	*		
22	Diethylene glycerol		*	
23	D-Fucose		*	
24	2-Furanacetaldehyde		*	
25	Arabino furanose		*	
26	D-lyxose			*
27	Lyxose		*	
28	Uridine		*	
29	Erythritol		*	
30	Geranylgeraniol		*	
31	Spinescence (Squalene)		*	
32	Octanal		*	
33	Tetra decanoic acid		*	
34	Carbodiimide			*
35	Butadiendioxd			*
36	Ethylene alcohol			*
37	p-Dichlorobenzene			*
38	Vitamin E			*
39	Geranylgeraniol		*	*
40	9.12.15-octadecatrienoic acid	*		*
41	trans-farnesol		*	
42	Trans-trans-farensol			
43	dehydroabietic acid			*
44	cinnamic acid			*
45	Farnesyl alcohol			*
46	L-arabinose			*
47	D-galactose			*
48	ionic acid			*

49	Decanol			*
50	Pentose			*
51	Benzaldehyde			*
52	Octanoic acid			*
53	Caffeic acid			*
54	Myristic acid			*
55	Butadiendioc acid		*	
56	Pentol			*
57	Ribtol			*
58	Trimethylamine			*
59	Palustric acid			*
60	Archidic acid			*
61	Hexadecyl glycerol			*
62	cis-5,8,11,14,17-Eicosapentaenoic acid		*	
63	m-Hydroxymandelic acid		*	
64	10,12-Tricosadiynoic acid		*	
65	Cinnamic acid			*

4.2. RT and Area percentage of propolis chemical compounds found in samples collected from different regions of Palestine

The chemical composition of propolis samples, RT of each compound and area percentages are shown in tables 3, 4 and 5. The chemical composition of propolis samples from three regions and RT were presented in figures 5, 6 and 7.

Among the examined propolis samples collected from Nablus region, 17 different chemical compounds were identified using the GC-MS analysis. GC-MS chromatograms show the amount of time each compound spent on the column after it has been injected into the column which is around 141 minutes. Propanoic acid was the first compound to appear and eluted after the initial propolis injection at 10.36 minutes. The compound 9,12,15-Octadecatrienoic acid appeared as the last compound and eluted after 141.57 minutes of sample injection. The following is a list of compounds found in Nablus only: 1-heptatriacotanol, androast-2,16-diene, 1-O-octadecylglycerol, Monolinoleoylglycerol, 1-naphthalenepropanol, flurbiprofen, eudemon, 9,12,15-octadecatrienoic acid and 10,12-docosadiynedioic acid (Table 2).

The total number of compounds identified from samples collected from Nablus region was 17, among these 6 compounds occupied an area in the chromatogram of more than 5%. These compounds include:

1-heptatriacotanol, pimaric acid, androst-2,16-diene, α-D-xylopyranose, D-ribose and 10,12-docosadiynedioic acid. Percentage of the area for the other 17 compounds were less than 5% (figure 8).

The total number of identified propolis chemical compounds found in examined samples collected from Jericho region (27) was higher than that found in samples collected from Nablus region and the total RT was 122 minutes. The RT for these compounds was shorter than that for compounds found in samples collected from Nablus region. Methyl keton was the first compound to appear and eluted after the initial propolis injection at 6.7 minutes. The compound D-lyxose appeared as the last compound and eluted after 122.79 minutes of sample injection. The followings is a list of compounds found in Jericho only: 5,8,11 eicosatriynoic acid, methyl keton, ribitol, diethylene glycerol, D-fucose, 2-furanacetaldehyde, tetra decanoic acid, arabino furanose, lyxose, uridine, erythritol, trans-frnesol, spinescence, octanal, cis-5,8,11,14,17-eicosapentaenoic acid, m-hydroxymandelic acid, 10,12-Tricosadiynoic acid and butadecanoic acid (Table 2).

The total number of compounds identified from samples collected from Jericho region was 27, among these eight compounds were with a percentage area more than 5%; these compounds were uridine, methyl keton, glucopyranose, arabino furanose, D-xylopyranose, pimaric acid, glycerol and

cis-5,8,11,14,17-eicosapentaenoic acid. The percentage of the area for the other 19 compounds was less than 5% (figure 9).

