Chemical composition, total phenol contents, antioxidant and antimicrobial activities of propolis produced by honeybee *Apismelliferajemenitica* from *Ficuspalmata*Forssk in Al-Baha, Saudi Arabia.

Noofal I. M. Bayaqoob[1], Ahmed I. Rushdi[3] and Ahmad A. Al-Ghamdi[2]

[1]Honey bee Center ,Hadramout University

[2]Chair of Engineer Abdullah Ahmed Bagshan for Bee Research, College of Food and Agriculture Sciences, King Saud University, Saudi Arabia

[3]ETAL Consulting, 2951 SE Midvale Dr., Corvallis, Oregon 97333, USA.

Corresponding author: nowfalbayaqoob7@gmail.com

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Abstract

Honeybee hives were setup in Feeg village of Al-Baha province- Saudi Arabia, where *Ficus palmata* plants are dominant in the *Juniperus procera* forest. Propolis samples were collected from these hives for over a year. The propolis samples were extracted using three different solvents including dichloromethane (DCM), mixture of dichloromethane and methanol (DCM:MeOH, v:v, 2:1) and methanol (MeOH). The chemical compositions of the different propolis extracts were determine by gas chromatography-mass spectrometry (GC-MS). The total phenol content (TPC) in each extract was quantified using the Folin-Ciocalteu method. The free radical-scavenge activities (FRSA) of the various propolis extracts were measured by the method of 1,1-Diphenyl-2-picrylhydrazyl (DPPH). The chemical analysis showed that the propolis extracts of the different solvents varied in composition and contained mainly diterepenoids, triterpenoids, fatty acids, n-alkane, and n-alkene. The TPC ranged from 30.5±7.8 for DCM to 168.5±23.3 mg GA/g for DCM:MeOH propolis extracts. The FRSA ranged from 6.56 % for the DCM to 19.22 % for the DCM:MeOH extracts of July 2014. The MeOH extracts of the propolis showed higher toxicity against *Escherichia coli* and *Staphylococcus aureus* than the DCM:MeOH propolis extracts. The latter extracts showed the highest toxicities against *Candida albicans* and *Aspergillus niger*.

Key words: Phenols, Antioxidants, *Apis mellifera jemenitica*, *Ficus palmata*, Saudi Arabia

Introduction

The family Moraceae comprises about 800 tree species (18) where most of them are long trees and shrubs and secret milky liquid when they are cut (19). *Ficus palmata* Forssk, which disperses in regions up to 1000 meters above the sea level, belongs to the Moraceae family in general and is known as Fegra Fig. They sometimes grow in forests but mainly in village borders (13, 38). Five species belong to genus *Ficus* grow wild in Saudi Arabia, including *Ficus palmata*, which is considered as a medicinal plant due to its therapeutic properties (34).

Honeybees are eusocial insect living in different habitats, due to their developed social organization, and exploit plant flora to produce healthy foods and unique valuable chemicals (4). Propolis is one of these valuable chemicals produced by honeybees to use within their nest to protect it from infectious microbes and other threats. Honeybee foragers have been observed by the researchers collecting organic materials from lower surfaces of the leaves of the wild plant *Ficus palmata* in Feeg Village of Al-Baha Province in Saudi Arabia. Many studies have been conducted on different species of genus *Ficus* due to its biological properties (35). Chemical analysis have shown that active compounds, such as sterols or terpenes, are present in the genus *Ficus* spp.(25, 26). Psoralen and bergapten were isolated from the leaves of the species *Ficus carrica* L.(13). Urocoumarin glycosideswas isolated from the leaves of *Ficus ruficaulis* Merr.Var. antaoensis (10). Flavones were isolated from the bark of *Ficus microcarpa* (29). Ficusal, ficusesquilignan a, b and

ficusolide diacetate were found in heartwood of *Ficus microcapra*.(28). Chromones, terpernoids and alkaloids have been isolated from *Ficus lyrata*, *Ficus benjamina* and *Ficus septica* leaves, respectively (5, 39, 52). *Ficus* extracts are used to treat infectious diseases caused by some microbes such as influenza whooping cough, epilepsy and jaundice (6, 37). *Ficus* species could be used as a medicine to treat anti-tumor, anti-inflammatory and tonic medicament (28) and its extracts are utilized as antioxidants (1, 8).

