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Research Article

Functional Potential and Chemical Profile Analysis of Propolis Oil Extracted from Propolis of Balochistan

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Propolis oil (PO) was examined for chemical composition, phenolic and flavonoid content, and antioxidant and antimicrobial potential. Phenolic and flavonoid contents were 2.388 ± 1.116 mg GAE/g and 0.579 ± 0.140 mg QE/g. Oil showed 64.59 ± 14.59% inhibition of DPPH radical and significant antibacterial activities against target bacteria. *Salmonella typhi* was found to be highly sensitive (27.23 ± 4.35 mm) to PO, compared to *Escherichia coli* (23.40 ± 3.21), *Staphylococcus aureus* (21.43 ± 2.80), and *Klebsiella pneumoniae* (21.26 ± 3.25). The MIC and MBS values of PO were 0.35 and 0.7 mg/mL for *S. typhi* and *E. coli*, whereas they were 0.7 and 1.4 mg/mL for *S. aureus*. Moreover, the PO was found to be bacteriostatic for *K. pneumoniae*. *Aspergillus flavus* was found to be highly sensitive to PO, with an effective growth inhibition percentage of 73%, followed by *Aspergillus niger* (70%), whereas *Aspergillus parasiticus* was less sensitive with 25% growth inhibition. Functional groups in PO were determined with an FTIR spectrophotometer, and alcohol, alkane, aldehydes, alkenes, and ketones groups were found to be present, whereas GC-MS analysis revealed the presence of 27 different medicinal compounds, among which α-copanene (29.85%), benzyl benzoate (26.8%), 2,4-bis[1-(4-hydroxyphenyl)isopropyl]phenol, acetophenone (14.92%), undecylenic aldehyde (7.46%), p-linalool (5.9%), and ethyl 3-phenylpropionate (4.47%) were found in abundance.

1. Introduction

Propolis is a natural resinous cementing substance mixed with pollens and bee enzymes collected from tree leaves, buds, and other plant parts by honey bees (*Apis mellifera ligustica*) [1]. Phenolic compounds are the important constituents of propolis, represented by phenolic acids, flavonoids, and their esters, having promising anti-inflammatory, antioxidant, antimicrobial, and other bioactivities. The propolis chemical composition usually varies but mostly is comprised of plant balm and resin (50%), volatile oils (10%), wax (30%), pollen (5%), and other substances (5%), for example, organic residues [2]. Diverse chemical compounds have been reported in different types of propolis, out of which phenolic acids, terpenes, hydrocarbons, organic acids, aldehydes, esters, cyclic compounds, alcohols, flavonoid aglycones and their esters, quinones, phenolic aldehydes, sesquiterpenes, coumarins, ketones, and steroids are predominantly found to be present in propolis. The chemical compounds' enrichment

in propolis is usually dependent on the season, flora, and plant biodiversity [3].

The propolis applications in pharma and health made it highly attractive for its complete chemical component exploration. It is rich in chemical compounds that are important for the treatment of chronic and metabolic diseases caused by oxidative stress, including metabolic disorders. The propolis is rich in antioxidant components that are important for body defense against free radicals. The antimycobacterium potential of propolis has been previously established, as it can inhibit the growth of *Mycobacterium tuberculosis* along with the use of isoniazid in synergistic effect [4]. Antibacterial and antifungal properties of propolis have also been reported. The active substances in the propolis may vary based on the seasons, regions, and flora, which makes it hard to standardize these components in propolis. Propolis and propolis oil are the byproducts of honeybee farming, which can be a potent source of functional compounds useful for therapeutics. Therefore, it is necessary to investigate the chemical compositions and functional potential of propolis from different regions collected in different seasons. The propolis of the Balochistan region has not been analyzed for its functional and bioactive components so far, and the present study is the first intervention for this purpose. This study has been designed to investigate the functional properties and chemical composition of oil extracted from propolis collected from the Balochistan region of Pakistan.

2. Materials and Methods

2.1. Sample Collection and Oil Extraction. Propolis samples were collected from different areas (Loralai, Ziarat, Musakhail, Naseerabad, Jaffarabad, Sibi, Khuzdar, and Uthal) of Balochistan. All the collected samples were processed in the Food Microbiology and Bioprocess Technology Laboratory in the Department of Microbiology, University of Balochistan, Quetta. The samples were kept for drying at room temperature in a dark place, avoiding direct sun exposure. The dried samples were crushed to make a relatively uniform particle size in a range of 10–80 μm using a clean electric grinder (Philips, Pakistan) [5]. Oil was extracted using Soxhlet apparatus from dried ground propolis materials. An amount of 25 g of propolis was kept in the thimble, and 250 mL of ethanol absolute was added to the round bottom flask; the apparatus was run for 8–12 h. The pure oil was separated from the mixture with the help of a rotary evaporator (IKA, Germany) and used further for analysis [6].