The total RT of the GC-MS analysis for the propolis samples collected from Hebron region was 109 minutes and the total number of identify chemical compounds in this rejoin was 33 carbodiimide was the first compound to appear and eluted after the initial propolis injection at 6.72 minutes. The compound farnesyl alcohol appeared as the last compound and eluted after 109.05 minutes of sample injection. The following is a list of compounds found in Hebron only: pentol, ribtol, trimethylamine, palustric acid, archidic acid, hexadecyl glycerol, trans-trans-farensol, dehydroabietic acid, cinnamic acid, Farnesyl alcohol, L-arabinose, D-galactose, Ionic acid, decanol, pentose, benzaldehyde, octanoic acid, caffeic acid,9.12.15-octadecatrienoic acid, carbodiimide, butadiendioxd, ethylene alcohol, P-dichlorobenzene, D-lyxose, D-ribofuranose, vitamin E and myristic acid (Table 2).

Seven of these compounds were with an area more than 5%, these compounds include: myristic acid, L-arabinose, D-ribofuranose, trans, trans-farnesol, Archidic acid, oleic acid and Pentol. The percentage of area for the other 26 compounds was less than 5% (figure 10). Although Hebron region showed the highest number of chemical compound species compared to the other regions, the total RT was lowest than Jericho and Nablus samples.

Table 3. RT and Area percentage of propolis chemical compounds found in samples collected from Nablus region

Name	R. T	Area	%Area
Propanoic Acid	10.36	242583872	0.507815356
Glycerol	19.74	1433890688	3.001649304
D-Ribose	40.9	2991077376	6.261401513
D-Xylopyranose	48.52	2948505600	6.172283463
Oleic Cid	59.16	202905075	0.424753353
Pimaric Acid	62.06	13605519360	28.48124899
1-Heptatriacotanol	65	5240982528	10.97126279
Androst-2,16-diene	66.39	16834110464	35.23985223
10,12-Docosadiynedioic acid	67.66	3037415680	6.358404261
Glucopyranose	70.24	441269472	0.923735829
1-O-Octadecylglycerol	74.77	399270080	0.835815985
Geranylgeraniol	87.81	122083832	0.255565401
Monolinoleoylglycerol	98.3	56069428	0.117373494
1-Naphthalenepropanol	109.19	67729320	0.141781844
Flurbiprofen	122.33	8362872	0.017506501
Eudesmol	136.38	127501784	0.266907125
9,12,15-Octadecatrienoic acid	141.57	10816371	0.022642558

Table 4. RT and Area percentage of propolis chemical componds found in samples collected from Jericho region

Name	R.T	Height	Area	% Hight	%Area
Methyl Keton	6.7	160606720	1059583296	13.5808803	7.761845133
propionicacid	10.73	5666443	119229792	0.47915358	0.873402954
Diethylene glycerol	18.46	6191737	114775488	0.52357236	0.840773506
Glycerol	19.79	113283480	1745293824	9.57923421	12.78493199
Butanedioc acid	21.44	5246659	91115160	0.4436567	0.667452728
Erythritol	29.69	11839417	17592816	1.00113934	0.128873977

2-Furanacetaldehyde	37.68	7983362	110462832	0.6750719	0.809181683
Arabinofuranose	40.87	117571208	1360073344	9.94180386	9.963047459
D-Ribose	41.19	64196956	663562752	5.42848505	4.860846086
D-Xylopyranose	44.59	160487824	1409895680	13.5708265	10.32801476
Ribitol	45.41	31539828	358465312	2.66700316	2.625892884
Glucopyranose	48.49	76983648	1093894912	6.50972582	8.013190591
Tetradecanoic acid	50.52	16975874	241748192	1.43547738	1.770896195
Oleic acid	56.06	27579950	401275968	2.33215647	2.939496999
5,8,11-Eicosatriynoic acid	59.23	30879406	403417408	2.61115798	2.955183851
Primaric acid	61.87	102484864	1500230272	8.66610485	10.98974954
cis-5,8,11,14,17-Eicosapentaenoic acid	66.12	125789592	176380928	10.6367492	12.92056465
Uridine	67.64	81355480	101677764	6.87940728	7.44827782
10,12-Tricosadiynoic acid	76.16	9393099	85985544	0.7942795	0.629876366
Geranylgeraniol	87.76	4567829	27012824	0.3862549	0.197879069
trans-Farnesol	100.2	2405825	8234805	0.2034362	0.060323036
m-Hydroxymandelic acid	104.19	881190	2621867	0.0745133	0.019206159
Spinacene	109.11	10480560	32300786	0.8862345	0.236615375
Octanal	110.74	792938	2381228	0.0670507	0.017443388
Tetradecanoic acid	115.38	364554	9240632	0.0308266	0.067691096
D-fucose	117.02	4010665	7713145	0.3391412	0.056501681
D-Lyxose	122.79	3035215	4483188	0.2566573	0.032841034

Table 5. RT and Area percentage of propolis chemical compounds found in samples collected from Hebron region

