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Chemical Characterization and *in-vitro* Antimicrobial Screening of Ethanolic Extract of Propolis Collected from Jazan, Saudi Arabia

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MAB conceived, designed and supervised the work and participated in data interpretation. HAA performed data analysis and revision of manuscript. DNR and AJAR collected the data and performed the experiment. SAJ and ZUR collected the samples, prepared and revised the manuscript.

Key words
Propolis, Ethanolic extract, GC-MS, Jazan, Antimicrobial activity.

ABSTRACT

Propolis is a resinous substance produced by honeybees and used to protect their hives from outside attackers including microorganisms. In present study, propolis sample was collected from adjacent to Jazan city, Saudi Arabia and the chemical constituents of the ethanolic extract was evaluated by GC-MS. The ethanolic extract was tested against selected strains of microorganisms for its antibacterial and antifungal activities. The major classes of compounds indentified in the extract were fatty acid esters and aliphatic hydrocarbons. Methyl-(7Z)-7-hexadecenoate, methyl-(9E)-octadecenoate and methyl tetracosanoate were found to be the principal fatty acid esters, while E-pentatriacont-17-ene and 2,4-dimethylpentane were identified as predominant aliphatic hydrocarbon compounds. The propolis extract has exhibited remarkable antimicrobial activity against certain strains of bacteria and yeasts. In general, significant antimicrobial activity was recorded against gram positive bacteria (zone of inhibition: 16.3±0.35–30.6±0.11 mm; MICs: 1–4 µg/ml); while gram negative bacteria (zone of inhibition: 10.1±0.23–17.2±0.13 mm; MICs: 4–16 µg/ml) and yeasts (zone of inhibition: 9.1±0.23–18.7±0.10 mm; MICs: 2–16 µg/ml) showed comparatively lower susceptibility to propolis extract. The antimicrobial potential of propolis extract could be therapeutically significant and may allow substituting for some antimicrobial agents or synergizing the antimicrobial action, when used in combination.

INTRODUCTION

Propolis is a resinous wax-like material of varying colors (green to brown and reddish) obtained from bee hives. Propolis has been used in several countries as traditional remedy due its diverse biological properties. Recently, it has gained popularity as an alternative medicine and used as one of the components of health foods (Petrova *et al*., 2010; Toreti *et al*., 2013). Propolis is collected by *Apis mellifera* (honey bee) from tree buds and flora of various tree species mainly poplar, birch, palm, pine, alder, willow, *Baccharis dracunculifolia* and other botanical sources. In the production of propolis, honeybees also use substances actively secreted by plants and plant wound exudates (Castaldo and Capasso, 2002; Bakar et al., 2018; Daugsch *et al*., 2008; Park *et al*., 2004). Honeybees use propolis as cement to seal the cracks, strengthen the comb borders to protect it from hive invaders including microorganisms and extreme weather (Ghisalberti, 1979; Piccinelli *et al*., 2011). Normally propolis is sticky, soft and pliable; however, when cooled at low temperature (near to freezing), it becomes brittle and hard and remains brittle even at elevated temperatures (Kuropatnicki *et al*., 2013).

The chemical constituents in propolis have been found to be highly variable according to the geographical location, weather of collection and even between the hives of the same region. It mainly depends on the species of the plants grown in the close vicinity of the collection site, which honeybees used to collect the exudates. Consequently, the biological properties of propolis also vary with the type of vegetation around the collection sites (Bertelli *et al*., 2012; Castaldo and Capasso, 2002; Kujumgiev *et al*., 1999; Kumazawa *et al*., 2004). The propolis is composed a complex mixture of resin (approximately 50%), wax (30%), essential oils

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(10%), pollens (5%) and about 5% of other substances (Thomson, 1990). Literature survey has revealed that a variety of chemical constituents were reported in propolis collected from different geographical locations and till now more than 300 compounds have been identified. The major components isolated from propolis include flavones, flavonoids, phenolic compounds, aliphatic and aromatic carboxylic acids and esters, aldehydes and alcohols, monoterpenes, diterpenes, triterpenes, sesquiterpenes and various hydrocarbon compounds (Abu-Mellal *et al*., 2012; Almutairi *et al*., 2014; Marquez *et al*., 2010; Petrova *et al*., 2010; Silici and Kutluca, 2005).