2.2. Total Flavonoid Content Determination. Total flavonoid contents were evaluated with the help of the colorimetric method using aluminum chloride [7]. The PO (250 μL) at a concentration of 100 and 1000 μg/mL was added to distilled water (1.25 mL) and 5% $NaNO_2$ solution (75 μL). The mixture was allowed to stand for 6 min; subsequently, 150 μL of 10% $AlCl_3.6H_2O$ solution was added. After 5 min, 0.5 mL of 1M NaOH was added. The amount of 275 μL of distilled water was added before measuring the absorbance at a wavelength of 510 nm using a UV-visible spectrophotometer (T60, PG, UK). Quercetin as a standard at a varying concentration from 0.8 to 0.1 mg/mL was used for the calibration curve construction. The results were expressed in milligrams of quercetin equivalent per gram of PO. All the determinations were carried out in triplicate.

2.3. Total Phenolic Content Determination. Folin–Ciocalteu (FC) method was used for total phenolic content determination in PO following [8] with slight changes. The test sample (0.5 mL) was mixed with FC reagent (0.25 mL) and left for 5 min; 10% sodium carbonate (1 mL) was poured into the solution and mixed vigorously. Upon completion of 30 minutes of incubation, the absorbance of the reaction was measured at an optical density of 750 nm against a blank containing 95% ethanol with the help of a UV-visible spectrophotometer (T60, PG, UK). Gallic acid at varying concentrations of 0.8–0.1 mg/mL was used for the construction of the standard calibration curve. The result was expressed in milligrams of gallic acid equivalent (GAE) per gram of fresh oil sample weight. All the experiments were carried out in triplicate.

2.4. Quantitative Assay for DPPH-Free Radical Scavenging Activity. 0.1 mM DPPH solution (0.0039432 g of DPPH reagents in 100 mL absolute ethanol) was used in this experiment. An amount of 1 mL of DPPH solution was added to 0.5 mL of oil, mixed vigorously, and left for 30 min at room temperature. Color changes in the reaction mixture were evaluated by observation at an absorbance of 517 nm. The OD value of the DPPH solution was taken as control, and ethanol was used as blank. Ascorbic acid was used as a positive control. All the experiments were conducted in triplicate, and the activities were calculated using the following equation:

$$\%\ \text{scavenging rate} = \left[\frac{(A1 - A2)}{A1}\right] \times 100, \quad (1)$$

where $A1$ is the absorbance of the control (DPPH) and $A2$ is the absorbance of oil [9].

2.5. Fourier Transform Infrared Analysis. The FTIR scan without sample was performed for background analysis before the sample processing. PO (2 μL) was placed in Fourier Transform Infrared (FTIR) analysis sample holder. The 32-scan sample reading was performed at a resolution of 4 cm^{-1}. Analysis was performed in multiple replications for each sample within the region 4000–400 cm^{-1} [10].

2.6. Antibacterial Activity. The antibacterial activity of PO was determined by using it against pathogenic bacterial strains (*E. coli*, *S. aureus*, *S. typhi*, and *K. pneumoniae*). The target bacterial strains were introduced to the freshly prepared sterilized culture media and incubated at 37°C for 24 h. The bacterial lawn was prepared by spreading the target

strains over the surface of presterilized Muller Hinton agar plates with the help of sterilized swabs, using a well diffusion assay. Wells (6 mm) were made in the agar plates with the help of a sterilized cork borer; PO (150 μL) was introduced into the wells aseptically. The DMSO was used as negative control and the antibiotic doxycycline as positive control. All the inoculated test and control plates were incubated at 37°C for 24 h. Upon completion of the incubation time, the clear zone around the wells was measured and recorded in millimeters (mm) [8].

2.7. Minimum Inhibitory and Bactericidal Concentration of Propolis Oil. Minimal inhibitory concentration (MIC) of PO was examined by a twofold dilution method using Muller Hinton broth media for bacterial growth. The dilution amount (1.4, 0.7, 0.35, and 0.175 mg/mL) of the PO was maintained in the test medium before the inoculation of target strains. The broth media at 100 μL containing different dilutions were distributed in 96-well plates for test, as well as a sterility and growth control (containing 5% of DMSO). Each test and growth control well was inoculated with 100 μL of a bacterial suspension (10^6 CFU/ml). The MIC value for PO was determined as the lowest concentration that completely inhibited the visible bacterial growth after 24 h of incubation at 37°C. Bacterial growth patterns in the test and control wells were examined by analyzing turbidity at optical density (OD_{630}) (RT-6000 Microplate Reader). The MBC was determined by subculturing 10 μL of liquid culture from each well that exhibited no visible growth over fresh culture medium for colonies growth determination. The concentrations showing no bacterial growth after subculturing were taken as MBC [11].