Name	R.T	Area	% Area
Carbodiimide	6.72	9167496	1.4509896
Ethylene alchohol	7.49	5399193	0.85455973
p-Dichlorobenzene	9.35	6330783	1.00200756
Vitamine E	10.13	756856	0.11979173
phenylpropionic acid	10.6	2465134	0.39017021
Decanol	11.1	309171	0.04893418
Pentose	13.24	551015	0.08721215
propionic acid	14.97	1937393	0.30664176
caffeic acid	18.31	2592594	0.410344
Benzaldehyde	18.51	4127806	0.65333037
octanoic acid	19.21	1879353	0.29745545
Glycerol	19.3	414045	0.06553316
Butadiendioc acid	29.71	18866478	2.98610039
cinnamic acid	30.99	2767425	0.43801545
Pentol	37.7	122966704	19.4626111
L-arabinose	40.85	51101596	8.08812837
D-galactose	44.6	18981248	3.00426567
Ribtol	46.07	3402175	0.5384808
Myristic acid	50.52	41127464	6.50946809
lionic acid	54.54	1339987	0.21208705
oleic acid	56.07	70688664	11.1882805
Trimethylamine	57.99	28587380	4.52468058
pimaric acid	58.85	6049032	0.95741329
Palustric acid	59.92	5483123	0.86784379
dehydroabiettic acid	60.84	25041620	3.9634738
Archidic acid	61.85	63929576	10.1184827
trans,trans-farnesol	64.92	58307860	9.22870308
D-Ribofuranose	67.63	52741300	8.34765327
D-Lyxose	70.24	3858659	0.61073101
9.12.15-octadecatrienoic acid	71.66	2754906	0.436034
Hexadecylglycerol	74.77	10952735	1.73354912
Geranylgeraniol	87.75	4257867	0.67391584
Farnesyl alcohol	109.05	2673265	0.42311223

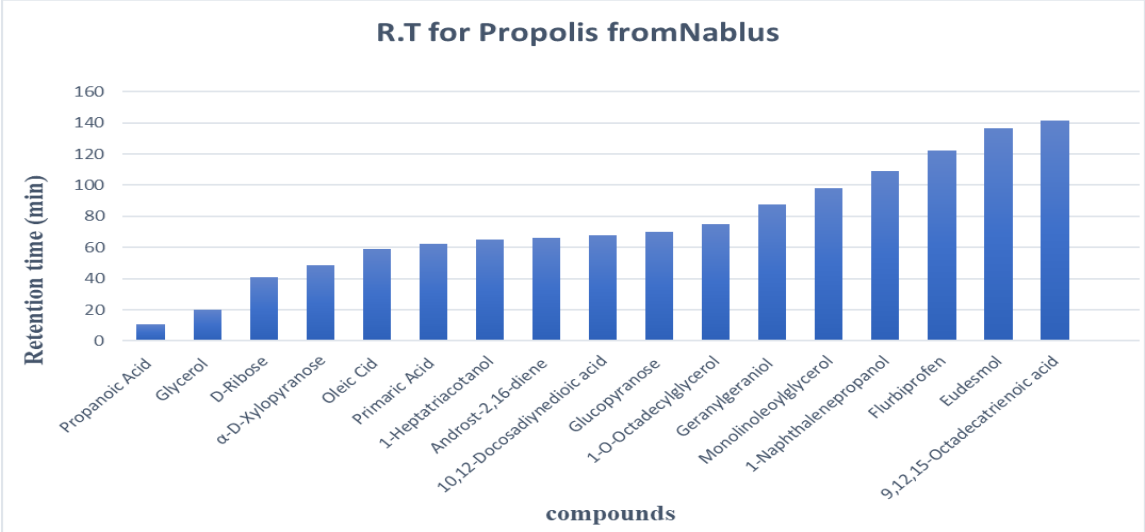

Figure 5. RT of propolis chemical compounds found in samples collected from Nablus region