Because of its diverse biological properties, propolis is being widely used as an ingredient in medicines, personal products, food products and beverages. In middle ages, it was used as mouth antiseptic and in the treatment of wounds by Arab physicians. Propolis was listed among the official drugs in London pharmacopoeia during seventeenth century; furthermore, it was popular as an antibacterial agent in Europe during seventeenth to twentieth centuries (Burdock, 1998; Lu *et al*., 2005; Silici and Kutluca, 2005). Recently, propolis has extensively been studied and found to exhibit a wide range of biological activities, such as antimicrobial (Kujumgiev *et al*., 1999; Moreno *et al*., 1999; Silici and Kutluca, 2005; Ugur and Arslan, 2004), anti-inflammatory (Park *et al*., 1996; Valenzuela-Barra *et al*., 2015), antioxidant (Abu-Mellal *et al*., 2012; Kumazawa *et al*., 2004), immunemodulatory (Dimov *et al*., 1991) and cytotoxic (Kimoto *et al*., 1998; Matsuno *et al*., 1997) activities. Among all the biological properties, propolis was mostly investigated for its antimicrobial activity. According to some of the antimicrobial screenings, propolis has exhibited higher activity against gram positive bacteria, while gram negative bacteria and fungi were reported to show comparatively lesser susceptibility (Davey and Grange, 1990; Dobrowalski *et al*., 1991; Silici and Kutluca, 2005). Antimicrobial activity of propolis depends on its chemical composition, as flavonoids, terpenoids and esters are considered to be mainly responsible (Sforcin *et al*., 2000). However, propolis samples without these constituents were also found to possess antibacterial property, suggesting that the mixture of different substances is required (Kujumgiev *et al*., 1999).

Despite the availability of substantial evidences of antimicrobial activity of honey bee propolis, to the extent of our comprehension, no report on chemical composition and *in-vitro* antimicrobial activity of the propolis samples from Jazan region of Saudi Arabia is available so far. Therefore, the current study was aimed to establish the chemical composition of ethanolic extract of propolis sample collected from Jazan region using gas chromatography tandem mass spectrometry (GC-MS) technique and to evaluate the extract for antibacterial and antifungal activities using pathogenic microorganisms.

MATERIALS AND METHODS

Chemicals and instruments

All chemicals used in this study were of high quality. Ethanol (95% v/v), absolute ethanol (≥99.8% v/v) and culture media for antibacterial and antifungal screenings were purchased from Sigma Aldrich, Germany. Ultrasonicator (Wisd, Dathan Scientific, Korea) used for occasional sonication of the mixture during extraction process. Rotary evaporator (Stuart, UK) was used for the evaporation of solvent. The GC-MS analysis was carried out using Agilent GC 6890N gas chromatographer coupled with Agilent MSD 5973 mass detector (Agilent Technologies, USA). Chromatographic separation was achieved using Agilent HP-5MS capillary column (30 m × 0.25 mm i.d. with a film thickness of 0.25 µm). The data was acquired using MSD ChemStation data system software.

Sample collection

Propolis sample was collected from the beehives belonging to *Apis mellifera* from the farms located in the midway between the city of Jazan and Sabya during the month of February 2018 (winter season). Jazan city is the capital of Jazan province, which is one of the thirteen provinces of Saudi Arabia. The southern border of the province is directly attached to the north-western border of Yemen. The Jazan region is stretching around 260 km along the coast of the Red Sea, and is generally hot with a brief and mild winter season. The average temperature remains 36°C during the months of May to October, whereas, it goes down to an average of 21°C between November to April. The rainfall is very scanty which can hardly support any appreciable vegetation in the region. The major vegetation around the sample collection site includes *Acacia ehrenbergiana, Tamarix nilotica, Hyphaene thebaica, Senna alexandrina, Phoenix dactylifera, Abutilon bidentatum* and *Desmostachya bipinnata*. The propolis samples from six different hives were collected in Teflon capped glass container using stainless steel spatula and directly transported to the laboratory. The individual samples were mixed to make the composite sample and stored in refrigerator until analysis.