2.8. Kill Time Assay. The kill time assay method was used for the determination of the PO effect over the target bacteria growth following [12] with slight modifications. Filtered sterilized oil was added to the presterilized nutrient broth to make a 1.4 mg/mL oil concentration in the test tubes. Fresh culture of test bacterial cultures (*E. coli*, *S. aureus*, *S. typhi*, and *K. pneumoniae*) at 10^6–10^7 CFU/mL was added to the test tubes and incubated at 37 ± 1°C for 24 h. The kill time curves were established by calculating the growth of the test bacteria at a specific time interval of 0, 2, 4, 8, 12, 16, and 24 h. Nutrient broth with test bacteria and without oil and broth with oil and no bacterial inoculum were used as positive and negative controls in the study. The test bacterial count was analyzed with the help of a standard plate count.

2.9. Antifungal Activity. Antifungal activity of PO was determined by introducing 2 mL of filtered sterile oil into the Petri plates containing 23 mL of molten Sabouraud dextrose agar in the flask after sterilization and poured into the Petri dishes and left for solidification. After solidification, 6 mm diameter wells were cut into the center of the agar plate with the help of a sterilized cork borer. Similarly, 6 mm parts from the freshly grown fungal culture (*Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus niger)* were cut with the help of cork borer and placed in the well previously bored in the PO inoculated SDA plates. Media plates without oil and inoculated with a similar amount of fungal inoculum were used as positive culture control, and only media mixed with oil and no fungal species inoculated were used as negative control. Fluconazole was used as an antifungal reference drug in this study. All the plates were incubated at 30°C for 3–7 days, and the growth of test plates was compared with the positive control fungal species growth by measuring the growth diameter zone formed around the wells. Results were calculated using the following equation:

$$\%\ \text{inhibition} = 100 - \frac{\text{linear growth in test (mm)}}{\text{linear growth in control (mm)}} \times 100. \quad (2)$$

2.10. Gas Chromatography-Mass Spectrometry Analysis. The PO was suspended in a 70% ethanol 1 : 10 (w/v) ratio and analyzed by GC-MS for identification and characterization of organic compounds present in the PO. The GC-MS analysis of various organic extracts isolated from propolis sample was performed using a Perkin Elmer GC-MS (Model Perkin Elmer Clarus 500, USA) equipped with a VF-5 MS fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 mm). GC-MS spectroscopic detection, an electron ionization system with ionizing energy of 70 eV, was used for this study. Helium gas (99.99%) pure was used as carrier gas at a constant flow rate ± 1 mL/min. Mass transfer line and injector temperature were set at 220°C and 290°C, respectively. Oven temperature was programmed from 50°C to 150°C at 3°C/min, then held isothermal for 10 min, and finally rose to 300°C at 10°C per min. The samples (1 mL sample diluted in 100 : 1 in hexane and similarly 1 mL sample diluted in 100 : 1 in chloroform) were injected in the split mode with a 1 : 10 ratio. The relative percentage of chemical constituents in PO was expressed as peak area normalization percentage [13].

3. Results

3.1. Determination of Total Flavonoids. The results of total flavonoid contents obtained by the aluminum chloride colorimetric method were calculated from the regression equation of the calibration curve and expressed as mg quercetin equivalents (QE) per gram of the sample. The total flavonoid content of oil was 0.579 ± 0.140 mg of quercetin equivalent per g^{-1} of PO presented in Table 1.

3.2. Determination of Total Phenolic Content. Folin–Ciocalteu reagent is often referenced in literature for the determination of phenolic compounds. The FC reagent reacts with the phenolic compounds, resulting in a blue color complex formation that absorbs radiation and allows quantification. The total phenolic content of PO was investigated by using a modified Folin–Ciocalteu assay. The phenolic contents present in the PO were estimated to be equal to 2.388 ± 1.116 mg of gallic acid equivalent per g^{-1} of PO presented in Table 1.

Table 1: Total phenolic content, total flavonoid content, and DPPH scavenging activity of propolis oil.

Sample	Total flavonoid content (mg QE/g) ± SD	Total phenolic content (mg GAE/g) ± SD	DPPH% scavenging activity
Propolis oil	0.579 ± 0.140	2.388 ± 1.116	64.59 ± 14.59

Note. Results are expressed as mean ± standard deviation.

3.3. Quantitative Assay for DPPH-Free Radical Scavenging Activity. The antioxidant activity of the PO was examined by comparing it with the known antioxidants. Ascorbic acid has been used as a standard antioxidant in this study. DPPH assay is predominantly in use for the estimation of free radical scavenging activity of natural products. The DPPH radical scavenging activity of the PO sample was found to be equal to 64.59 ± 14.59%, as presented in Table 1. These results showed that the chemical nature of the phenolic compounds and possibly the presence of other compounds are the contributing agents to the total antioxidant potential of the oil [9].

3.4. Fourier Transform Infrared Analysis (FTIR). The FTIR is commonly used for the determination of functional groups and types of chemical bonds present in compounds. Different types of functional groups in PO were determined by comparing the vibration frequencies obtained from an FTIR scan analysis. The absorption spectra band in the range of 3500–3200 cm^{-1} showed the O–H stretch presence and the H-bonded for alcohol and phenolics, and the spectra band at 3000–2850 cm^{-1} indicated the C–H stretch for alkane. The spectra band present at 1666.90 cm^{-1} represents the $C = O$ bond presence for the presence of ketones. Wide peak for the aldehydes was observed due to the influence of conjugation and aromatic ring. The strong band from 900 to 675 cm^{-1} showed the presence of aromatic compounds $C = C$. The relatively weak spectra band at 1680–1600 cm^{-1} is for alkenes $C = C$ stretch (Table 2).