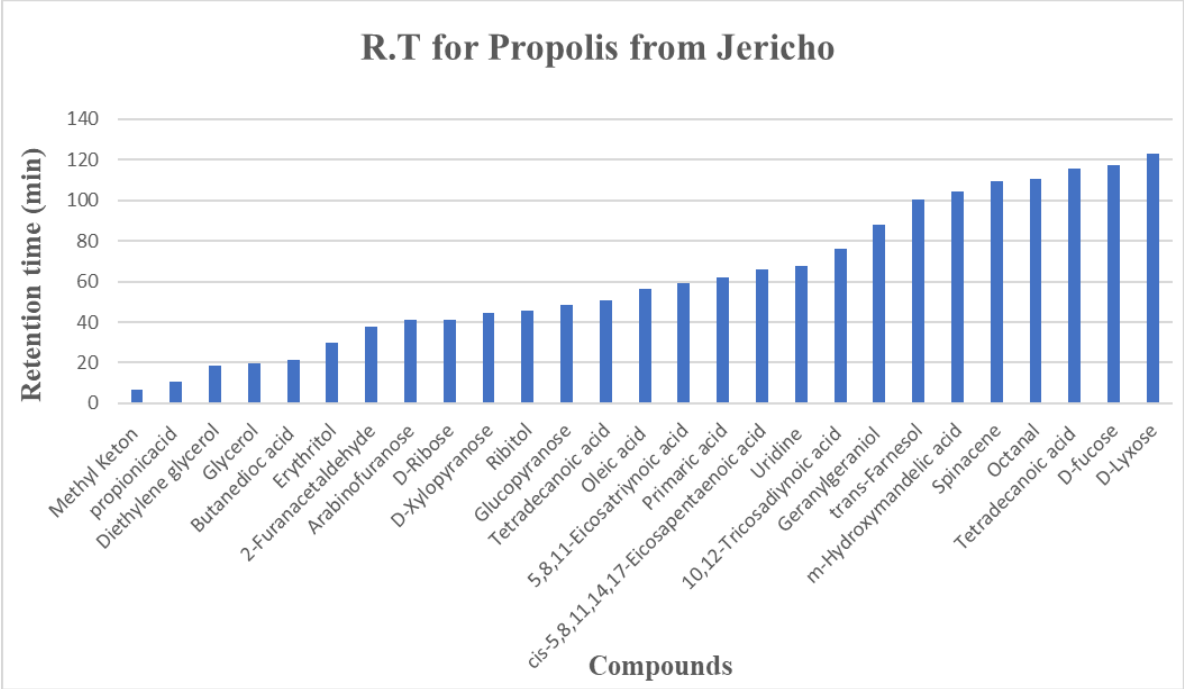

Figure 6. RT for propolis chemical compounds found in samples collected from Jericho region

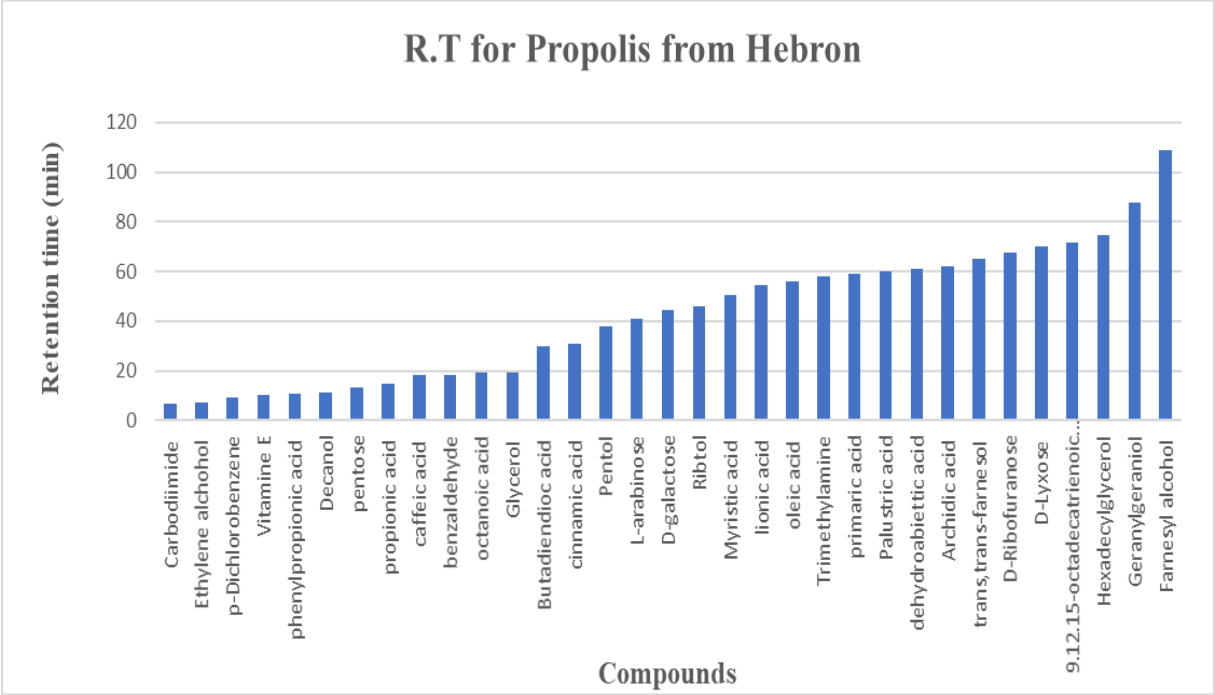

Figure 7. RT of propolis chemical compounds found in samples collected from Hebron region