Extraction of propolis sample

The composite sample was cooled in a freezer for 24 h at a temperature of -20°C and crushed using mortar and pestle into fine powder. The powdered propolis sample (10

g) was extracted in a pre-cleaned conical flask with 200 ml of 95% ethanol for 24 h with occasional sonication. The undissolved solid particles in the extract were removed by filtration using annealed glass fiber filter and the solvent was evaporated from the filtrate using a rotary evaporator under reduced pressure at 35°C to obtain a viscous semisolid residue, which was dried by using a freeze dryer. The ethanolic extract of propolis was analyzed by using gas chromatography tandem mass spectrometry (GC-MS) after making a very dilute solution in absolute ethanol.

GC-MS conditions

The chemical composition of the ethanolic extract of propolis was investigated by GC-MS analysis. The chromatographic separations of the chemical constituents were established using Agilent HP-5MS capillary column (30 m × 0.25 mm i.d., with film thickness of 0.25 µm). Helium (99.99%) was utilized as carrier gas at a flow rate of 1.0 ml/min. The sample injection was performed at split mode; temperature was maintained at 260°C with split ratio 1:10. The GC run was accomplished at a column temperature range of 50-280°C, where, initially the column was held for 5 min at 50°C, followed by an increment of 5°C/min upto 140°C, where it was kept for 2 min and then finally increased to 280°C at 3°C/min heating rate (isotherm at 280°C for 60 min). MS-detector was operated in the electron impact ionization mode at 70eV and the mass spectra were recorded in the range of 50-500 m/z. The data was acquired and processed by using MSD ChemStation Software. The separated compounds were identified by comparing their chromatographic retention behavior with the mass spectral reference libraries for GC-MS data and the data reported in the literature. The percentage of individual constituents was calculated using the respective peak area.

Antimicrobial susceptibility test

The ethanolic extract of propolis sample was screened for antimicrobial activity by using two techniques; the disk diffusion method and broth dilution technique. The susceptibility test was performed according to the guidelines of National Committee for Clinical Laboratory Standards (Cavalieri *et al*., 2005; NCCLS, 2003).

Microbial strains

A total of 17 strains of microorganisms including six gram positive, five gram negative bacteria and six strains of fungi were screened in this study. The gram positive stains were *Bacillus cereus*, *Bacillus megaterium*, *Staphylococcus aureus*, *Staphylococcus epidrermis*, *Micrococcus flavus* and *Enterococcus faecalis*. Gram negative strains were *Enterobacter cloacae*, *Escherichia coli*, *Acinetobacter baumannii*, *Proteus mirabilis* and *Salmonella enteritides*, whereas *Candida albicans, Aspergillus flavus, Aspergillus fumigates, Alternaria alternaria*, *Cladosporium herbarum* and *Fusarium oxysporum* were the fungal strains used in this study to perform the antimicrobial susceptibility testing of propolis sample. The cultures of all the bacterial and fungal strains were procured from Prince Mohammed Bin Naser Hospital, Jazan, Saudi Arabia and stored at 4°C. The inoculums for bacterial strains were prepared by suspending the stock cultures in Mueller-Hinton broth (Sigma-Aldrich) and fungal strains were cultured in Sabouraud dextrose broth (Sigma-Aldrich). The turbidity of all the inoculums was adjusted equivalent to 0.5 Mc Farland turbidity standards (approximately 1.5×10^8 CFU/ml). The microbial suspensions were used as inoculum within 15 min.

Disk diffusion method

The propolis extract was screened by disk diffusion technique against all the bacterial and fungal strains. The suspensions of respective microorganisms were inoculated on the surface of the test plates (100 mm diameter) containing 20 ml Mueller-Hinton agar media (Sigma-Aldrich) for bacterial strains and Sabouraud dextrose agar (Sigma-Aldrich) for fungal strains. The inoculums were applied with a sterile cotton swab by streaking it back and forth. The disks impregnated with 10 µg of propolis (ethanolic extract) and control (prepared with ethanol without propolis) were placed on the inoculated plates. The test plates were incubated at 37 °C for 24 h for bacterial and 30°C for 48 h for fungal strains and growth inhibition zone around the disks were measured. The disks containing gentamicin and amphotericin B (10 µg/disk each) were applied as reference standards for bacterial and fungal strains, respectively. The screening was performed in triplicate.

