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Extraction of Egyptian Propolis by Different Solvents and Their Antibacterial Activities

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Abstract
Objectives: *Propolis, a resinous product produced by honeybees, has been known for its pharmacological bioactivities particularly its antimicrobial potential. This study aimed to analyze chemical composition and antibacterial activity of four Egyptian propolis samples collected from different governorates.*
Methods: *four propolis samples named Q, F, L and G, were extracted by three solvents; 70% ethanol, absolute methanol and chloroform. The resulting extracts were analyzed by gas chromatography– mass spectrometry (GC/MS), which revealed a range of bioactive compounds. Antibacterial activities of each extract was evaluated against multi-drug resistant (MDR) bacteria, including, E. coli, Serratia sp., Klebsiella spp., Proteus spp., Pseudomonas aeruginosa and Staphylococcus aureus.*
Results: *The ethanol extract of propolis (EEP) yielded the highest number of compounds: 36, 57, 35, and 45 compounds for propolis samples Q, F, L, and G respectively, followed by the methanol extract of propolis (MEP). Sample F contained the highest number of compounds among the four samples. Major identified bioactive compounds included; Benzo-Pyran derivatives, α-amyrin; Hexanedioic acid, Octacosane, Palmitic acid, Pyrido[2,3-b] indole, Lup-20(29)-en-3-ol acetate (3α)-, Heptacosane; Methyl-3-Oxurs,12-en-23-oate, and Dotriacontane. Both MEP and EEP demonstrated the notable antibacterial activity against the tested MDR bacteria, while chloroform extract of propolis (CEP) was efficient against Staphylococcus aureus.*
Conclusion: *The Egyptian propolis contains a diverse of bioactive compounds, and its antibacterial activity is influenced by the solvent used for extraction. The findings supported its potential as alternative agent against MDR bacteria.*
Keywords: Propolis extraction; GC/MS; Bioactive compounds; Multidrug-resistant bacteria; Antibacterial activity.

INTRODUCTION

Honeybee products have been documented for their nutritional value and therapeutic potentials, with propolis being one of the most widely studied in folk medicine for thousands of years [1], particularly in wound healing [2], with people worldwide appreciating its therapeutic properties [3]. Honeybees (Apis mellifera species) produce propolis, a resinous material derived from a variety of plant sources combined with salivary enzymes, waxes and pollen [4]. Propolis plays an essential role in maintaining the beehive health: Bees use it in sealing the cracks, protect the beehives from predators [5], and act as a natural disinfectant [6]. Due to its sticky nature, raw propolis is frequently extracted using solvents, which help preserve its bioactive polyphenolic compounds [7]. These compounds, particularly phenolics and flavonoids, have been linked to various therapeutic effects [8]. The therapeutic ability of propolis largely depends on its chemical composition, which is influenced by botanical origin and extraction methods. Solvent extraction, particularly using ethanol or methanol, is widely employed to isolate its active constituents, mainly flavonoids and phenolic acids [8, 9]. These bioactive compounds have been related to a range of pharmacological effects, including antibacterial, antioxidant, and wound-healing activities [10, 11].

The global rise of antibiotic-resistant pathogens has become a life-threatening public health issue; as conventional antibiotics continue to lose effectiveness against multidrug-resistant (MDR) bacteria [12]. Hospital-acquired infections, frequently caused by MDR strains such as E. coli, Klebsiella spp., and Staphylococcus aureus, contribute significantly to morbidity and mortality worldwide. The lack of new

antibiotics has incited the search for alternative therapies that can either replace or potentiate current antimicrobial agents [13]. Recent studies have highlighted propolis potential against multidrug-resistant bacteria, making it a promising candidate for developing new antibacterial agents [14, 15]. So, this study aimed to extract different Egyptian propolis samples by different solvents and identify the resulted compounds by GC/MS. These different extracts were tested against multi-drug resistant bacterial strains to overcome this serious health concern.

2. MATERIALS AND METHODS:

2.1 Sampling sites and collection

Four propolis samples named **(Q, F, L** and **G)** were collected from four Egyptian governorates **(Fig.1)** and provided by honeybee keepers from Apis mellifera sp. Hives by the traditional scraping technique. The first sample **(Q)** was obtained from Qina, where camphor and acacia plants predominate. The second sample **(F)** came from Al Fayyum, where the predominant plants were citrus trees; the third sample **(L)** was obtained from Luxor, where the predominant plants were camphor, clover, and acacia. The fourth sample **(G)** was collected in Giza, where camphor, mango, and citrus trees were the predominant plants. The propolis samples were collected during the autumn season of 2019, stored in dark, sterile polyethylene plastic bags that were kept in the freezer (- 4°C) until further use.

Fig. (1): Map of Egypt showing the four collection sites of propolis samples.

2.2. Chemicals used:

The solvents; absolute ethanol, methanol and chloroform were purchased from BDH (British drug houses, UK) While pure DMSO (dimethyl sulfoxide) was purchased from Sigma Aldrich (France). BSTFA (Bis-trimethylsilyl trifloroacetamide) was purchased from LOBA CHEMIE (India) and pyridine was sourced from Sigma-Aldrich (Germany).

2.3. Preparation and extraction of propolis samples:

About 40 gm of each raw propolis sample (Q, F, L and G) was ground and added to 1000 ml of each solvent; (70% ethanol, absolute methanol and absolute chloroform). the flasks were sealed, wrapped with aluminum foil and stored at room temperature (25°C) in the dark for two weeks with occasional shaking. After incubation, the extracts were filtered twice using Whatman No. 1 filter paper (Sigma-Aldrich, Germany), and the solvents were evaporated under reduced pressure at 50°C using a rotary evaporator. The resulting semi-solid residues were labeled as ethanol extract of propolis (EEP), methanol extract (MEP), and chloroform extract (CEP). The dry extracts were collected with a spatula, stored in polyethylene bags, and kept at – 4°C until further use [16].

2.4. Preparation and GC/MS Analysis of Propolis Samples:

According to [17], about 5 mg of each dry propolis extract (Q, F, L, and G) was derivatized by adding 75 µl of pyridine and 50 µl Bis (trimethyl, silyl) trifluoroacetamide (BSTFA) in dark glass vials. The mixtures were heated at 80 °C for 20 minutes, after which the supernatants were subjected to gas

chromatography–mass spectrometry (GC-MS) analysis. The analysis was carried out using a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a TG–5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The GC oven was programmed to start at 50 °C, then ramped at 5 °C per minute to 250 °C and held for 2 minutes. A second ramp increased the temperature to 300 °C at 30 °C per minute, also held for 2 minutes. Injector and MS transfer line temperatures were set to 270 °C and 260 °C, respectively. Helium was used as the carrier gas with a constant flow rate of 1 mL/min. A 4-minute solvent delay was applied. One microliter of each diluted sample was injected automatically using an AS1300 auto sampler in split mode. Electron ionization (EI) was performed at 70 eV, and spectra were acquired in full scan mode over a mass range of m/z 50–500. The ion source temperature was maintained at 200 °C. identification of compounds was achieved by comparing the obtained mass spectra with entries in the WILEY 09 and NIST 14 spectral databases. GC/MS analysis was conducted at Nawah scientific, Mokattam, Cairo, Egypt.

2.5 Bacterial strains:

Twenty-seven clinical multidrug-resistant (MDR)bacterial strains, previously collected from Egyptian hospitals, were kindly provided by Dr. Mervat Aly Abo-state.[18, 19, 20].