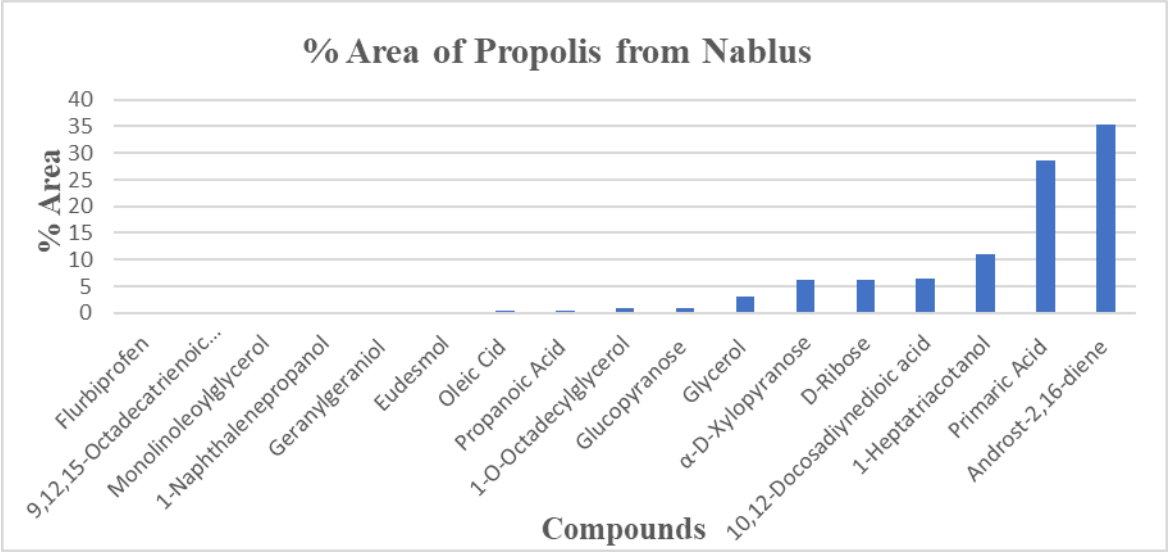

Figure 8. Area percentage of propolis chemical compounds found in samples collected from Nablus region

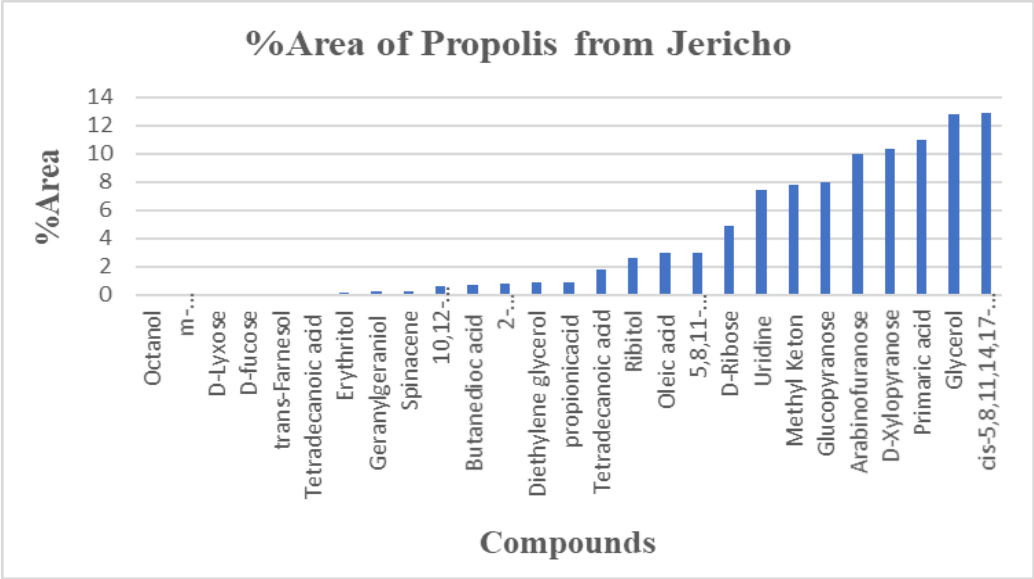

Figure 9. Area percentage of propolis chemical compounds found in samples collected from Jericho region