Therefore, the objectives of this study were to investigate the chemical compositions, total phenol contents, antioxidant and antimicrobial activities of the different solvent extracts of the propolis produced by local honeybees from *Ficus* species. This work could be considered as the first work to show that *F. palmata* is the source of propolis components.

Materials and Methods

Apiary site:

Al-Baha Province in Saudi Arabia, which situated between longitude 41˚and 42˚ E and latitude16˚ and 20˚ N occupies 12.000 km^2 and is described as a high region with a diverse vegetation cover comprising about 190 plant species belonging to 59 families (2). These plant species include *Juniperus procera*, *Acacia tortilis* and *Ficus palmata* and others (2), which are also the major plant species of the study area. The Feeg Village (study area) is an apiary site located between Baljrashi Governorate and the center of Al-Baha. It is situated between longitude 41˚30'0'E and latitude 20˚0'0"N. This area is of temperate climate in summer and cold in wet winter and also exhibits fog and rainfall most of the year (14).

Sample collection:

Honeybees were observed collecting materials from the lower surface of the *F. palmata* leaves to produce propolis. Propolis samples were collected over two years from May to September 2014 and from July to September 2015. They were collected from bee hives of *Apis mellifera jemenitica* that were assembled in the Feeg Village. Net plastic was used as a trap for propolis collection from bee hives. The collected propolis samples were usually green in color and they were sticky at high temperature and rigid at low temperature. The propolis samples were kept in glass vials with Teflon caps (Thermo scientific). The vials were marked with the sample names, collection dates and plant sources. All samples in the vials were stored in a refrigerator at -20˚C until further analyses.

Sample Extractions:

For chemical analysis, each sample of propolis was cut into small pieces. About 1g of each sample was extracted separately in 20 ml of three different solvents, including dichloromethane (DCM), mixture of dichloromethane:methanol (DCM:MeOH 2:1, v:v) and methanol (MeOH). Each mixture of the sample and solvent was placed in a shaker for 24 hours then sonicated by using ultra sonication bath at 25˚C for 30 minutes. Glass microfiber filters (47mm) was used to filter each extract, which was transferred to pre-weighed vials. Then the extract was blown by nitrogen gas to dryness and re-weighed to obtain the yield of the extraction. Finally, exactly 0.5 ml of the relevant solvent was added to the vial for chemical analysis by gas chromatography-mass spectrometry (GC-MS), (43).

Derivatization:

Derivatization was performed only to samples that were extracted by DCM:MeOH and MeOH. An exact volume of 20 µl of each sample was added to a 1.5 ml glass vial, then it was evaporated to dryness under nitrogen gas. About 100µl of [N, O-bis (trimethylsilyl) trifluoroacetamide, BSTFA, Pierce Chemical Co.] was added to the aliquot, then placed inside oven for three hours. After cooling down, the aliquot was evaporated to dryness under nitrogen gas and, after dryness, 20 µl of hexane was added for each sample before GC-MS analysis (21).

Chemical analysis:

Instrumental analysis was carried out by Agilent 6890 gas chromatograph coupled to a 5973 Mass Selective Detector (GC-MS) , using a DB-5MS (Agilent) fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) and helium as carrier gas. The GC was temperature programmed from 65°C (2 min initial time) to 310°C at 6°C min^{-1} (isothermal for 55 min final time) and the MS was operated in the electron impact mode at 70 eV ion source energy. Mass spectrometric data were acquired and processed using the GC-MS ChemStation data system.

Total phenol contents:

Folin-Ciocalteu method was used to determine the total phenol contents (TPC) in different extracts of the propolis samples according to the procedure of (48) with some modification. Three different dilutions (5, 10 and 15µl) from each extract of the propolis were mixed with 50µl Folin-Ciocalteu reagent in 96 wells and left for five minutes to stand. To adjust the volume to come 65µl, 10µl of dimethyl sulfoxide (DMSO) was added to 5µl and 5µl DMSO to 10µl, then 80µl of 7.5% sodium carbonate was added and left in dark for two hours under room temperature allowing blue color to develop. The absorbance was measured at 630nm using micro plate reader (MR-96A, SHEZHEN MINDRAY BIO-MEDICAL ELECTRONICS CO., LTD. CHINA). Each volume was performed in three replicates. The TPC in the propolis sample was calculated based on a standard curve (ranged from 25µg/ml to 100µg/ml) using the formula:

$$\text{Absorbance} = 1562.5\times \text{Gallic acid (µg)} - 16.9 \quad (R^2=0.9938)$$

and expressed as milligram of galic acid equivalent per gram (mg GA/g) of propolis.