2.6: Antibacterial assays:

All dried propolis extracts were weighed under sterilized conditions and transferred into sterile, dark vials. Each sample (0.5 gm) was dissolved in 10 mL of DMSO to prepare stock solutions. Antibacterial activity was assessed using a modified Kirby–Bauer disc diffusion method on LB agar plates, following the guidelines of the Clinical Laboratory Standards Institute [21], as adjusted by [22]. A bacterial suspension of each multidrug-resistant (MDR) strain was prepared in LB broth at a concentration of 1×10^5 CFU/mL and uniformly spread on the surface of LB agar plates. The inoculated plates were allowed to rest for one hour at room temperature to allow evaporation of excess moisture. Sterile filter paper discs (9 mm diameter) were carefully placed onto the surface using sterile forceps, and each disc was loaded with 50 µl of each propolis extract. Plates were incubated at 37 °C for 24 hours, after which the diameter of the inhibition zones around the discs was measured. All tests were conducted in triplicate, and chloramphenicol discs served as the standard antibiotic control.

3. RESULTS:

Four Egyptian propolis samples from different regions were analyzed in this study. Three solvents were used to extract these propolis samples. GC/MS was used to analyze the extracted propolis samples. The GC/MS analysis of ethanolic extract (EEP) revealed a diverse range of bioactive compounds across the samples. Sample Q yielded 36 compounds, as indicated in **Fig. 2 (a).** The predominant compounds in sample Q were pyrido[2,3-b] indole (12.34%), 4H-1-benzopyran-4-one, 2, 3-dihydro-5,7-dihydroxy-2-phenyl-s- (22.16%), and benzo[j] fluoranthene-10-ol (23.33%). Other compounds in lower concentrations, linoleic acid ethyl ester (3.35%); hexanedioic acid bis(2-ethyl-hexyl) ester (2.84%).

Propolis sample F exhibited the highest number of 57 compounds as indicated in **Fig. 2 (b)**. The predominant compounds in sample F were guanidine (6.94%); guaiol (8.96%); 4H-1-benzopyran-4-one 2,3-dihydro-5-hydroxy-7-methoxy-2-phenyl (12.46%); dihydrochrysin (10.76%); 4H-1-benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl (17.44%); benzo[b] phenanthro [2,3-d] thiophene (4.13%); and lup-20(29)-en-3-ol, acetate (3α)- (3.55%). In addition to, benzeneethanol (1.36%), benzofuran 2, 3-dihydroxy (coumaran) (1.49%), oleic acid (0.18%) and linoleic acid ethyl ester (1.79%). In case of sample L extracted by ethanol, GC/MS analysis revealed 35 compounds as shown in **Fig. 2 (c)**. The most prevalent compounds were hexanedioic acid, bis(2-ethylhexyl) ester (19.42%); dotriacontane (10.48%); 9, 12-octadecadienoic acid (z, z)-2,3-bis[(trimethylsilyl) oxy] propyl ester (5.61%) and trilinolein (10.73%). Amongst acetamide, n, n-diethyl (2.79%); tetrasiloxane, decamethyl (3.59%); cyclotetrasiloxane, octamethyl (4.48%); benzoic acid, 2-hydroxy-methyl ester (1.72%); hexadecanoic acid ethyl ester (1.87%); oleic acid (0.73%) and ethyl oleate (4.93%). Sample G, extracted using ethanol, yielded a total of 43 identified compounds, as shown in **Fig. 2(d)**. The major constituents involved lup-20(29)-en-3-ol acetate (3α)- (15.84%), hexadecanoic acid methyl 2-ethylhexyl ester (9.83%) and trilinolein (7.25%). Other notable compounds detected in moderate to lower concentrations were 9,12,15-octadecatrienoic [(trimethyl silyl) oxy]-1-acid [(trimethyl silyl) oxy] methyl ethyl ester (Z, Z, Z)- (5.35%), ethyl oleate (4.71%), dotiacontane (4.42%), 1,25-dihydroxy vitamin D_3 – TMS derivative (4.26%), tetrasiloxane, decamethyl (3.55%), and lupeol (3.36%). Minor components included linoleic acid methyl ester (2.39%), benzoic acid, 2-hydroxy-methyl ester (1.83%) and hexadecanoic acid ethyl ester (1.05%).

GC/MS analysis of methanol-extracted propolis samples revealed a diverse profile of bioactive compounds, as illustrated in **Fig. 3(a–d)**. In Sample Q **Fig. (3a)**, 28 compounds were identified. The most abundant constituents were α-amyrin (34.71%), lup-20(29)-en-3-ol-acetate (3α)- (17.73%), and 12-oleanen-3-yl acetate (3α)- (15.39%). Other detected compounds included 2H-pyran, 2-[7-heptadecyl-nyloxy] tetrahydro (4.34%), 1-heptatriacontanol (3.22%), and thunbergol (1.41%). In Sample F, **Fig. (3b)**, 51 compounds were detected. The most predominant compounds were pyrido[2,3-b] indole (11.26%), 4H-1-benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl (14.57%), α-amyrin (7.68%), and lup-20(29)-en-3-α-acetate (3α)- (7.65%). Among these, notable components were α-guaiene (1.25%), benzene, 1-(1,5-dimethyl-4-hexenyl), 4-methyl (2.83%), guaiol (3.24%), hexadecanoic acid trimethyl ester (2.89%), 4H-1-benzopyran-4-one, (5,7-dihydroxy)-2-phenyl (5,7-dihydroxy-2-phenyl-4H-chromen-4-one) (3.15%), and oleane-12-en-3-ol acetate (3α)- (3.23%). For Sample L, Fig. (3c), 17 compounds were identified. The major compounds were 4H-1-benzopyran-4-one, 2,3-dihydro-5,7-dihydroxy-2-phenyl-, (s)- (23.10%), 4H-1-benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl (11.33%), olean-12-en-3-ol, acetate (3α)- (13.06%), and lup-20(29)-en-3-ol-acetate (3α)- (14.61%). While the minor compounds included hexadecanoic acid (0.83%), oleic acid (1.16%), 4H-1-benzopyran-4-one, 5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy (2.11%), 2-propenoic acid, 3-(4-hydroxy-3-methoxyphenyl) (4.19%), pyrido[2,3-b] indole (5.59%), and lupeol (2.60%). As for Sample G **Fig. (3d)**, 46 compounds were detected. The most abundant compounds in this sample were palmitic acid-TMS derivative (9.13%), 11-octadecenoic acid (Z)-cis-vaccenic acid (10.41%), and 9,12,15-octadecatrienoic acid, bis[(trimethylsilyl)oxy] propyl ester (Z, Z, Z)- (7.98%). Among the identified compounds were hexadecanoic acid (4.31%), 9-octadecenoic acid (E)-TMS derivative (4.04%), 4H-1-benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl (2.23%), and ethyl iso-allocholate (3.06%).

Extraction of propolis samples by chloroform revealed various compounds in **Fig. (4)**, in sample Q, 28 compounds were detected as shown in **Fig. (4a)**. Among these compounds were palmitic acid TMS derivative (6.75%); stearic acid TMS derivative (3.34%), hexanedioic acid, bis-(2-ethylhexyl)-ester-(3.58%); thunbergol (5.19%), and oleic acid, eicosyl ester (2.61%). The predominant compounds were heptacosane (13.93%), dotriacontane (14.13%), and nonacosane (6.22%). For sample F extracted by chloroform, as indicated in **Fig. (4b)**, 42 compounds were identified in sample F. These compounds included guaiol (2.25%); hexadecanoic acid (3.16%); hexadecanoic acid trimethylsilyl ester- (1.44%); oleic acid (3.57%); and 9-octadecenoic acid (z)-(1.54%). The major compounds were pyrido[2,3-b] indole (18.29%); 4H-1-benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl-(20.34%) and isochiapin B (4.40%). Results of GC/MS analysis of sample L extracted by chloroform revealed 17 compounds, as shown in **Fig. (4c)**. Among these compounds, palmitic acid TMS derivative (1.24%); 2-propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)- (1.34%); dotriacontane (1.35%); 4H-1-benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl (naringenin) (5.41%) and heptacosane (5.11%) have been recorded. The major compounds were Olean-12-en-3-ol, acetate (3α)- (13.86%); methyl 3-oxours-12-en-23-oate (34.98%) and lup-20(29)-en-3-ol, acetate (3α)- (16.52%).