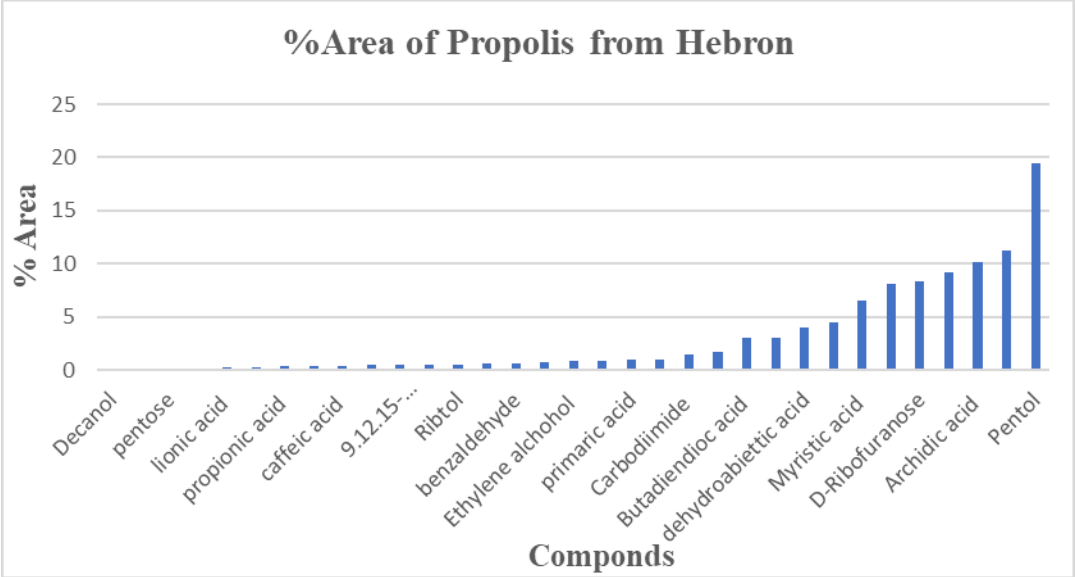

Figure 10. Area percentage of propolis chemical compounds found in samples collected from Hebron region

4.3 The Area size for each common shared compound

Data presented in table 7 show the area size for each of the four common shared compounds by the three regions (glycerol, propanoic acid, oleic acid and pimaric acid). Differences in area size between regions of each of the four compounds: glycerol area was 1.43, 1.74, 0.0004 in Nablus, Jericho and Hebron, respectively. Propanoic acid area was 0.24, 0.119, 0.119in Nablus, Jericho and Hebron, respectively. oleic acid area was 0.20, 0.40, 0.07 in Nablus, Jericho and Hebron, respectively. With respect to pimaric acid area size was 13.60, 1.50, 0.006 in Nablus, Jericho and Hebron, respectively. It is worth noting that pimaric acid area was the largest in samples collected from Nablus region contains significant amount compared to the area size of propanoic, oleic and pimaric acid. pimaric acid area size was also found to be the largest in the tested samples of the three regions.

Data presented in table 7 show the area size of the three shared compounds (D-robose, glucopyranose and D-xylopyranose) found in Nablus and Jericho regions. Differences of area size between the two regions of each of the three compounds: D-ribose area was 2.99, 0.66 in Nablus and Jericho, respectively. glucopyranose area was 0.44, 1.09 in Nablus and Jericho, respectively. D-xylopyranose area size was 2.94, 1.40 in Nablus and Jericho, respectively. D-

xylopyranose area was the largest reflecting variations in the concentration of these product compared to D-ribose and glucopyranose. It was also found that glucopyranose concentration was higher in samples collected from Jericho compared with those samples collected from Nablus region, on the other hand, both D-robose and D-xylopyranose were with higher concentration in the samples collected from Nablus region.

Data presented in table 8 shows the area size for the compound geranylgeraniol, found in collected propolis samples, is shared by Jericho and Hebron regions. Geranylgeraniol area size was 0.027, 0.0042 in Jericho and Hebron respectively, indicating higher concentration of these compound in samples collected from Jericho compared to that among the samples collected from Hebron.

Table 6. Area size for common chemical compounds found in samples collected from all study regions

Study region	Propanoic Acid	Glycerol	Oleic Acid	Pimaric Acid
Nablus	242583872	143389069	20290508	13605519360
Jericho	119229792	174529382	40127597	1500230272
Hebron	119229792	000041405	7068866	6049032

Table 7. Area size for the three common shared compounds found in samples collected from Nablus and Jericho regions

Study region	D-robose	Glucopyranose	D-Xylopyranose
Nablus	299107738	441269472	29485056
Jericho	66356275	1093894912	140989568

Table 8. Area size for the share compounds found in samples collected from Hebron and Jericho regions

Study region	Geranylgeraniol
Jericho	27012824
Hebron	4257867

CHAPTER V

DISCUSSION

Discussion

It is well known that identification of mixtures of natural products by mass spectral analysis alone is difficult due to the occurrence of the number of isomers for various compounds with minor differences in their mass spectra (Pereira et al., 2000).In current study time limitation, lack of equipment and financial support are the major obstacles that restrict the use of different methodologies for identification and characterization of chemical compounds and that's why our methodology was restricted to the use of GC-MS analysis alone.