Free radical-scavenge activity:

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method was used to evaluate the antioxidant activities of propolis extracts according to (7). The DPPH reagent was provided by (SIGMA-ALDRICH, CO., 3050 Spruce Street, SL Louis, MO 63103 USA). Three concentrations from each extract were used to evaluate the antioxidant activities. A one milligram was dissolved in 1 ml DMSO, then 500µl was taken from the main solution to dilute with 500 µl DMSO. Three different dilutions (4, 8 and 12µl) from each extract of the propolis were mixed with 180 µl DPPH reagent in 96 well and incubated in the dark for 30 minutes. The wavelengths from 490 to 630 nm were used to measure the absorbance of each reaction by using micro plate reader (MR-96A, SHEZHEN MINDRAY BIO-MEDICAL ELECTRONICS CO., LTD. CHINA). Each volume was performed in three replicates. Methanol was used as a blank. Galic acid was used as a standard to calculate the antioxidant activity. To determine percentage inhibition, the flowing formula was used:

$$\text{Percentage inhibition} = [(A_0-A_1/A_0) \times 100]$$

where A_0= Absorbance of negative control and A_1= Absorbance of sample.

Antimicrobial activity:

Disc diffusion method was used to evaluate antimicrobial activities of the propolis samples against four human pathogens including gram-negative *Escherichia coli* ATCC 25922, gram positive *Staphylococcus aureus* ATCC 25923, *Aspergillus niger* AUMC 8777 and *Candida albicans* ATCC 66193. All pathogen strains were obtained from the Microbiology Laboratory in the Botany and Microbiology Department, College of Science, King Saud University. Nutrient agar was used to grow bacteria strains (*E.coli* and *Staphylococcus aureus*) at 37 ˚C for 24 hours in an incubator. Potato dextrose agar was used to grow *Candida albicans* and *Aspergillus niger* at 25 ˚C for 48 hours. To adjust the turbidity of 0.5 McFarland standards (108 CFU/mL), saline solution (0.089% NaCl) was used to prepare suspension for *Candida albicans*, while *Aspergillus niger* was applied directly by using sterile cotton applicator where spores have been picked up from colonies to inoculate the media in petri dishes. Sterile blank discs (6mm in diameter) were submerged with 60 µl of each extract and laded on the surface of plate. Inhibition zone diameter was used to evaluate the antimicrobial activities of propolis extracts. Each extract was performed in triplicates.

To determine the susceptibility of both gram positive and negative bacteria, ampicillin 10µg/disc as positive control, and nystatin 100 µg/disc were used as a standard for yeast and fungus (30).

Statistical analysis:

SAS9.2® software was used for data analysis. Means and standard deviations of the results were calculated using general linear model (GLM). Variance tables were constructed using T-test significant difference method at $P < 0.05$. Correlation coefficients between total phenolic contents and antioxidant activity were calculated using PROC CORR. and their levels of significance at $P<0.05$.

Results

Chemical analysis:

The means of yields of propolis extracts are listed in Table 1. The yields ranged from 0.42 to 1.4 mg (Mean = 0.78±0.4mg) for DCM, 0.18 to 0.5 mg (Mean = 0.45±0.2 mg) for DCM:MeOH, 0.18 to 0.32 mg (mean = 0.41±0.3 mg) for MeOH extracts in the year 2014. In the year 2015, they ranged from 0.77 to 1.61 mg (Mean = 1.29±0.5 mg) for DCM, 0.71 to 1.19 mg (Mean = 1.01±0.3 mg) for DCM:MeOH and 0.07 to 1.2 mg (Mean = 0.76±0.6 mg) for MeOH.