Analysis of Sample propolis G extracted by chloroform was shown in **Fig. (4d)**. Sample G contained 27 compounds, among them hexadecanoic acid (1.44%); palmitic acid TMS derivative (2.21%); 10-octadecenoic acid methyl ester (2.66%); hexanedioic acid, dioctyl ester (2.10%); 4H-1-benzopyrem-4-one, 5-hydroxy-7-methoxy-2-phenyl- (1.06%) and 9, 12, 15- octadecatrienoic acid 2, 3 bis[(trimethylsily)oxy] propyl ester -(z, z, z)- (5.78%). While the predominant compounds in sample G were hexatriacontane (6.29%); octacosane (21.32%); heptacosane (10.20%) and oleic acid eicosyl ester (8.86%). The predominant compounds across different propolis extracts were summarized in **Fig. (5)**.

Fig. (2): GC/MS chromatograms of ethanolic extracts of propolis samples (Q, F, L and G).

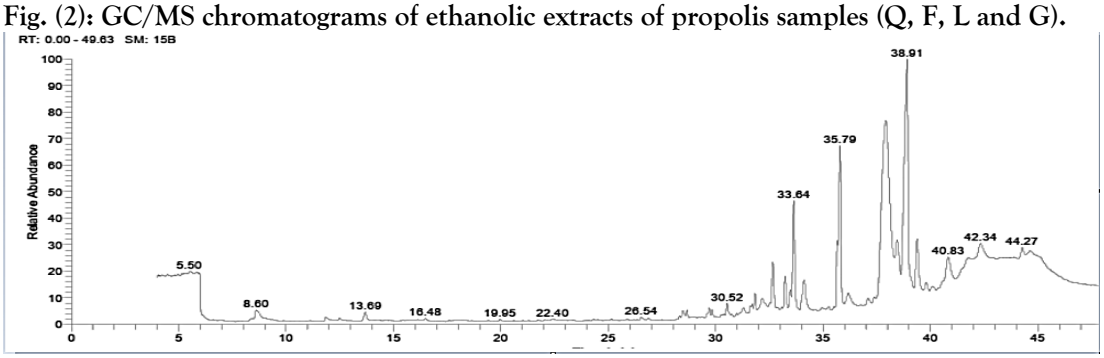


Fig. 2a. GC/MS chromatogram of the ethanolic extract of propolis sample Q.

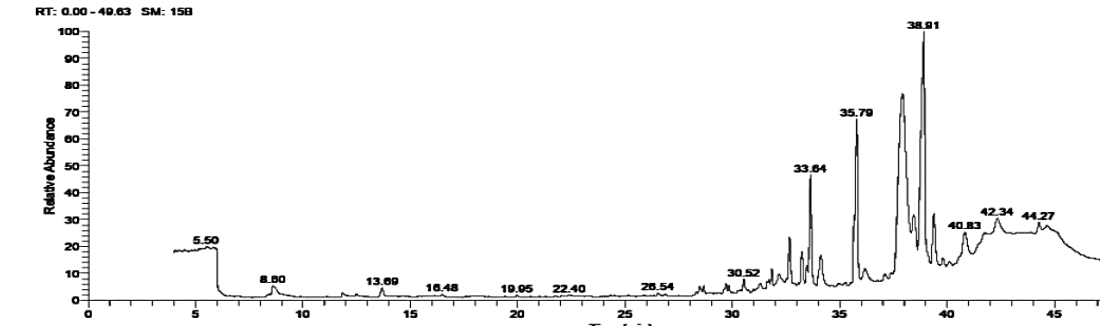


Fig. 2b. GC/MS chromatogram of the ethanolic extract of propolis sample F.

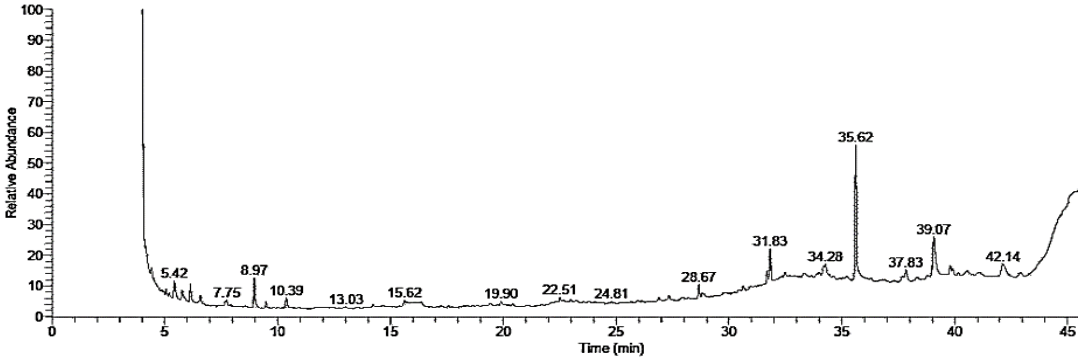


Fig. 2c. GC/MS chromatogram of the ethanolic extract of propolis sample L.

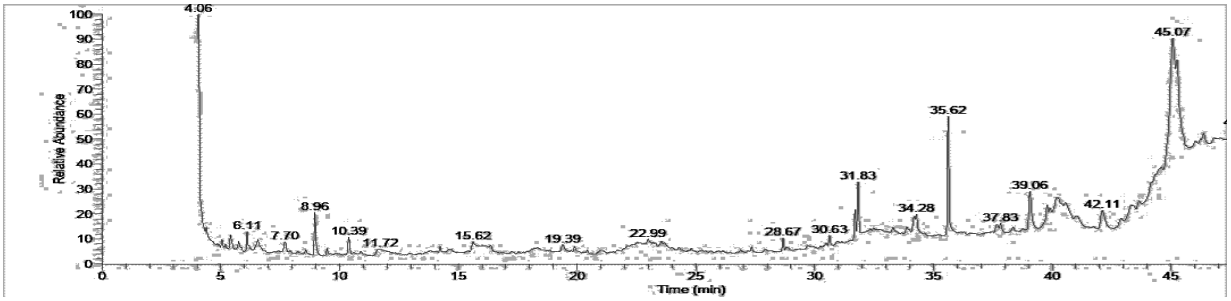


Fig. 2d. GC/MS chromatogram of the ethanolic extract of propolis sample G.

Fig. (3): GC/MS chromatograms of methanolic extracts of propolis samples (Q, F, L and G).

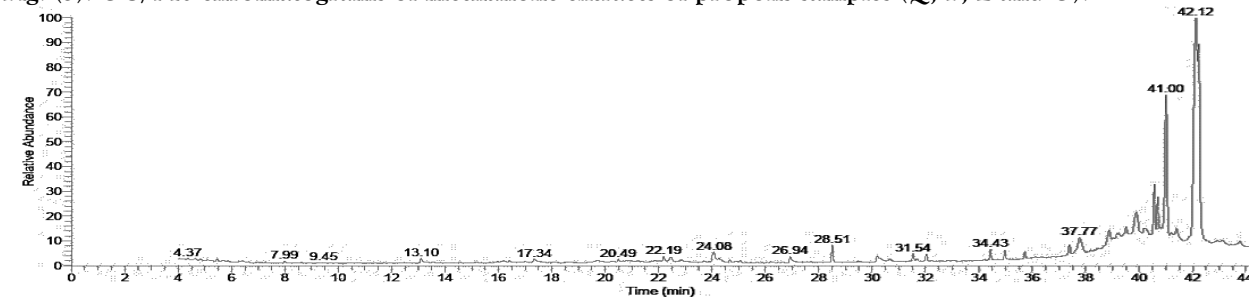


Fig. 3a. GC/MS chromatogram of the methanolic extract of propolis sample Q.