MIC determination by broth dilution method

Antimicrobial susceptibility test for propolis extract against all the selected microbial strains was also performed by broth dilution technique. The stock solution of propolis sample was prepared in 95% ethanol (1000 µg/ml). A two-fold dilution series in the respective broths were prepared. Mueller-Hinton broth (Sigma-Aldrich) and Sabouraud dextrose broth (Sigma-Aldrich) were used for bacterial and fungal strains, respectively. A measured amount of inoculums standardized to 0.5 Mc Farland turbidity standards was transferred to the broth tube containing test compounds to achieve an inoculum density of 5×10^5 and 2×10^6 CFU/ml for bacterial and fungal strains, respectively. One tube for each microorganism was prepared as positive growth control (broth plus culture

inoculum) and one served as negative growth control (only broth). The tubes were incubated for 24 h at 37°C for bacterial and for 48 h at 30°C for fungal strains. The minimum inhibitory concentration of test samples were recorded by comparing each tube with the respective positive growth controls. The MIC of the test samples was considered as lowest concentration that inhibited the appearance of visible growth of the microorganisms under the defined time period. The MIC was expressed in µg/ml.

Statistical analysis

In disk diffusion method, all values of zone of inhibitions were expressed as mean ± SD and the statistical differences between test and standard drugs were tested by Student's *t*-test. A value of $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Chemical composition of propolis

Propolis sample was collected from the hives belonging to *Apis mellifera* from Jazan region, Saudi Arabia. The sample was extracted with ethanol and the residue obtained after solvent evaporation was investigated for its chemical composition by using gas chromatography tandem mass spectrometric analysis. Overall, 25 chemical constituents were identified and listed along with their retention times and percent abundance in the extract in Table I. The representative GC chromatogram has been presented in Figure 1, while the mass spectrums of representative compounds have been depicted in Figure 2. In this study, the principal constituents identified in the ethanolic extract of propolis sample from Jazan region were fatty acid esters and aliphatic hydrocarbons. The fatty acid esters were found to be the major compounds detected in the sample. The relative proportions of these compounds ranged from 0.10% to 13.27%, with an average value of 3.43% of the total extract. Among the 15 fatty acid esters identified, the predominant compounds were methyl tetracosanoate (13.27%), methyl-(7Z)-7-hexadecenoate (11.59%), methyl-(9E)-octadecenoate (11.53%), methyl octadecanoate (5.86%) and methyl hexacosanoate (4.10%). Long chain aliphatic hydrocarbons were also detected in the significant amount from the propolis sample, the relative percentage of these substances ranged between 0.16%-18.06%, with a mean value of 5.15% of the total extract. E-Pentatriacont-17-ene (18.06%) and 2,4-dimethylpentane (11.83%) were the major aliphatic hydrocarbons identified in the propolis sample. Other types of chemical constituents including alcohol (3-tetradecylox-1,2-propanediol), aromatic hydrocarbon (p-xylene) and glycol ether (octaethylene glycol monododecyl ether) were also found to present. However, these substances were detected in considerably lower proportions.