3.5. Antibacterial Activity. The agar well diffusion method was used to evaluate the antibacterial potential of PO. The oil was found to be active against all tested bacteria due to the higher zone of inhibition around the well [14]. It was found that *S. typhi* is highly sensitive to PO compared to other Gram-positive and Gram-negative bacteria with the maximum zone of inhibition diameter of 27.23 ± 4.35 mm, followed by *E. coli*, 23.40 ± 3.21 mm. The *S. aureus* showed sensitivity to PO with a good zone of inhibition of 21.43 ± 2.80 mm, whereas the inhibition zone for *K. pneumoniae* was 21.26 ± 3.25 mm. Comparing our results with the reference drug doxycycline, where the test bacteria *E. coli*, *S. aureus*, *S. typhi*, and *K. pneumoniae* showed a zone of inhibition of 20.4 ± 1.51, 23.8 ± 1.73, 23.5 ± 1.53, and 23.4 ± 2.14, respectively, the oil was found to be active against all tested bacteria, and the results are presented in Figure 1.

3.6. Antifungal Activity. Antifungal activity of PO was analyzed by measuring the growth percentage inhibition against three target fungi. The PO was found to be active against all fungal species. According to the obtained results, *A. flavus* was highly sensitive to PO with a percentage of zone of inhibition of 73%, followed by *A. niger* with 70% zone of inhibition, while *A. parasiticus* was less sensitive to oil with 25% growth inhibition compared to the standard drug fluconazole (Figure 2).

3.7. Minimum Inhibitory and Minimum Bactericidal Concentration. The PO showed potential antibacterial activity against different species at different concentrations. Positive control showed sufficient growth after incubation of the assay plates for 24 h, while no turbidity and growth were observed in the negative control. The presence of growth confirmation was done by visible turbidity, optical density, and colony formation on the plates. The concertation of 0.35 and 0.7 mg/mL of PO was counted as MIC and MBCs for *S. typhi* and *E. coli,* respectively, whereas 0.7 and 1.4 mg/mL were the MIC and MBC for *S. aureus*. The *K. pneumoniae* growth seemed to stunt during the oil and bacterial interaction, but its colony formation was found positive upon subculturing of test tubes at all concentrations, which shows that the concentrations of PO used in this study are bacteriostatic for this bacterial species at the highest used concentration.

3.8. Kill Time Assay. The kill time assay of target bacterial species showed that *S. aureus*, *E. coli*, *S. typhi*, and *K. pneumoniae* are sensitive to the oil used with different intensity for different pathogens. The *K. pneumoniae* growth was decreased by the PO with the passage of time but could not reduce significantly after 24 h of incubation compared to the other test bacterial species. On the other hand, the *S. typhi* was found to be sensitive to the oil, and the growth was reduced from log 7 to log 3 CFU/mL in the first eight hours of incubation, which were not detectable after 16 h. Similarly, *S. aureus* and *E. coli* growth were reduced to the minimum in the first 16 hours, which were undetectable after 24 h of incubation, whereas the control group's growth was observed as normal in the absence of propolis oil. These results confirmed the antibacterial activities of the propolis oil against these common and important pathogens (Figure 3).

3.9. Gas Chromatography-Mass Spectrometry

3.9.1. Propolis Oil Chemical Components. The GC-MS analysis of the PO enabled the identification and confirmation of compounds present in the oil. The GC-MS analysis results revealed the presence of 27 different compounds belonging to different chemical classes. The active principles of the identified compounds with their retention

Table 2: Fourier Transform Infrared (FTIR) spectroscopy analysis of propolis oil.

Range (cm^{-1})	Type of signal	Type of link	Main attribution
3740–3550	Elongation	O-H and N-H	Hydroxyls and amino acids
3366–3333	Stretching	O-H	Phenolic groups
3000–3200	Stretching	C-H and aromatics	Flavonoids and aromatic rings
2971–2830 and 2730–2066	Elongation symmetric and asymmetric	C-H	Hydrocarbons
1699–1610	Asymmetric bending vibrations	C = O	Lipids, flavonoids, and amino acids
1560–1505	Elongation	C = C and aromatics	Flavonoids and aromatic rings
1450–1415	Bending vibration	C-H, $C-H_2$, and $C-H_3$	Flavonoids and aromatic rings
1399–1310	Bending vibration	C-H	CH_3 group of flavonoids
1232–1200	Bending vibration (O-H) and asymmetrically bending (C-CO)	O-H and C-CO	Hydrocarbons
1198–1000	Stretching vibration (C-C) and bending (C-OH)	C-C and C-OH	Flavonoids and secondary alcohol groups