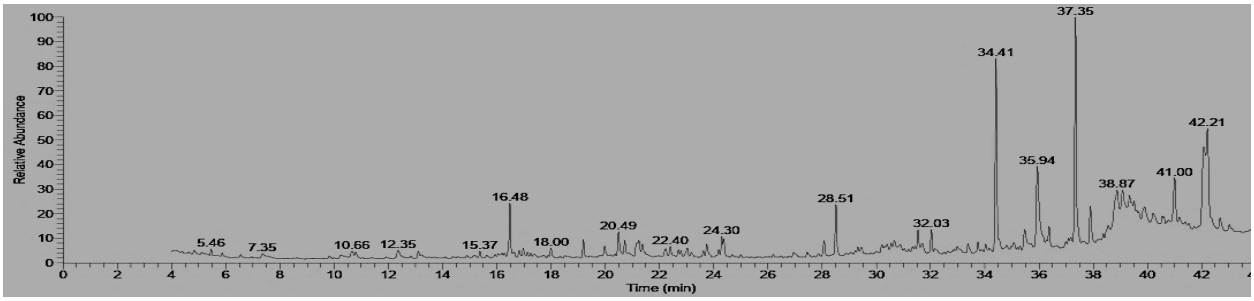


Fig. 3b. GC/MS chromatogram of the methanolic extract of propolis sample F.

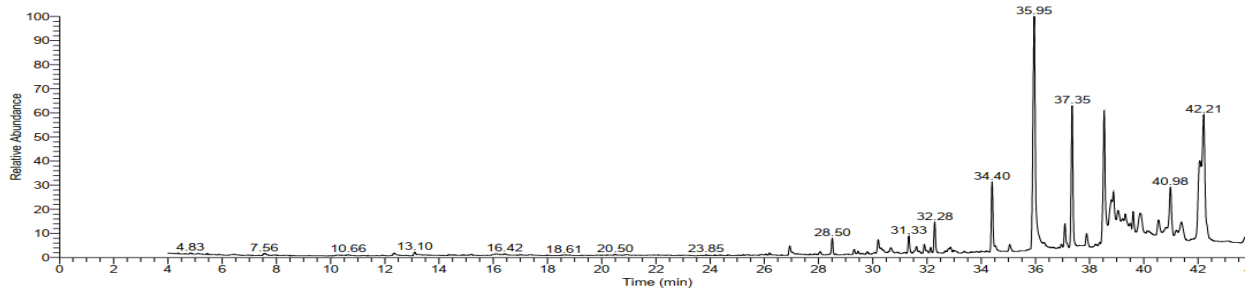


Fig. 3c. GC/MS chromatogram of the methanolic extract of propolis sample L.

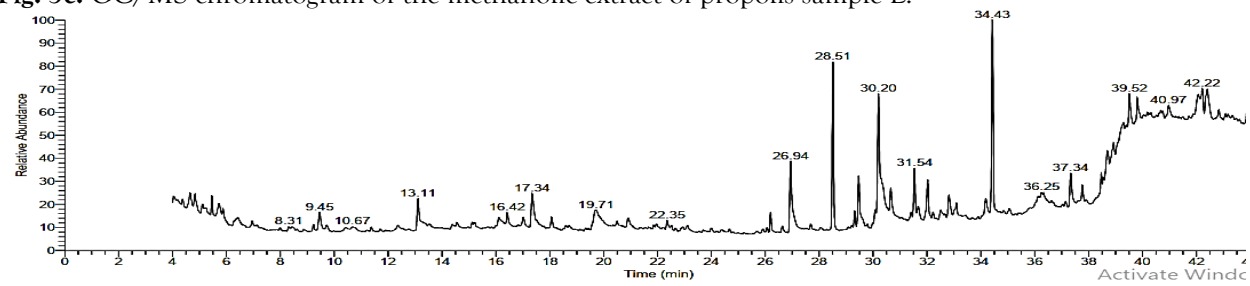


Fig. 3d. GC/MS chromatogram of the methanolic extract of propolis sample G.

Fig. (4): GC/MS chromatograms of chloroform extracts of propolis samples (Q, F, L and G).

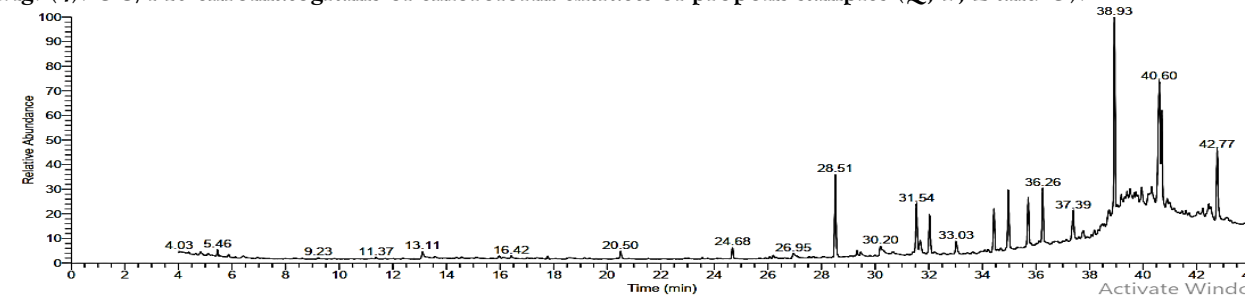


Fig. 4a. GC/MS chromatogram of the chloroform extract of propolis sample Q.

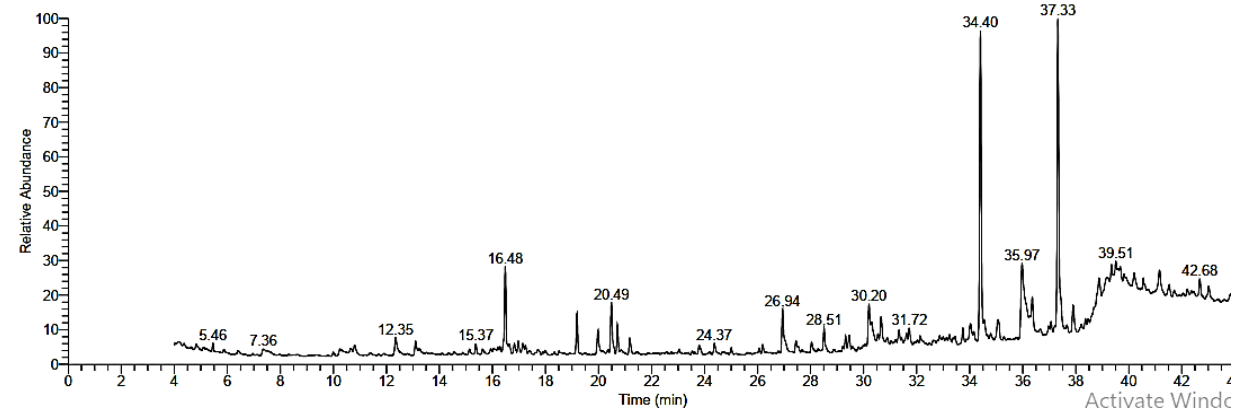


Fig. 4b. GC/MS chromatogram of the chloroform extract of propolis sample F.