The chemical compositions of the different propolis extracts are listed in Table 2. The major compounds are trierpenoids, diterpenoids, n-alkanes, n-alkanoic acids and n-alkenes for DCM; diterpenoids, triterpenoids, n-alkanoic acids, n-alkanes, n-alkenes, n-alkanols and minor amounts of abietane diterpenoids, phenolic acids carbohydrates and sterols for DCM:MeOH, diterpenoids, triterpenoids, carbonhydrates, n-alkanoic acids, abientane diterpenes sterols and minor levels of monoterpenes and sesquiterpenes for MeOH.

Total phenol contents:

The TPC values of the propolis extracts, during the period May - September 2014, are shown in Table 3. The TPC of the DCM:MeOH extract during September 2014, was significantly greater than that of May - August 2014 ($P < 0.05$). The overall mean TPC values of the 2014 DCM, DCM:MeOH, and MeOH extracts of propolis ranged from 38.0±15.5 - 102.0±24.0, 50.0±7.1 - 168.5±23.3, and 30.5±7.8 - 62.0±32.5 mg GA/g, respectively. In addition, the mean TPC values of the propolis extracts of May - July 2014 were significantly different ($P < 0.05$) than those of August - September 2014, and the TPC mean of the DCM:MeOH extracts of September 2014 was higher than that of May - August, 2014. However, there was no significant difference in the TPC values of the propolis MeOH extracts of May - September 2014 ($P < 0.05$; Table 3). In 2015 (July, August, and September), The mean TPC values of the DCM, DCM:MeOH, and MeOH propolis extracts of July - September 2015 ranged from 48.5±23.3 - 108.5±29.0, 32.0±8.5 - 53.5±20.5, and 40.5±14.8 - 93.5±10.6 mg GA/g, respectively. The TPC of the MeOH extracts of September 2015 was significantly different from that of the MeOH extracts of July and August (Table 3).

Free radical-scavenging activity:

All propolis samples collected during the two successive years, exhibited free radical scavenging activity (FRSA). The FRSA value (13.5%) of the DCM extracts of propolis of September 2014 was greater than those (6.6 – 10.6%) of the DCM extracts of May - August 2014 (Table 3). Meanwhile, the FRSA values (8.0 – 19.2%) of May - September 2014 DCM:MeOH proplis extracts were greater than those (5.6 – 17.0%) of the 2014 MeOH extracts. There were also significant differences in the FRSA of the propolis extracts of July - September 2015. The FRSA values of the DCM and DCM:MeOH propolis extracts of September 2015 (11.9 for DCM and 12.6 % for DCM:MeOH) were greater than the corresponding extracts of July (4.8 for DCM and 7.0 % for DCM:MeOH) and August 2015 (6.9 for DCM and 7.7 % for DCM:MeOH) (Table 3). However, there were no significant differences among the MeOH extracts of propolis (Table 3).

Antimicrobial activity:

The results of different propolis extracts of May 2014 showed no significant difference in the zone of inhabitation (ZOI) against *E.coli*, *S. aureus* and *C. albicans*; while the MeOH extract of July showed a significant inhibitory activity against *A. niger* ($P < 0.05$; Table 4). The MeOH extracts of June 2014 showed a significant ZOI against *E. coli* and *S. aureus* ($P < 0.05$), whereas all extracts showed the same inhibitory activity against *C. albicans* and *A. niger* (Table 4). DCM extract of propolis of July 2014 showed a significant inhibitory activity against *E. coli* ($P < 0.05$), while the DCM: MeOH and MeOH extracts showed significant inhibitory activity against *S. aureus* ($P < 0.05$: Table 4). These propolis extracts showed same inhibitory activities against *C. albicans* (Table 4). The MeOH extract showed a strong inhibitory activity against *A. niger* ($P < 0.05$; Table 4). The MeOH extract of propolis of August 2014 showed a significant inhibitory activity against *E. coli* and *S. aureus* ($P < 0.05$), whereas the DCM:MeOH extract showed a significant inhibitory activity against *C. albicans* and *A. niger*. ($P< 0.05$: Table 4). The extracts of propolis of September 2014 showed no significant difference between inhibitory activity against *E. coli*, *C. albicans* and *A. niger*, whereas the DCM:MeOH extract showed inhibitory activity against *S. aureus* ($P < 0.05$; Table 4). The propolis samples obtained in the second year (July to September 2015) showed a significant inhibitory activity against the above mentioned human pathogens. The DCM:MeOH and MeOH propolis extracts of July 2015 showed significant inhibitory activity against *E. coli* ($P < 0.05$), whereas the DCM extracts showed a significant inhibitory activity against *S. aureus* ($P < 0.05$; Table 4). In addition, these extracts showed a significant inhibitory activity against *A. niger* and *C. albicans* ($P < 0.05$; Table 4). The propolis extracts of August 2015 using DCM and DCM:MeOH showed a significant inhibitory activity against *E. coli* and *S. aureus* ($P < 0.05$). In addition, the MeOH and DCM:MeOH extracts showed a strong ZOI against *C. albicans* and *A. niger* ($P < 0.05$; Table 4). The MeOH extracts of September 2015 showed a significant inhibitory activity against *E. coli*, *S. aureus*, and *A. niger* ($P < 0.05$), whereas the DCM:MeOH extracts showed a significant inhibitory activity against *C. albicans* ($P < 0.05$; Table 4).