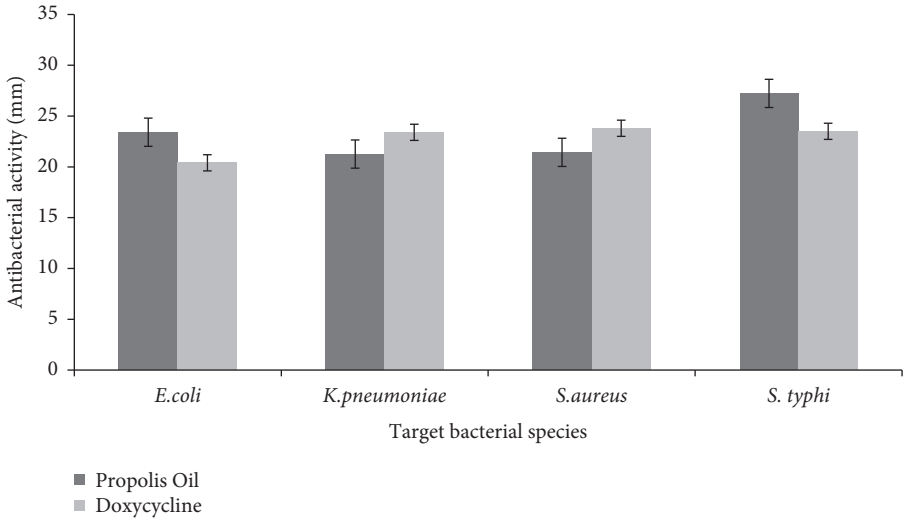

Figure 1: Antibacterial activity of propolis oil against pathogenic bacteria.

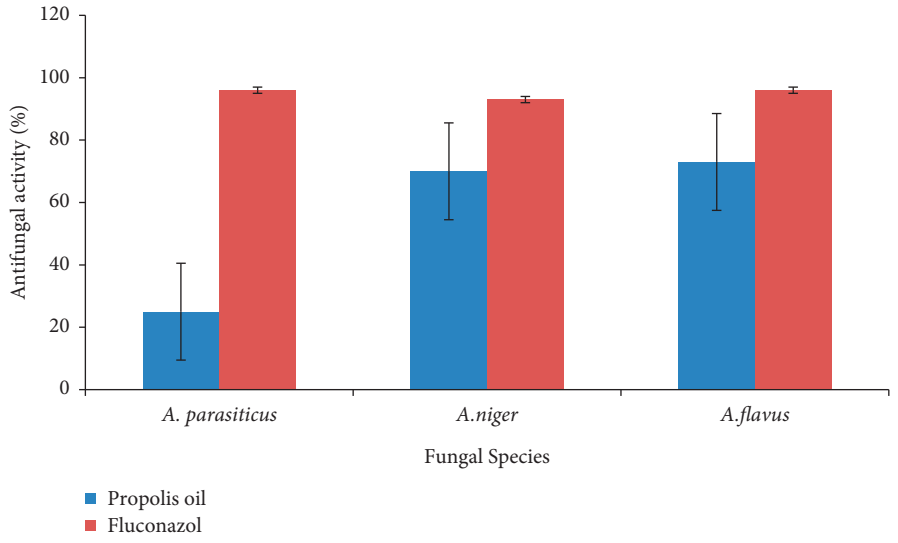

Figure 2: Antifungal activity of propolis oil against fungal species.