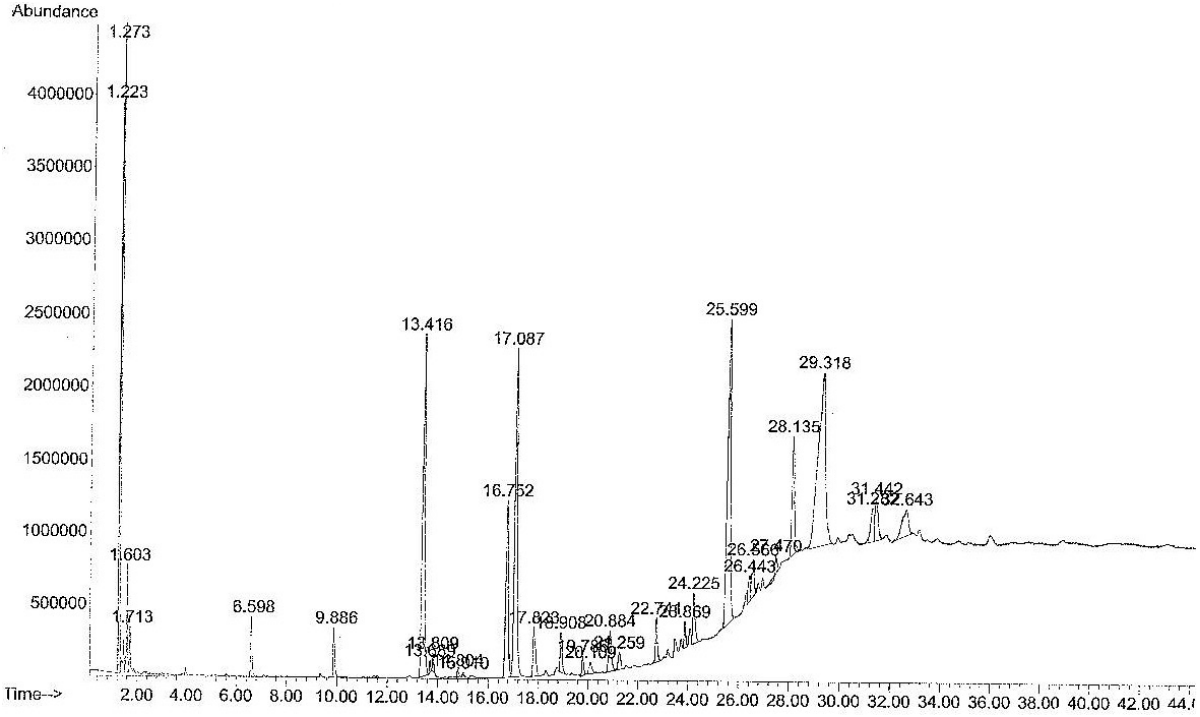

Fig. 1. GC-MS chromatogram of the ethanolic extract of propolis collected from Jazan region, Saudi Arabia.