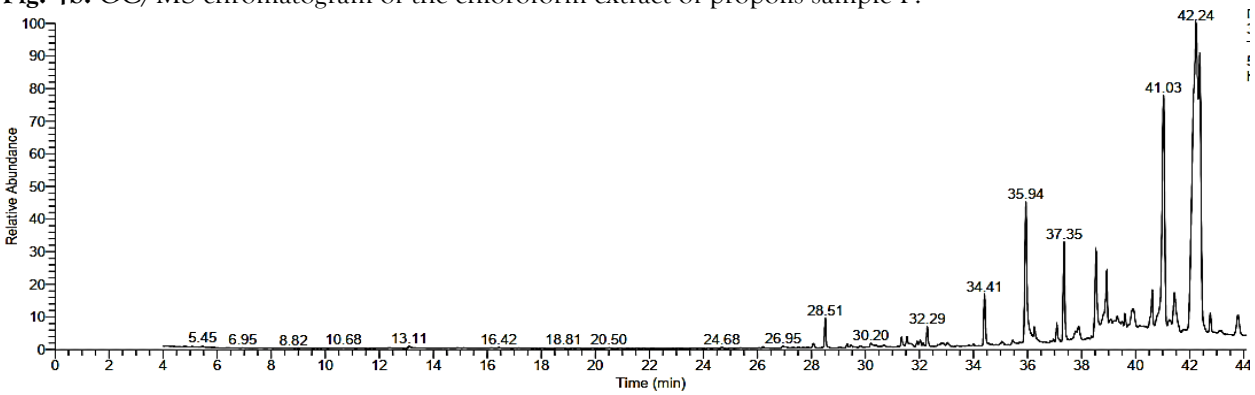
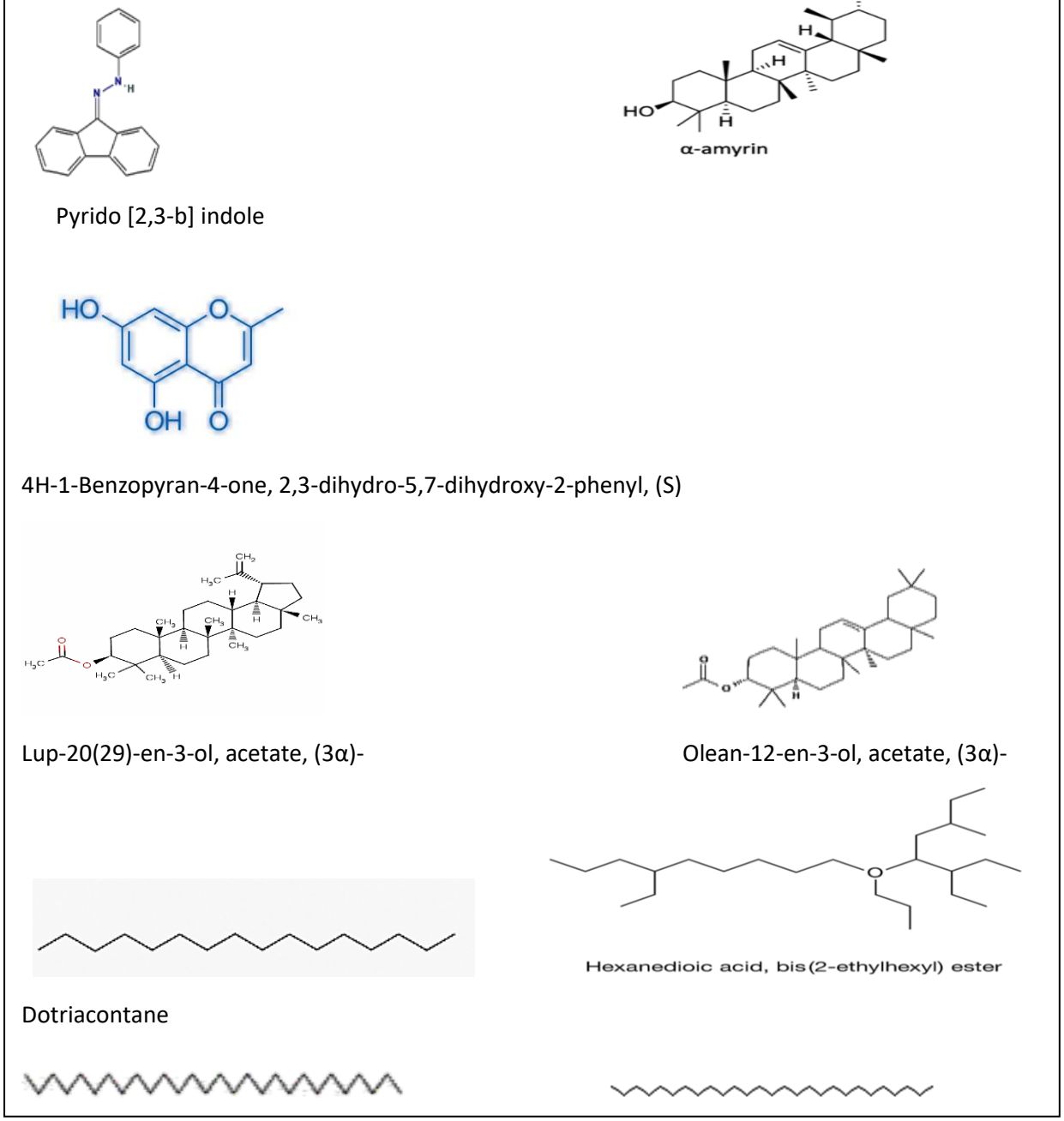


Fig. 4c. GC/MS chromatogram of the chloroform extract of propolis sample L.



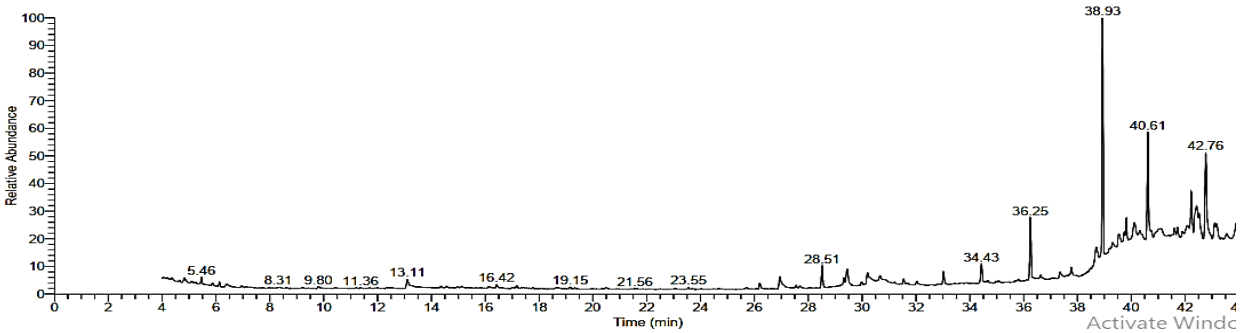


Fig. 4d. GC/MS chromatogram of the chloroform extract of propolis sample G.
Fig (5): The predominant compounds of different propolis extracts identified in this study

The antibacterial activities of propolis samples extracted using different solvents (ethanol, methanol and chloroform) are indicated in **Table (1)**. Among the 13 E. coli strains tested. 6 strains showed sensitivity / intermediate activities to ethanolic extract of propolis (EEP), 9 strains for methanolic extract of propolis (MEP) and 4 strains for chloroform extract of propolis (CEP). Both EEP and MEP showed moderate to high activity against Serratia sp. MAM-5, Klebsiella sp. MAM-6, Proteus sp. MAM-7 and MAM-8,

Pseudomonas aeruginosa MAM-9. While CEP demonstrated effective to intermediate inhibition against Staph. aureus MAM-10 and Staph sp. MAM-20 and Klebsiella sp. MAM-6, MAM-22 and MAM-27. The most efficient propolis extract overall was the methanolic extract (MEP), which produced the highest recorded inhibition zone (24 mm) for E. coli MAM-14 by MEP, while CEP showed a notable inhibition zone (23 mm) for Staph. aureus MAM-10. For comparison, the Chloramphenicol standard disc generally demonstrated a range of inhibition zones from 0 to 27 mm for certain MDR- bacterial strains tested.