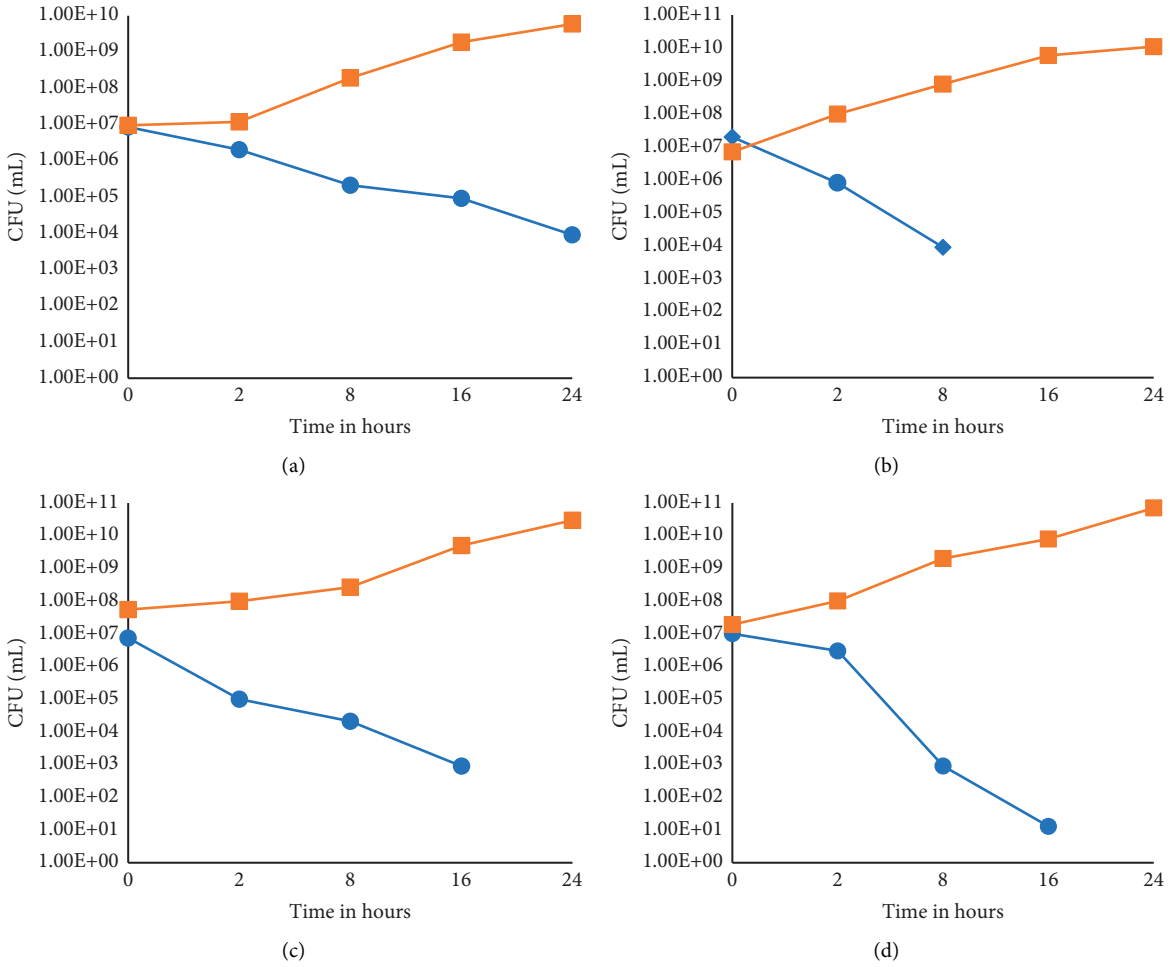

Figure 3: Kill time assay of *Klebsiella pneumonia* (a), *Salmonella typhi* (b), *Staphylococcus aureus* (c), and *Escherichia coli* (d) in the presence of propolis oil. Black circle (●) represents the test bacterial growth, and black square (■) represents the control bacteria growth.

time (RT), peak %, and molecular formula are presented in Figure 4 and Table 3. The 27 identified constituents represented approximately 67% of the total oil, where α-copanene (29.85%), benzyl benzoate (26.8%), 2,4-bis[1-(4-hydroxyphenyl)isopropyl]phenol, acetophenone (14.92%), decaylenic aldehyde (7.46%), p-linalool (5.9%), and ethyl 3-phenylpropionate (4.47%) were the most abundant, and all these compounds are known for their medicinal and biotechnological applications.

4. Discussion

The phenolic and flavonoid contents are known to have significant antimicrobial, antioxidant, anti-inflammatory, and antidepressant activities. These compounds comprise good reducing agents, making them good antioxidants. The total phenolic and flavonoid contents (2.388 ± 1.116 mg GAE/g and 0.579 ± 0.140 mg QE/g) in propolis oil expressed comparatively lower activity than those in other reported studies [15]. The obtained results also showed lower total phenolic and flavonoid content as compared to other studies in China [16], Algeria [17], Brazil [18], and Iran [19]. The differences in the polyphenols may be because of the harvesting site variation of the propolis samples. It has been reported that the local flora greatly influences the chemical composition of propolis. The obtained results are in agreement with the recently published data [20].

The results of the free radicals scavenging percentage of propolis oil showed good antioxidant activity, with a scavenging rate of $64.59 \pm 14.59\%$. It has been reported that essential oil from different plant extract presented comparatively lower antioxidant activities varying from 16.19 to 4.01% than the propolis oil in our study [21]. The higher total phenolic and flavonoid contents are directly proportional to the higher antioxidant potential. The comparatively low antioxidant potential of the PO may be due to the absence of some compounds, such as flavonoids, which are considered one of the major elements responsible for the antioxidant activity of natural products [9].

The FTIR analysis of PO was carried out to characterize the chemical structures and functional groups. According to [22], the FTIR results of the propolis showed the O–H and $C=O$ at (~1168 cm^{-1}) and C–O and C–C at (~1000 cm^{-1}), and the frequency (2848 cm^{-1} – 2915 cm^{-1}) showed the presence of O–H stretch and C–H bonded for alcohol. Recently, reference [23] reported the FTIR spectra between