Discussion

The current study is considered as the first report to investigate *Ficus palmata* as a source of propolis. The major compounds of the different propolis extracts included diterpenoid, triterpenoid, sesquiterpene, fatty acids, monoterpenoid, sesquiterpenoid and carbohydrates. Studies on different species of *Ficus* spp. have detected similar compounds such as fatty acids (19) polysaccharides (58) phenolic compounds (14, 50, 50, and 54). Trans-caryophyllene has been found in Brazilian propolis (30), whereas β-amyrin, and lupeol were detected in leaf extracts of *F. benghalensis* and *F. religiosa* (45, 49, and 55). Moreover, different compounds have been detected in different types of propolis collected from various geographical areas, such as caryophyllene oxide and hexadecanoic acid detected in propolis produced by stingless bees in Yucatan, Mexico (42), δ-cadinene and cedrol has been found in propolis produced by honeybee in Italy (16, 41), cedrene has been found in propolis from China (13), sesquiterpene alcohol has been identified in different propolis samples collected from Albania, Bulgaria and Mongolia (3), and α-pinene, β-pinene and β-eudesmol were found in propolis samples collected from Brazil and China (22, 31).These compounds have been also detected in the different propolis extracts of the current study. Compounds; such as (+)-manool, totarol, which were found in significant amounts in our propolis samples, were also detected in propolis samples produced by stingless bees (40). Phenolic compounds are available in both edible and non-edible plants and act as antioxidant, antimicrobial (20). The presence and variation of the phenolic compounds in propolis, which influence their biological activities, are related to plant sources. The variation of TPC levels of the different propolis extracts may be due to the type of solvent, solubility of compounds, plant source, geographic area and time of collection. For instance, the TPC in the mixture of DCM:MeOH extracts of propolis of September, June and July 2014, were higher than the MeOH extracts, which may affect the biological properties of propolis such as antioxidant and antimicrobial. Free radicals

contribute or play normal physiology in human body but in, specific conditions, their presence of more than normal make it reactive with oxygen species and induce cellular damage causing some diseases for human such as cancer, arteriosclerosis and inflammatory disorders (1). However, free radical scavenging is important to treat chronic diseases (51, 56). Also, the a range of total phenolic contents of this study agrees with other levels in different propolis samples from different countries such as Korea, Brazil, China and Australia in literatures (57).

In this study the DCM:MeOH propolis extract of September 2014 showed high level of TPC, but the free radical scavenging activity was low. Despite some compounds were present in low concentration, such as 4,6-Dioxoheptanoic acid, Succinic acid-bis in DCM:MeOH propolis extract of July 2014, but this extract exhibited strong free radical scavenging activity (19.22%). This indicated that compounds may play a role in free radical scavenging activity. Also the MeOH propolis extract of July 2014 exhibited a significant free radical scavenging activity, which may be due to the presence 5-epi-neointermedeol, ferruginol, succinic acid-bis and vanilic acid which act as antioxidant (9, 36). Other compounds act as antioxidants, including camphene, carveol and artemetin, which have been found in the DCM and DCM:MeOH propolis extracts of September 2015. Moreover, the FRSA and TPC of the propolis extracts; prepared in May, June, July, August, and September 2014, were significantly correlated ($r = 0.90$), as were those of the propolis extracts prepared in July, August, and September 2015 ($r = 0.74$).The correlation between phenol contents and antioxidant activity of the propolis extracts of DCM, DCM:MeOH and MeOH was highly significant ($P< 0.05$).