The samples of propolis were taken from Nablus, Jericho and Hebron the most important propolis-producing regions in Palestine. The total ethanolic mixtures were isolated from the samples and their chromatograms were taken from single capillary column as described in methodology section. GC-MS chromatogram revealed very complex compositions and provided evidence for the presence of 65 different of chemical compounds in all tested samples.

Based on the very complex chemical compositions of propolis and its pharmacological and therapeutic properties, it seems that propolis is a very powerful natural product produced by bees with a great potential for the

treatment of human and veterinary diseases with great success. The great problem with propolis, as with some other hive products, its composition varies with the flora of a given area, the time of collection and the inclusion of wax contaminants.

A study conducted in two regions of Turkey documented 45 different chemical compounds of the propolis samples in these regions using the GC-MS analysis (Kartal et al.,2002). Variation between number of chemical compounds in both studies is most likely to reflect biodiversity and climatic variations. This is an expected finding as Palestine is known for its great biodiversity.

The highest number of chemical compounds in propolis was found in samples collected from Hebron region (33). Such high variations in chemical composition of propolis could be attributed to plant biodiversity, which constitute a major factor that affect propolis composition (Khan et al., 2018). This is also expected as Hebron region well known for it is wide biodiversity resulted from the climatic condition of the region. The lowest number of different chemical compounds was found in propolis samples collected from Nablus region (17). With respect to number of chemical compounds (27) in propolis samples collected from Jericho region; the number is higher than that found in samples collected from Nablus region and lower than that found in

sample collected from Hebron region. Differences in compounds number could be due to different climatic conditions, time of sample collection, plant biodiversity and cultivation practices in these regions. In this respect, Jericho region is well-known for land use for agricultural purposes compared to Nablus region which could be classified as semiarid region and land use for cultivation is limited compared to the other two studied regions.

RT is a measure of the time taken for a solute to pass through a chromatography column (time from injection to detection). Here we present data for RT for samples collected from Nablus region as a representative example (see tables 3, 4, 5). GC-MS chromatograms show the amount of time each compound spent on the column after it has been injected onto the column which is around 141 minutes. Propanoic acid was the first compound to appear and eluted after the initial propolis injection at 10.36 minutes. The compound 9,12,15-Octadecatrienoic acid appeared as the last compound and eluted after 141.57 minutes of sample injection.

Variations in RT for different samples from different regions is expected as propolis chemical composition vary between regions and affected by the nature of flora and fauna of the studied regions. This is clear from the finding of different number of chemical compounds found in each region.

Using the same columns and the same G-C system for identification of propolis composition from different regions, one should expect similar if not identical RT for any specific compound. That is well known fact about the RT for a compound is not fixed as many factors can influence it even if the same GC-MS and column are used. To clarify this idea, Glycerin RT was used as an example which showed a RT of 19.74, 19.79, 19.3 in Nablus, Jericho and Hebron, respectively. RT for this compound is almost identical, however noticeable differences in RT for other product were found such as RT for Propionic acid (10, 10, 14). Both compounds were selected as examples for their RT as both compounds shared by the three studied regions.

The finding on RT reflects consistency of the used the GC-MS chromatography system. This finding also ensures the validity and importance of using this system for the purpose separation and identification of chemical composition of materials such as propolis.

Combining data for RT and area size for the four common compounds shared by all studied regions, we found that all these compounds have similar RT, however great variations in area size was found as shown in (figure 10). In this figure pimaric acid showed a pronounced increase in area size compared to the rest of the common shared compounds. variations in area size reflects compound concentration and dealing with different regions it is expected to

see variations in concentration of different products because of the differences in climate, geographic location and the source of the plant that the bees feeds on.

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CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

Conclusions and Recommendation

In the current study, propolis samples were collected from three different regions known for their biodiversity. The biodiversity was reflected because of climatic variations, which plays a major rule in the high biodiversity reported for Palestine.

GC-MS revealed very complex compositions and provided evidence for the presence of 65 different of chemical compounds in all tested samples in our area. The finding of large number of compounds in the studied areas in comparison with studies reported from other countries like Turkey is most likely due to high plant biodiversity seen in Palestine.

Several studies showed that propolis is a very powerful natural product with a great potential for the treatment of human and veterinary diseases with great promising success.