Table (1): Antibacterial activities of propolis samples extracts against MDR bacterial strains.

Bacterial Codes	Inhibition zones diameters (mm)												
	EEP				MEP				CEP				C.
	Q	F	L	G	Q	F	L	G	Q	F	L	G	
E. coli **MAM-1**	14	0	0	0	0	18	0	18	0	0	14	13	NT
E. coli **MAM-2**	16	16	20	14	17	18	21	16	0	15	16	0	0
E. coli **MAM-3**	15	14	0	0	0	16	18	15	15	15	15	14	27
E. coli **MAM-4**	0	0	0	0	0	0	0	0	0	14	0	0	18
Serratia sp. **MAM- 5**	14	15	14	0	15	16	15	0	0	0	0	0	17
Klebsiella sp. **MAM-6**	13	14	16	16	18	16	18	18	16	20	0	17	0
P. mirabilis **MAM-7**	14	17	16	15	18	17	23	13	0	0	0	18	0
Proteus sp. **MAM-8**	13	14	16	16	13	16	17	14	0	0	0	0	Nt
Continue Table (1):													
Pseudom. **MAM-9**	15	16	18	13	0	15	18	14	0	0	0	0	Nt
Staph. aureus **MAM-10**	0	0	0	0	0	0	0	0	14	23	17	0	17
E. coli **MAM-11**	0	0	15	0	20	17	19	0	0	0	0	0	Nt
E. coli **MAM-12**	13	0	0	0	0	0	16	15	0	0	0	0	Nt
E. coli **MAM-13**	0	15	15	13	14	15	16	0	0	0	0	0	Nt
E. coli **MAM-14**	0	0	0	0	24	19	21	0	0	0	0	0	0
E. coli **MAM-15**	0	0	0	0	13	0	0	0	0	0	0	0	Nt
Staph. sp. **MAM-16**	0	0	0	0	0	14	0	0	0	13	0	0	Nt
E. coli **MAM-17**	0	0	0	0	0	0	0	0	0	15	0	0	Nt

Proteus sp. **MAM-18**	15	16	0	13	15	13	14	0	0	0	0	0	Nt
E. coli **MAM-19**	18	18	17	0	18	16	16	0	17	18	0	0	0
Staph. sp. **MAM-20**	17	15	14	0	0	0	0	0	17	17	17	0	Nt
Staph. sp. **MAM-20**	17	15	14	0	0	0	0	0	17	17	17	0	Nt
E. coli **MAM-21**	18	0	0	13	0	0	0	15	0	0	0	13	Nt
Klebsiella sp. **MAM-22**	0	0	0	0	0	0	0	0	0	14	0	0	Nt
Continue Table(1):													
Pseud. sp. **MAM-23**	0	0	0	0	0	0	17	0	0	0	0	0	Nt
Klebsiella. Sp **MAM- 24**	0	0	0	16	0	15	0	0	0	0	0	0	Nt
Klebsiella sp. **MAM- 25**	0	0	0	0	0	0	0	0	0	0	0	0	Nt
E. coli **MAM-26**	0	0	0	0	17	0	0	0	0	0	0	0	Nt
Klebsiella sp.**MAM-27**	0	0	0	0	0	0	0	0	0	14	0	0	Nt

EEP: Ethanol exctract of propolis; MEP: Methanol extract of propolis; CEP: Chloroform extract of propolis; C: Chloramphenicol (control); NT: Not tested; 0: No detectable inhibition zone.
Note: Inhibition zones <13 mm indicate resistance, 13–17 mm indicate intermediate sensitivity, and >17 mm indicate sensitivity.

4-DISCUSSION:

The complex and diverse chemical makeup of propolis, which included phenols, flavonoids, terpenes, aromatic acids, alkaloids and other bioactive compounds, was largely responsible for its successful ability to fight out bacterial infections. The chemical composition and biological activity of propolis are influenced by various factors such as the regional plant flora, bee species producing it and the geographical area. These factors contribute to significant variability in its biological properties [10, 23]. This study provides a comparative analysis Egyptian propolis samples from specific regions (Qina, Al-Fayyum, Luxor and Giza) using different solvents to identify unique bioactive compounds. By analyzing the chemical profile of these regional propolis extracts and evaluating their antibacterial activities against multidrug-resistant bacterial (MDR) strains. This research provides new valuable insights into the antimicrobial potential of Egyptian propolis. Similarly, [24] studied Egyptian propolis and its biological activity, highlighting that its functional properties and bioactive compounds content are considerably influenced by geographical area, season, and harvesting time. Their findings align with our study, emphasizing the impact of regional variability on the antimicrobial and antioxidant potential of propolis.

In another study, [25] classified Egyptian propolis into two main types (orange and blue) and one mixed subtype (green) using chemometric techniques (UPLC-MS/MS), that identified 65 compounds. While their research linked bioactive compounds such as pinocembrin, kaempferide, and galangin to significant antioxidant and xanthine oxidase (XO) inhibitory activities, the current study focused on the antimicrobial potential of propolis. These complementary findings emphasized the diverse therapeutic applications of Egyptian propolis across various biological activities.

A study by [26] analyzed the chemical profile of Egyptian propolis collected from different regions and seasons, along with commercial propolis from local market, using GC/MS. They identified 57 compounds in Egyptian propolis compared to 44 in commercial propolis, highlighting the greater chemical diversity of propolis. The key compounds identified in both Egyptian and commercial propolis included aliphatic acids (e.g., linoleic acid, palmitic acid), aromatic acids (e.g., benzoic acid derivatives), and phenols (e.g., phenol, 2-isopropyl-4-methyl), which are known for their antimicrobial properties. As a comparison, the propolis sample from El-Minya, (Egyptian governorate) demonstrated activity against a broad range of bacterial strains, including Staph. aureus, Bacillus cereus, Serratia sp., Pseudomonas sp., and E. coli [27]. This spectrum of activity aligned with our findings, where flavonoid-rich extracts showed effectiveness against similar Gram-positive and Gram-negative bacteria. The variation in bacterial strains targeted by different regional samples underscored the impact of chemical diversity influenced by geographic and botanical factors, emphasizing that the efficacy of propolis is not solely dependent on a uniform chemical profile but also on the synergy of its diverse bioactive compounds. Such commonalities suggest a foundational similarity in their antimicrobial potential, probably due to the presence of these main bioactive components. The use of different solvents in this study (ethanol 70%, methanol, and chloroform) allowed for a comprehensive examination of the bioactive compounds in Egyptian propolis. More flavonoids and phenolic compounds were found in the propolis extract made with 70% ethanol as the extraction solvent [28]. Canadian propolis is abundant in fatty acid esters, chrysin, and naringenin, whereas Spanish propolis contains naringenin, chrysin, and other hydrocarbons [11]. This variety underlines the different chemical profiles of Egyptian propolis and the impact of regional flora. Lup-20(29)-en-3,28-diol, is commonly known as Lupeol, a triterpenoid in propolis, that has strong antimicrobial, antioxidant and wound healing properties as reported by [29]. Palmitic acid TMS derivative is also known as hexadecanoic acid trimethylsilyl ester, that exhibited notable antibacterial activity against Pseud. aeruginosa and E. coli. [30]. 4H-1-Benzopyran-4-one, 2, 3-dihydro-5, 7-dihydroxy-2-phenyl is commonly known as dihydrochrysin, which is a flavonoid derivative, dihydrochrysin possessing antioxidant, anti-inflammatory and antimicrobial properties against Gram-negative and Gram-positive bacteria [31].