Not all phenolic compounds were effective against all human pathogens even though their concentrations were high. For example, the DCM:MeOH propolis extract of September 2014 exhibited a significant activity against *Aspergillus niger* and may be attributed to the presence of sandaracopimaric acid, ferruginol, sclareol and β-lupeol. On the contrary, the same extract showed low inhibitory activity against *Candida albicans*. In contrast, MeOH extract of August 2014 exhibited a significant inhibitory activity against *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus niger*, although the TPC concentrations were low. This can be attributed to the presence of totarol, dehydroabietan, 7-ketototarol, iso-communic acid, sandaracopimaric acid, β-lupeol and β-amyrin. This finding is consistent with different studies in literatures which have shown that antimicrobial and antioxidant activities depend on the availability of certain compounds in propolis (23, 33, and 46). The variable of inhibitory activity of different propolis extracts from different months may due to the variation in concentrations of certain compounds, such as ferruginol, which possess antimicrobial activity. The concentration variability of these compounds may also due to their different solubility in different solvents. Extraction method play a role in the concentration of compounds, which reflects on biological properties of the compounds (47). Moreover, other compounds are present in low concentrations of specific extracts, which may act as synergic with other compounds such as 13-epi-manool, (+)-manool, totarol, β-Amyrin, lupeol, cedrene and cedrol. For example, the MeOH extracts of the propolis of September 2014 exhibited a strong inhibitory activity against *E. coli*, *S. aureus*, *C. albicans* and *A. niger*, despite the low concentrations of the these compounds. The MeOH extract of propolis of May 2014 showed a strong inhibitory activity *E. coli*, *S. aureus*, *C. albicans* and *A. niger*, this could be attributed to the presence of totarol, 7-ketototarol, communic acid, isopimaric acid, β-amyrin and β-lupeol. This finding is consistent with the results of Runyoro et al., (44), where ethanolic extract of propolis has a strong inhibitory activity with the presence of antimicrobial agents. Moreover, the DCM:MeOH and MeOH propolis extracts of July 2015 exhibited strong inhibitory activity against *E. coli*, *S. aureus*, *C. albicans* and *A. niger*; this activity may be due to the presence of high concentration of 7-ketototarol. The DCM propolis extracts of July 2015 exhibited a significant inhibitory activity against *S. aureus*, which may be attributed to the presence of different compounds such as ferruginol, cis-franesol and α-amyryl acetate. This may indicate that the level of TPC is not the important factor for biological property of the propolis rather than specific compounds present in

propolis that have certain property such as antioxidant agent or antimicrobial activity. This finding is compatible with the finding of Kumazawa et al., (23).

Conclusion

This work can be considered as the first study to report that honeybees collect lipophilic materials from lower surface of *Ficus palmata* leaves. The major compounds of the different extracts of the propolis samples, produced in different months, included diterpenoids, triterpenoids, fatty acids, n-alkane and n-alkene. The results revealed that a high level of TPC was found in DCM extracts, while the high percentage of free radical scavenging activity was detected in the DCM:MeOH and MeOH propolis extracts. The MeOH extracts exhibited a strong inhibitory activity against all human pathogens. Some samples showed low antioxidant capacity and negative results indicating that DPPH method was not sufficient. Therefore, using more than one method is important to evaluate the antioxidant activity of propolis in future studies. Further studies are needed to investigate this type of propolis.

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Table 1. The yields (mg) of propolis extracts of different solvents for the samples collected from May 2014 to September 2015.

	Year 2014							Year 2015				
Solvent	May	June	July	August	September	Mean	SD	July	August	September	Mean	SD
DCM	0.42	0.46	0.48	1.1	1.4	0.78	0.4	0.77	1.61	1.5	1.29	0.5
DCM:MeOH	0.18	0.49	0.46	0.44	0.68	0.45	0.2	1.12	1.19	0.71	1.01	0.3
MeOH	0.18	0.32	0.18	0.58	0.78	0.41	0.3	1.0	1.2	0.07	0.76	0.6

Table 2.The chemical compound groups of the different solvent extracts of propolis collected from May 2014 to September 2015.