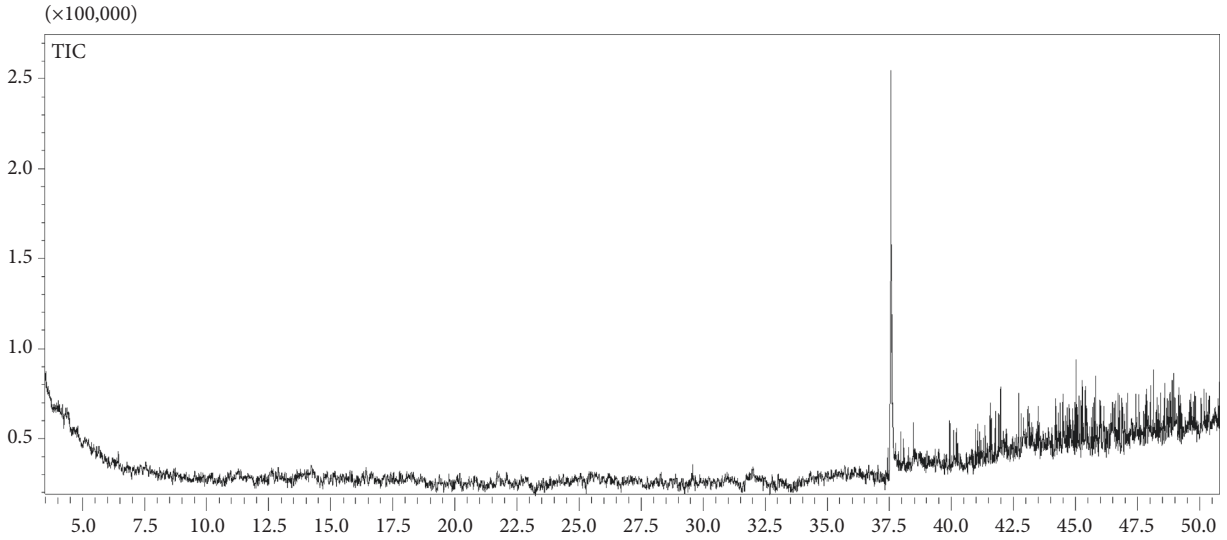

Figure 4: GC-MS chromatography of propolis oil.

Table 3: The major constituents of the propolis oil analyzed by GC-MS.

No.	Compounds	RT	Peak (%)	Formula
1	Cyclohexane-4-hydroxy	3.735	0.02	$C_6H_{10}O_2$
2	Ethyl 3-phenylpropionate	3.835	0.02	$C_{11}H_{14}O_2$
3	Undecylenic aldehyde	5.671	0.07	$C_{11}H_{18}O$
4	Crotonic Acid	5.585	0.02	C_4H_6O2
5	Capric aldehyde	5.508	0.04	C_6H_2O
6	Isoprenol	7.994	0.07	$C_5H_{10}O$
7	Fluorobenzyl alcohol	8.670	0.03	C_7H_7FO
8	p-Linalool	8.670	0.02	$C_{10}H_{18}O$
9	Acetophenone	10.762	0.03	C_8H_8O
10	Iprofenfos	12.358	0.07	$C_{13}H_2O_3PS$
11	Retinal aldehyde	15.279	0.23	$C_{20}H_{28}O$
12	Isopinocarveol	16.000	0.06	$C_{10}H_{16}O$
13	Pulegone	17.642	0.13	$C_{10}H_{16}O$
14	Alpha-copanene	18.451	0.8	$C_{15}H_{24}$
15	Maleic monoamide	19.720	0.02	$C_4H_5NO_3$
16	L-Phenylalanine	19.910	0.08	$C_{10}H_{20}O_2$
17	2,5-Anhydro-D-manose	21.869	0.116	$C_6H_{10}O_5$
18	Phenylephrine	22.339	0.11	$C_9H_{13}NO_2$
19	Scillarenin	28.272	0.02	$C_{24}H_{32}O_4$
20	Pyrethrin-2	28.245	0.02	$C_{22}H_{28}O_5$
21	Allymalonic	29.125	0.03	$C_6H_8O_4$
22	Cyclodecanol	29.170	0.02	$C_{10}H_{20}O$
23	Isopropyl silicate	41.505	0.04	$C_{12}H_{28}O_4S_I$
24	2,4-Bis[1-(4-hydroxyphenyl)isopropyl]phenol	42.405	0.17	$C_{24}H_{26}O_3$
25	P-ethyl benzoate	42.680	0.29	$C_9H_{10}O_2$
26	e-Caprolactone	44.069	0.15	$C_6H_{10}O_2$
27	Pipermargine	47.320	0.07	$C_{12}H_{16}O_3$

3,550 and 3,540 cm^{-1} representing the occurrence of OH group and asymmetric CH_2 methyl group at 2,900 cm^{-1}. The composition and constituents of essential oils may vary and are dependent on the chemical composition of soil available nutrients to the plant cultivation area. Usually, the essential oils are composed of terpenes, for example, terpinol, cineole, citronellal, and other constituents.