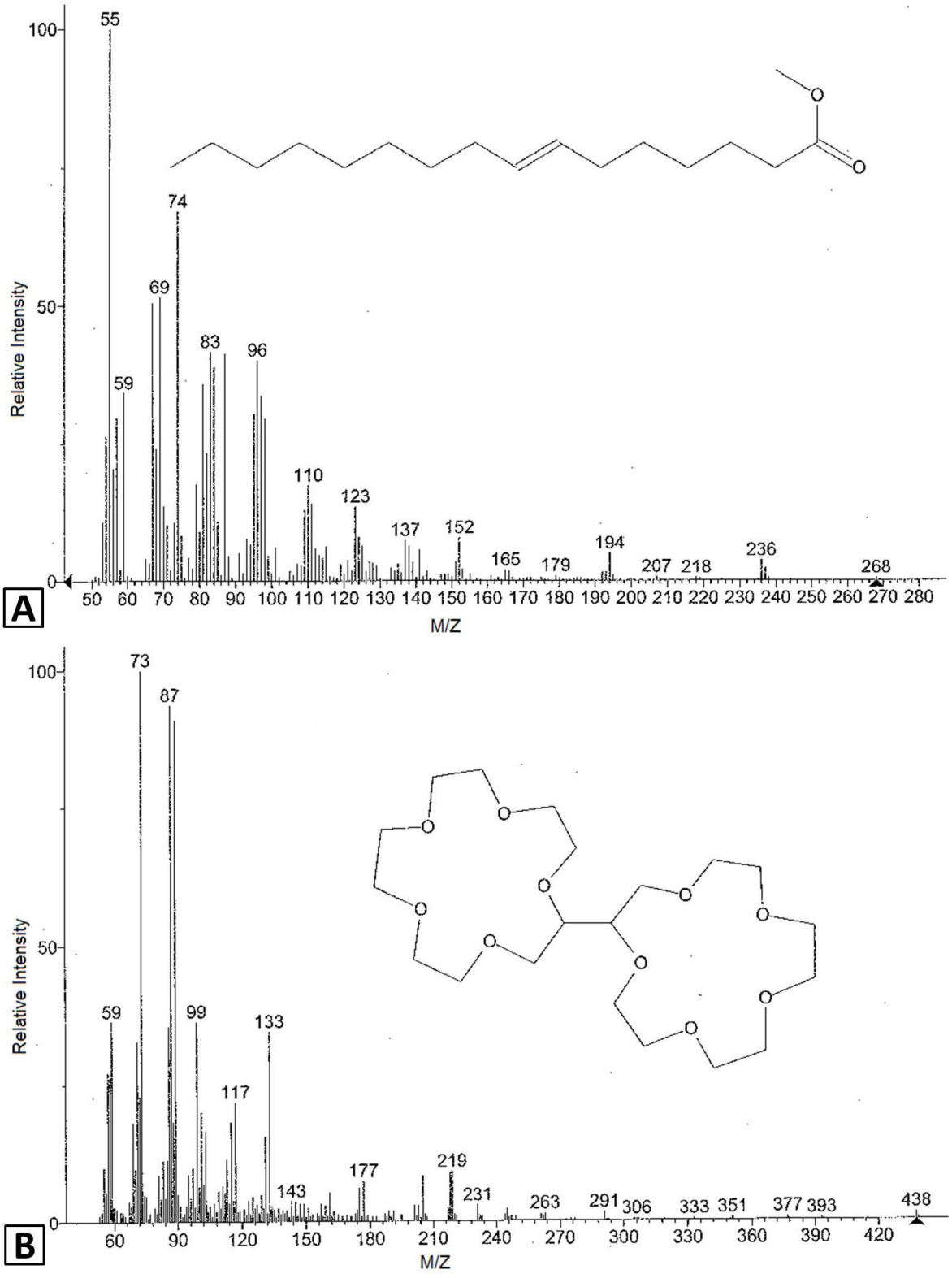

Fig. 2. Representative mass spectrum of the selected two chemical constituents detected in ethanolic extract of propolis collected from Jazan region, Saudi Arabia. **A,** Methyl (7Z)-7-hexadecenoic acid; **B,** (2S, 2S)-2,2-Bis [1,4,7,10,13]-pentaoxacyclopentadecane.

Table I.- Chemical compounds identified by GC-MS analysis of ethanolic extracts of propolis sample collected from Jazan, Saudi Arabia.

Compound identified	Retention time (min)	Molecular formula	Molecular weight	Percent abundance
Fatty acid esters				
Methyl dodecanoate	1.713	$C_{13}H_{26}O_2$	214	0.54
Methyl tetradecanoate	6.598	$C_{15}H_{30}O_2$	242	1.05
Methyl (7Z)-7-hexadecenoate	13.416	$C_{17}H_{32}O_2$	268	11.59
Methyl (9Z)-9-hexadecenoate	13.689	$C_{17}H_{32}O_2$	268	0.20
Methyl hexadecanoate	13.809	$C_{17}H_{34}O_2$	270	0.30
Methyl heptadecanoate	15.010	$C_{18}H_{36}O_2$	284	0.10
Methyl octadecanoate	16.752	$C_{19}H_{38}O_2$	298	5.86
Methyl (9E)-9-octadecenoate	17.087	$C_{19}H_{36}O_2$	296	11.53
Methyl (9Z, 12Z)-9,12-octadecadienoate	17.823	$C_{19}H_{34}O_2$	294	1.68
Methyl (9Z, 12Z, 15Z)-9,12, 15-octadecatrienoate	18.908	$C_{19}H_{32}O_2$	292	1.01
Methyl eicosanoate	19.786	$C_{21}H_{42}O_2$	326	0.51
Methyl -9-eicosenoate	20.109	$C_{21}H_{40}O_2$	324	0.47
Methyl docosanoate	22.741	$C_{23}H_{46}O_2$	354	1.19
Methyl 3-hydroxyoctadecanoate	24.225	$C_{19}H_{38}O_3$	314	1.54
Methyl tetracosanoate	25.599	$C_{25}H_{50}O_2$	382	13.27
Methyl hexacosanoate	28.135	$C_{27}H_{54}O_2$	410	4.10
Hydrocarbons				
3-Methyl pentane	1.223	C_6H_{14}	86	1.34
2,4-Dimethyl pentane	1.273	C_7H_{16}	100	11.83
p-Xylene	1.603	C_8H_{10}	106	1.54
Nonadecane	14.804	$C_{19}H_{40}$	268	0.16
n-Heptacosane	20.884	$H_{27}H_{56}$	380	1.56
E-Pentatriacont-17-ene	29.318	$C_{35}H_{70}$	490	18.06
(2S, 2S)-2,2-Bis[1,4,7,10,13]-pentaoxacyclopentadecane	31.282	$C_{20}H_{38}O_{10}$	438	1.55
Miscellaneous				
3-Tetradecylox-1,2-propanediol	21.259	$C_{17}H_{36}O_3$	288	0.62
Octaethylene glycol monododecyl ether	26.566	$C_{28}H_{58}O_9$	538	1.29