Because of its special chemical components, strong pharmacological, properties and low toxicity. This wide spectrum of therapeutic effects makes propolis a potential candidate in several clinical scenarios. We were encouraged by this fact and evaluation of the identified compounds for their future use as pharmaceutical products was within the scope of our study. However, due to time and cost limitations we were unable to follow this aim.

In this respect, Propanoic acid was found to be one of the four major components in all studied samples. This compound was reported to have a potential promising effect for the treatment and prevention of diseases and to their use for preparing medications for the treatment and prevention of cardiovascular disease. Glycerol was another major component in our samples. This compound is also known for its potential use in pharmaceutical, medical and personal care preparation.

Based on our finding and previous studies on the importance and potential use of the various propolis compounds, it is reasonable to encourage further studies on the major compounds of propolis, especially for challenging human diseases like cancer. We also encourage beekeepers for periodic change of hive location between various regions to increase the chance of production of propolis with different composition. Changes in propolis composition through this process might result in the production of propolis with high potential value in therapeutic use.

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الملخص باللغة العربية

يعتبر العكبر (البروبوليس) من المركبات العضوية التي ينتجها النحل وذلك من خلال امتصاص المواد العديدة من براعم الأشجار وخلطها باللعاب. يستخدم النحل هذه المادة لإغلاق الثقوب في الخلايا وكذلك كمادة معقمة ومقاومه للبكتيريا والفطريات والفيروسات. تم مؤخرا ملاحظة أهمية هذه المادة وقيمتها باعتبارها واحدة من المصادر القيمة للمركبات العلاجية حيث أثبتت مركبات البروبوليس العضوية أن لها خصائص بيولوجية وصيدلانية عديدة مثل الأنشطة المضادة للبكتيريا والمضادة للسرطان والمضادة للالتهابات.

هدفت هذه الدراسة استكشاف المكونات العضوية للعكبر من خلال دراسة عينات مختلفة جمعت من مناطق جغرافية ممثلة لبيئة منطقة فلسطين ولتحقيق هذا الهدف تم جمع عينات البروبوليس من مناطق مختلفة من كل من مناطق مدن أريحا والخليل ونابلس في العام 2018-2019. تم تحضير مستخلصات العكبر باستخدام الإيثانول وحفظت العينات لحين التحليل الكيميائي وذلك باستخدام كروماتوجرافيا الغاز إلى جانب الطيف الكتلي (GC-MS).

تم تحديد سبعة وعشرين مركب من العينات التي جمعت من منطقة الخليل وتم تحديد اثنين وعشرين مركبة من عينات منطقة أريحا كما تم التعرف على ثمانية عشر مركبا من العينات التي جمعت من منطقة نابلس. وبالتالي فان مجموع المواد المختلفة والتي لوحظ تواجدها في فلسطين هو 65 مركبا.

تبين وجود تشابه في تركيب عينات البروبوليس في مناطق الدراسة ممثلا بوجود اربع مواد مشتركه عند كافة مناطق الدراسة وهي: حمض الأسكوربيك ، الجلسرين ، حمض الأوليك وحمض البيمريك. لوحظ كذلك احتواء العينات ألمجموعه من كل من منطقة أريحا والخليل على نسب جلسرين أكثر من تلك العينات المجموعة من منطقة نابلس. لوحظ وجود مركبات مشتركة بين كل من منطقة نابلس و اريحا مثل:(D-robose, Glucopyranose, D-Xylopyranose). بالاضافه الى ذالك لوحظ وجود Geranylgeraniol مشترك بين كل من منطقه اريحا و منطقه الخليل. ان هذه الاختلافات التركيبية في المناطق المختلف هي نتائج متوقعة نظرا لاختلاف العوامل البيئية والتي تنعكس على طبيعة الغطاء النباتي. تبين نتائج التحليل للفترة الزمنية التي استغرقتها المستخلصات من مناطق الدراسة المختلفة للمرور عبر أعمدة التحليل وجود اختلافات وكانت الفترات على النحو التالي: 141

و 122 و 104 في كل من نابلس و لريحا و الخليل على التوالي. حيث تعكس هذه الاختلافات اختلافات تركيبية كانت واضحة من حيث أعداد المركبات وطبيعتها في كل منطقه.

للاستفادة من نتائج الدراسة الحالية هنالك حاجة لدراسة المركبات المختلفة والتي تم العثور عليها ومقارنتها في بيئات عالمية وذلك بهدف الاستفادة منها في المجالات الصناعية الدوائية وغيرها من الصناعات حيث أن العديد من الدراسات تشير إلى امكانات كبيرة في مركبات العكبر.