The antibacterial activity of PO is of great importance for the bee-keeping community; independently of their origin, the propolis always shows potential health-enhancing activity. Propolis is a rich source of polyphenols and flavonoids having great antimicrobial potential against pathogenic microorganisms without causing any adverse action [24, 25]. The essential oil of propolis inhibits bacterial growth by inhibiting the enzymatic activity of bacteria. Both Gram-positive and Gram-negative bacteria are susceptible to propolis. According to [26], the strong antimicrobial effect is due to the propolis constituents, mainly phenols, flavonoids,

phenolic acids, and their esters, and also to the mixture of volatile constituents. Propolis oil exhibited antibacterial activity against *E. coli*, *S. aureus*, *S. typhi*, and *K. pneumoniae*. The inhibition zone diameters calculated were 23.40 ± 3.21(*E. coli*), 21.43 ± 2.80 (*S. aureus*), 27.23 ± 4.35 (*S. typhi*), and 21.26 ± 3.25 (*K. pneumoniae*). The antibacterial effect of propolis oil against *S. aureus*, *S. typhi*, and *E. coli* is in agreement with [15]. Serval studies confirmed the antimicrobial activity of propolis collected from countries like Mongolia, Albania, Egypt, and Brazil [15]. The inconsistency in the antibacterial activities is probably due to the variation in extraction procedures and seasonal and flora variations, which usually influence the collection of compounds by bees and make a diverse chemical composition of the propolis [27]. It is not so clear whether the antibacterial effect may be caused by a single active component or by the synergy of many active constituents found in the essential oil. However, reference [28] concluded that the mechanism involved in antimicrobial activity is much complex and attributed due to the synergistic association between terpenes and flavonoids hydroxyl acids and found that essential oil with high terpenoids percentages is probably more effective in antibacterial activity.

The antifungal activity of propolis oil was analyzed in terms of the percentage of inhibition against three filamentous fungi (*A. flavus*, *A. niger*, and *A. parasiticus*). The oil was found to be highly active against the three fungi. The PO has strong antifungal activity against *A. flavus* with 73% of inhibition zone and 70% against *A. niger* but relatively less effect on the growth of *A. parasiticus* with 25% of the inhibition zone. A great potential for antifungal activity and trend was found to be consistent with the literature [29, 30]. The antifungal activity of oils in the present study may be due to the presence of antifungal compounds (linalool, eugenol, and other phenolics) reportedly present in different plant extracts.

GC-MS analysis was carried out to quantify the significant components present in propolis oil extract as it contains many phytochemicals having several therapeutic activities. From the results, it was observed that 27 different compounds (Table 3) have been identified. These compounds represent approximately 67% of the total oil, where α-copanene (29.85%), benzyl benzoate (26.8%), 2,4-bis[1-(4-hydroxyphenyl)isopropyl]phenol, acetophenone (14.92%), decaylenic aldehyde (7.46%), p-linalool (5.9%), and ethyl 3-phenylpropionate (4.47%) were the most abundant. Furthermore, pulegone, crotonic acid, and maleic monoamide were found in fewer amounts. The detected compounds such as polyphenols, terpenoids, steroids, sugars, and amino acids were also reported in propolis by previous researchers [31]. Our results support the presence of α-copanene as a major compound of essential oil [22]. Previously, reference [32] reported the presence of carboxylic acid (20.4%), steroids (11.5%), terpenoids (15.0%), hydrocarbons (9.6%), and sugars (6.4%) in propolis. According to [33], propolis water extracts are characterized by groups such as carboxylic acids (17.1%), sugars (31.4%), sugar alcohols (11.4%), terpenoids (14.3%), and hydrocarbons (5.7%). Additionally, another study revealed that the most abundant compound α-calacorene together with cadinene, cadinol, manoyl oxide, caryophyllene oxide, camphor, copaene, bourbonene, eucarvone, and cedrol has been identified as a volatile constituent of Turkish and Anatolian propolis [13]. Correspondingly, tricyclene, α-calacorene, n-alkanes, bourbonene, thymol, cadinol camphene, camphor, caryophyllene oxide, copaene, terpinene, cadinene, spathulenol, cedrol, and bisabolene have been described in Portuguese propolis volatiles [34]. However, some studies analyzed variation in components that can play an important role in discrepancy of chemical composition comprising environment, type of bee, climate, vegetation, flora accessed, geographical area of collection, and management [35]. Moreover, many other propolis volatile compounds were also studied; however, overlapping of the gas-chromatographic peaks and lower concentrations did not permit their quantification.

5. Conclusion

It is concluded in this study that several bioactive compounds are present in PO extracted from propolis of Balochistan, having good antioxidant, antibacterial, antifungal, and functional potential. The broad antimicrobial and significant functional potential of propolis oil supports the use of propolis and its oil for the treatment of various infectious diseases. The compounds found in this study, benzyl benzoate, isopinocarveol, acetophenone, decaylenic aldehyde, linalool, ethyl 3-phenylpropionate, and so on, are common pharmaceutical and industrial ingredients used in different medicinal and industrial applications. The presence of these compounds makes propolis of this region an important candidate for food and pharma applications.

Data Availability

Most of the data are already presented in the paper. The remaining data can be made available upon reasonable request.

Conflicts of Interest

The authors declare that they have no known competing financial or personal conflicts of interest.

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