The chemical components identified in this study are expected to be from the vegetation in the surrounding of the beehive location, however, it is difficult to specify that a particular component is from which plant species. To correlate the chemical composition of the propolis sample observed in this study, the constituents present in the neighboring flora should be established. It is well known that the chemical composition of propolis vary from one region to another, mainly due to different types of vegetation in the surrounding. Accordingly, the composition of propolis sample observed in this study was found to be different from the propolis samples from other part of the world. For example, flavonoids, typical constituent in poplar-type propolis, are one of the principal components of Brazilian, European, Russian, Turkish and Chinese propolis, and were not detected in the present investigation (Bankova *et al*., 1983, 2014; Chang *et al*., 2002; Park *et al*., 1997; Silici and Kultuca, 2005; Volpi and Vergonzizni, 2006). Furthermore, a study has reported triterpenoids as one of the major components in prolpolis samples from Yemen, was also not detected in the present study. The difference in the chemical constituents of the propolis is mainly due to different vegetation around beehives of the two regions along with other factors such as geographical locales, weather of sample collection and bee selecting behavior (Al-Ghamdi *et al*., 2017).

Antimicrobial susceptibility test

The ethanolic extract was screened for antimicrobial susceptibility against selected strains of gram positive, gram

negative bacterial strains and yeasts. The antimicrobial property was tested by two methods, the disk diffusion and broth dilution methods. By the disk diffusion technique, antibacterial and antifungal activities of propolis extract were screened by recording the zone of growth inhibition against each microorganism and comparing the potency with those produced by standard antimicrobial drugs (gentamicin for bacteria and amphotericin B for yeasts). The minimum inhibitory concentrations (MICs) of propolis extract were estimated by broth dilution technique using two fold dilutions in 95% ethanol. The measured inhibition zones and MIC values of the extract was summarized in Table II. Among the tested bacterial strains, maximum potency of propolis extract was observed against gram positive bacterial strains *Micrococcus flavus* (inhibition zone: 30.6±0.11 mm) and *Staphylococcus aureus* (inhibition zone: 29.4±0.16 mm), the minimum inhibitory concentrations against both the strains were found to be 1.0 µg/ml. The lowest susceptibility among the tested bacterial strains was observed from *Proteus mirabilis*, a gram negative bacteria (inhibition zone: 10.1±0.23 mm and MIC: 16.0 µg/ml), followed by *Enterobacter cloacae* (inhibition zone 13.2±0.21 mm, MIC: 8.0 µg/ml). Among the tested gram negative bacterial strains, the highest susceptibility was shown by *Escherichia coli* (inhibition zone: 17.2±0.13 mm and MIC: 4.0 µg/ml). *Alternaria alternaria* (inhibition zone: 18.7±0.10 mm and MIC: 4.0 µg/ml) and *Fusarium oxysporum* (inhibition zone: 18.2±0.14 mm and MIC: 4.0 µg/ml) have exhibited the highest susceptibility towards the propolis extract, among the selected fungal strains, whereas *Aspergillus fumigates* (inhibition zone 9.1±0.23 mm, MIC: 16 µg/ml) showed least susceptibility. Fatty acid esters and hydrocarbons are the major constituents detected in the present study and were considered to be mainly responsible for antimicrobial activity of the propolis sample. Several studies have reported antibacterial activity of propolis samples possessing fatty acid esters and phenolic compounds as their main chemical constituents (Greenaway *et al*., 1998; Kujumgiev *et al*., 1999). However, it has usually been seen that combination of different compounds are essential for biological activities of propolis.