Further research into the antibacterial mechanisms of propolis suggests that it may inhibit ATP production, enhance membrane permeability, impair bacterial motility, and disrupt membrane potential [32]. Chinese red propolis was rich in flavonoids as chrysin that exhibited potent antibacterial activity against Staph. aureus and MRSA [33].

Amyrin derivatives, especially α-amyrin and β-amyrin and methyl 3-oxours-12-en-23-oate are pentacyclic triterpenoids found in propolis, which exhibited antibacterial properties [34]. Recent studies found that amyrin derivatives not only inhibited bacterial proliferation but also improved the overall antimicrobial properties of propolis, rendering it a promising candidate for alternative microbial therapies [35]. Aliphatic acids in propolis were linked to breaking down the bacterial cell membrane and inhibiting vital enzyme activity found in bacteria [13]. Guaiol exhibited a variety of biological activities: (antioxidant, antimicrobial, anti-inflammatory and anticancer properties) [36]. The broad-spectrum antibacterial activity observed in this study is likely attributed to the high concentrations of flavonoids present in the ethanolic and methanolic extracts. Specifically, the significant activity against multidrug-resistant E. coli and Staph. aureus aligns with the high levels of flavonoids such as dihydrochrysin and naringenin in these extracts. These compounds have been shown to disrupt bacterial cell walls and inhibit essential enzymes, as proved by previous research [31]. These results, supported with previous findings, highlighted the importance of solvent selection in propolis extraction, depending on the target bacterial strains and desired biological activities.

[37] stated that ethanolic extracts of Acacia trees had strong antibacterial action against multidrug-resistant (MDR) bacteria such as E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staph. aureus, and Streptococcus sp. This activity was linked to the presence of a wide spectrum of secondary metabolites, notably tannins, alkaloids, and flavonoids, which have been shown in vitro to have strong antibacterial activities [38]. These findings are consistent with our investigation, which found alkaloids in Egyptian propolis, including pyrido[2,3-b] indole. The percentage of alkaloids detected in propolis samples supports their possible contribution to reported antibacterial activity, highlighting the importance of these bioactive chemicals in treating MDR infections. [39] demonstrated that pyrido[2,3-b] indole derivatives effectively target the ATPase domains of these enzymes, disrupting DNA replication and transcription in Gram-negative pathogens. Propolis is known to exhibit multiple antibacterial

mechanisms, including the inhibition of cell division, disruption of microbial cytoplasmic membranes and cell walls, suppression of bacterial motility, inactivation of enzymes, induction of bacteriolysis, and inhibition of protein synthesis [40]. This study's results, besides those of earlier research, underscored the potential of Egyptian propolis as a natural antimicrobial agent, particularly in the context of multidrug-resistant bacteria. The variability in chemical composition, influenced by geographical differences, and the use of appropriate extraction solvents were critical factors in maximizing propolis' therapeutic value [9].

Conflict of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES:

[1] Martinello, M., and Mutinelli, F., 2021, "Antioxidant and Antimicrobial Activity of Propolis: A Review," Antioxidants, 10, p. 71. https://doi.org/10.3390/antiox10010071.

[2] Pasupuleti, V. R., Sammugam, L., Ramesh, N., and Gan, S. H., 2017, "Honey, Propolis, and Royal Jelly: A Comprehensive Review of Their Biological Actions and Health Benefits," Oxid. Med. Cell. Longev., 2017, p. 1259510. https://doi.org/10.1155/2017/1259510.

[3] Hossain, R., Quispe, C., Khan, R. A., Saikat, A. S. M., Ray, P., Ongalbek, D., Yeskaliyeva, B., Jain, D., Smeriglio, A., Trombetta, D., et al., 2022, "Propolis: An Update on Its Chemistry and Pharmacological Applications," Chin. Med., 17, pp. 1–60.

[4] Sforcin, J. M., 2016, "Biological Properties and Therapeutic Applications of Propolis," Phytother. Res., 30, pp. 894–905.

[5] Wagh, V. D., 2013, "Propolis: A Wonder Bee Product and Its Pharmacological Potentials," Adv. Pharm. Sci., 2013, p. 308249. https://doi.org/10.1155/2013/308249.

[6] Stojanović, D., Pavlović, I., Ljević, M., and Savić, S., 2020, "The Use of Propolis in Veterinary Medicine," J. Vet. Med., 67, pp. 110–118. https://doi.org/10.2478/jvetmed-2020-0015.

[7] Rebiai, A., Lanez, T., and Belfar, M. L., 2014, "Total Polyphenol Contents, Radical Scavenging, and Cyclic Voltammetry of Algerian Propolis," J. Pharm. Pharm. Sci., 6, pp. 395–400.

[8] Irigoiti, Y., Navarro, A., Yamul, D., Libonatti, C., Tabera, A., and Basualdo, M., 2021, "The Use of Propolis as a Functional Food Ingredient: A Review," Trends Food Sci. Technol., 115, pp. 297–306. https://doi.org/10.1016/j.tifs.2021.06.041.

[9] Kasote, D., Bankova, V., and Viljoen, A. M., 2022, "Propolis: Chemical Diversity and Challenges in Quality Control," Phytochem. Rev., 21, pp. 1887–1911.

[10] Falcão, S. I., Vale, N., Cos, P., Gomes, P., Freire, C., Maes, L., and Vilas-Boas, M., 2014, "In Vitro Evaluation of Portuguese Propolis and Floral Sources for Antiprotozoal, Antibacterial and Antifungal Activity," Phytother. Res., 28, pp. 437–443. https://doi.org/10.1002/ptr.5013.

[11] Huang, S., Zhang, C. P., Wang, K., Li, G. Q., and Hu, F. L., 2014, "Recent Advances in the Chemical Composition of Propolis," Molecules, 19(12), pp. 19610–19632.

[12] Alotaibi, M. A., 2024, "The Rising Challenge of Antimicrobial Resistance in Asia: Public Health Implications," Asian J. Public Health, 18, pp. 50–62. https://doi.org/10.1016/j.ajph.2024.03.005.

[13] Chen, Y., Chen, X., Liang, Z., Fan, S., Gao, X., Jia, H., Li, B., Shi, L., Zhai, A., and Wu, C., 2022, "Epidemiology and Prediction of Multidrug-Resistant Bacteria Based on Hospital Level," J. Glob. Antimicrob. Resist., 29, pp. 155–162. https://doi.org/10.1016/j.jgar.2022.03.003.

[14] Abdelrazeg, S., Hussin, H., Salih, M., and Shaharuddin, B., 2020, "Propolis Composition and Applications in Medicine and Health," Int. Med. J., 25, pp. 1505–1542. https://www.researchgate.net/publication/340335240.

[15] Afata, T. N., Nemo, R., Ishete, N., Tucho, G. T., and Dekebo, A., 2022, "Phytochemical Investigation, Physicochemical Characterization, and Antimicrobial Activities of Ethiopian Propolis," Arab. J. Chem., 15, p. 103931. http://doi.org/10.1016/j.arabjc.2022.103931.

[16] Cunha, I., Sawaya, A. C., Caetano, F. M., Shimizu, M. T., Marcucci, M. C., Drezza, F. T., Povia, G. S., and Carvalho, P. O., 2004, "Factors That Influence the Yield and Composition of Brazilian Propolis Extracts," J. Braz. Chem. Soc., 15, pp. 964–970.