In general, the propolis sample screened in this investigation was found to possess good antibacterial and antifungal activities; however, it has been recognized that gram positive bacterial strains have shown considerably greater antimicrobial potency than gram negative bacteria and yeasts. The results of present study was found to be in good agreement with previous studies, where the propolis samples exhibited greater antimicrobial potency against gram positive bacteria than gram negative bacterial and fungal strains (Kujumgiev *et al*., 1999; Slici and Kutluca, 2005; Stepanovic *et al*., 2003; Ugur and Arslan, 2004). However, it is difficult to compare the antimicrobial results of different investigations, due to different chemical composition of propolis samples collected from different regions and/or variations in methods used to evaluate antimicrobial activity. The antimicrobial potential shown by propolis could prove to be therapeutically significant mainly for topical application. Furthermore, Stepanovic *et al*. (2003), have reported synergistic effect of propolis with other antibacterial and antifungal agents, which suggested that the combination of different antimicrobial agents with propolis extract may potentiate the antimicrobial activity and hence would allow reducing the dose of the selected antimicrobial agent.

Table II.- Antimicrobial activities of ethanolic extracts of of propolis from Jazan, Saudi Arabia.

Micro-organisms	Disk diffusion method		Broth dilution method
	Inhibition zone ± SD (mm)		MIC (µg/ml)
	Propolis extract (10µg/disk)	Standard drug# (10µg/disk)	Propolis extract
Gram positive bacteria			
Bacillus cereus	23.5±0.17*	28.3±0.19	2.0
Bacillus megaterium	16.3±0.35*	19.2±0.32	4.0
Staphylococcus aureus	29.4±0.16*	31.4±0.22	1.0
Staphylococcus epidermis	24.1±0.28*	28.2±0.06	2.0
Micrococcus flavus	30.6±0.11	30.4±0.14	1.0
Enterococcus faecalis	17.2±0.12*	24.1±0.18	4.0
Gram negative bacteria			
Enterobacter cloacae	13.2±0.21*	21.5±0.16	8.0
Escherichia coli	17.2±0.13*	19.5±0.13	4.0
Acinetobacter baumannii	14.4±0.24*	21.8±0.11	8.0
Proteus mirabilis	10.1±0.23*	18.3±0.12	16.0
Salmonella enteritides	15.1±0.32*	23.3±0.08	4.0
Yeasts			
Candida albicans	17.5±0.28*	21.4±0.14	2.0
Aspergillus flavus	15.5±0.19*	19.0±0.13	8.0
Aspergillus fumigates	9.1±0.23*	26.2±0.09	16.0
Alternaria alternaria	18.7±0.10*	24.4±0.22	4.0
Cladosporium herbarum	15.6±0.07*	22.3±0.11	8.0
Fusarium oxysporum	18.2±0.14*	20.1±0.13	4.0

#Gentamicin and amphotericin B were taken as standard antimicrobial agents for bacterial and fungal strains, respectively. The experiments were performed in triplicate. SD, Standard deviation. *P < 0.05; n = 3.

CONCLUSION

In this study, the propolis sample was collected from

beehives of *Apis mellifera* from Jazan, Saudi Arabia. Chemical composition of ethanolic extract of propolis was established by GC-MS analysis. Fatty acid esters and long chain aliphatic hydrocarbons were the major constituents identified. The predominant fatty acid esters were methyl-(7Z)-7-hexadecenoate, methyl-(9E)-octadecanoate and methyl tetracosanoate, whereas E-pentatriacont-17-ene and 2,4-dimethylpentane were identified as major aliphatic hydrocarbon compounds. Certain strains of gram positive and gram negative bacteria and yeasts have shown significant susceptibility to ethanolic extract sample. However, in general greater antimicrobial activity was displayed against gram-positive bacteria in comparison to gram negative bacterial and fungal strains. The antimicrobial potential of poroplis would be of great therapeutic interest and may allow substituting some antimicrobial agents especially for topical application or may be helpful to potentiate the antimicrobial agents when used in combination.

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Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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