[17] Gençay, S., and Salih, B., 2009, "Preparation and Analysis of Propolis Extracts by GC/MS," Anal. Methods, 1, pp. 171–175. https://doi.org/10.1039/b813832j.

[18] Abo-State, M. A. M., El-Salam, S. S. A., and Assi, R., 2017, "Prevalence of Multidrug-Resistant (MDR) Among Clinical Bacterial Isolates in El-Gharbia Governorate and Efficiency of Antibiotic," World J. Pharm. Pharm. Sci., 6, pp. 116–133. https://doi.org/10.20959/wjpps201710-10304.

[19] Abo-State, M. A. M., Saleh, Y. E. S., and Ghareeb, H. M., 2018, "Prevalence and Sequence of Aminoglycosides Modifying Enzymes Genes Among E. coli and Klebsiella Species Isolated from Egyptian Hospitals," J. Radiat. Res. Appl. Sci., 11, pp. 408–415. https://doi.org/10.1016/j.jrras.2018.08.005.

[20] Saleh, Y. E., Abo-State, M. A. M., Helal, N. L., and Ghareeb, H. M., 2017, "Aminoglycoside and Beta-Lactam Resistance Pattern Among Gram-Negative Bacilli (GNB) Isolated from Egyptian Hospitals," Asian J. Microbiol. Biotechnol., 2, pp. 20–29.

[21] CLSI, 2012, Performance Standard for Antimicrobial Disc Susceptibility Tests: Approved Standard, 11th ed., Clinical and Laboratory Standards Institute (CLSI), Document M02-A11.
[22] Martin, P. A., Lohr, J. R., and Dean, D. H., 1981, "Transformation of Bacillus thuringiensis Protoplasts by Plasmid Deoxyribonucleic Acid," J. Bacteriol., 145, pp. 980–983. https://doi.org/10.1128/jb.145.2.980-983.1981.
[23] dos Santos, L., Hochheim, S., Boeder, A. M., Kroger, A., Tomazzoli, M. M., Dal Pai Neto, A., et al., 2017, "Chemical Characterization, Antioxidant, Cytotoxic and Antibacterial Activity of Propolis Extracts and Isolated Compounds from the Brazilian Stingless Bees Melipona quadrifasciata and Tetragonisca angustula," J. Apic. Res., 56, pp. 543–558. https://doi.org/10.1080/00218839.2017.1371535.
[24] Shehata, M. G., Ahmad, F. T., Badr, A. N., Masry, S. H., and El-Sohaimy, S. A., 2020, "Chemical Analysis, Antioxidant, Cytotoxic, and Antimicrobial Properties of Propolis from Different Geographic Regions," Ann. Agric. Sci., 65, pp. 209–217.
[25] Ghallab, D. S., Mohyeldin, M. M., Shawky, E., Metwally, A. M., and Ibrahim, R. S., 2021, "Chemical Profiling of Egyptian Propolis and Determination of Its Xanthine Oxidase Inhibitory Properties Using UPLC–MS/MS and Chemometrics," J. Chromatogr. B, 136, p. 110298.
[26] Abozid, M. M., and Ahmed, A. A., 2013, "Chemical Composition of Egyptian and Commercial Propolis and Its Effects on Liver Function and Lipid Profiles in Albino Rats," J. Biol. Chem. Environ. Res., 8, pp. 323–340.
[27] El-Bassuony, A., and Abouzid, S., 2010, "A New Prenylated Flavonoid with Antibacterial Activity," Nat. Prod. Commun., 5, pp. 43–45.
[28] Mavri, A., Abramovič, H., Polak, T., Bertoncelj, J., Jamnik, P., Smole, M. S., and Jeršek, B., 2012, "Chemical Properties and Antioxidant and Antimicrobial Activities of Slovenian Propolis," Chem. Biodivers., 9, pp. 1545–1558. https://doi.org/10.1002/cbdv.201100337.
[29] Pereira Beserra, F., Sergio Gushiken, L. F., Vieira, A. J., Augusto Bérgamo, D., Luísa Bérgamo, P., Oliveira de Souza, M., et al., 2020, "From Inflammation to Cutaneous Repair: Topical Application of Lupeol Improves Skin Wound Healing in Rats by Modulating the Cytokine Levels, NF-κB, Ki-67, Growth Factor Expression, and Distribution of Collagen Fibers," Int. J. Mol. Sci., 21, p. 4952.
[30] Tatlısulu, S., and Özgör, E., 2023, "Identification of Cyprus Propolis Composition and Evaluation of Its Antimicrobial and Antiproliferative Activities," Food Biosci., 51, p. 102273
[31] Goker, H., Boykin, D. W., and Yildiz, S., 2005, "Synthesis and potent antimicrobial activity of some novel 2-phenyl or methyl-4H-1-benzopyran-4-ones carrying amidinobenzimidazoles," Bioorg. Med. Chem., 13, pp. 1707–1714.
[32] Przybyłek, I., and Karpiński, T. M., 2019, "Antibacterial properties of propolis," Molecules, 24, p.2047. https://doi.org/10.3390/molecules24112047.
[33] Zhang, W., Margarita, G. E., Wu, D., Yuan, W., Yan, S., Qi, S., Xue, X., Wang, K., and Wu, L., 2022, "Antibacterial activity of Chinese red propolis against Staphylococcus aureus and MRSA," Molecules, 27, p.1693. https://doi.org/10.3390/molecules27051693.
[34] Yam-Puc, A., Santana-Hernández, A. A., Yah-Nahuat, P. N., Ramón-Sierra, J. M., Cáceres-Farfán, M. R., Borges-Argáez, R. L., and Ortiz-Vázquez, E., 2019, "Pentacyclic triterpenes and other constituents in propolis extract from Melipona beecheii collected in Yucatan, México," Rev. Bras. Farmacogn., 29, pp. 358–363. https://doi.org/10.1016/j.bjp.2019.01.006.
[35] Tamfu, A. N., Ceylan, O., Cârâc, G., Talla, E., and Dinica, R. M., 2022, "Antibiofilm and anti-quorum sensing potential of cycloartane-type triterpene acids from Cameroonian grassland propolis: Phenolic profile and antioxidant activity of crude extract," Molecules, 27, pp. 1–19.
[36] Choudhary, M., Batool, I., Atif, M., Hussain, S., and Atta-ur-Rahman, 2007, "Microbial transformation of (-)-guaiol and antibacterial activity of its transformed products," J. Nat. Prod., 70, pp. 849–852.
[37] Khan, R., Islam, B., Akram, M., Shakil, S., Ahmad, A. A., Ali, S. M., Siddiqui, M., and Khan, A. U., 2009, "Antimicrobial activity of five herbal extracts against multidrug resistant (MDR) strains of bacteria and fungus of clinical origin," Molecules, 14, pp. 586–597.
[38] Lewis, K., and Ausubel, F. M., 2006, "Prospects of plant derived antibacterials," Nat. Biotechnol., 24, pp. 1504–1507. https://doi.org/10.1038/nbt1206-1504.
[39] Hu, Y., Shi, H., Zhou, M., Ren, Q., Zhu, W., Zhang, W., et al., 2020, "Discovery of pyrido[2,3-b] indole derivatives with Gram-negative activity targeting both DNA gyrase and topoisomerase IV," J. Med. Chem., 63, pp. 9623–9649.
[40] Fernandes Júnior, A., Balestrin, E. C., Betoni, J. E. C., Orsi, R. O., Cunha, M. L., and Montelli, A. C., 2005, "Propolis: anti-Staphylococcus aureus activity and synergism with antimicrobial drugs," Mem. Inst. Oswaldo Cruz, 100, pp. 563–566. https://doi.org/10.1590/S0074-02762005000500018.