Solvent	Chemical group	Relative concentration (%) Year 2014					Relative concentration (%) Year 2015		
		May	June	July	August	September	July	August	September
DCM:MeOH	Monoterpene alcohol	0	0.05	0	0	0	0	0	0
	Neolignan biphenol	0	0.5	0	0	0	0	0	0
	Lipids	0	0.3	0	0	0	0	0	0
	Cycloalkane	0	0.14	0	0	0	0	0	0
	Noncyclic triterpenoid	0	0.07	0	0	0	0	0	0
	Flavonoid	0	0	0.03	0.01	0	0	0	0
	Bicyclic sesquiterpene	0	0	0.02	0	1.19	0	0	0
	Bicyclic diterpenoid	0	0	0.63	0	1.19	0	0	0
	Cyclic diester	0	0	0.07	0	0	0	0	0
	Sugar	0	0	0	12.4	9.56	1.55	0.08	0.34
	Tricyclic sesquiterpene	0	0	0	0	0	0.4	0.06	0
MeOH	Diterpenoids	83.7	13	1.44	7.8	0.98	38.8	33.5	48.6
	Triterpenoids	1.75	0.59	0	0.33	0.17	0	0	0
	Fatty acids	1.89	0.06	0.19	0.42	0.48	0	0	0
	Abietane diterpene	1.31	0.08	0	0.07	0.04	0	0	0

Continue table 2:

Solvent	Chemical group	Relative concentration (%) Year 2014					Relative concentration (%) Year 2015		
		May	June	July	August	September	July	August	September
	Monoterpene	1.98	0	0	0	0	0	0	0
	Steroids	0.69	0	0.36	0.3	0	0	0	0
	Sugar	25.98	0	6.1	6.3	9.62	0	0	0
	Lipids	0	0.14	0	0	0	0	0	0
	Sesquiterpene	0	0	0.06	0	0	0.07	0	3.1
	Bicyclic diterpene	0	0	2.46	3.83	0	0	0	0
	Alcohol	0	0	2.1	0.17	0	0	0	0
	Phenols	0	0	0.02	0	0	0	0	0
	Vitamin A	0	0	0	2.94	0	0	0	0
	Tricyclic sesquiterpene	0	0	0	0	0	5.62	10.32	0
DCM	Diterpenoids	4.9	5.24	2.53	1.25	0.37	19.83	0.20	0
	Triterpenoids	11.0	11.13	0.91	0.40	0	0.58	0	1.17
	Fatty acids	14.2	0	0	0	0.58	0	0	6.23
	n-alkane	11.51	17.93	10.32	23.0	22.94	9.42	27.36	23.2
	n-alkene	1.32	0	2.28	1.39	2.92	2.83	5.56	2.64
	Sesquiterpene	0	0	0	0	0	1.5	2.1	0.68
	Monoterpenoids	0	0	0	0	0	7.89	0.73	1.61
	Bicyclic monoterpene	0	0	0	0	0	0	0.95	0.14
	Bicyclic sesquiterpen	0	0	0	0	0	0	0	0.27
	Flavonoids	0	0	0	0	0	0	0	0.59
	Benzofuran	0	0	0	0	0	0	0	2.38

Continue table 2:

Solvent	Chemical group	Relative concentration (%) Year 2014					Relative concentration (%) Year 2015		
		May	June	July	August	September	July	August	September
DCM:MeOH	Diterpenoids	42.5	27.29	0.24	2.12	0.86	19.83	24.64	0
	Triterpenoids	61.0	1.81	0	0	0.1	0	0	0
	Fatty acids	12.12	2.0	0.25	0.68	0.71	0	0	0
	n-alkane	2.42	13.5	0	1.6	0	0	0	0
	n-alkene	2.82	0	0	0	0	0	0	0
	Hydrocarbone	1.36	0.41	0	0.15	0.17	0	0	0
	Abietane diterpenoid	1.1	0	0	0.08	0	0	0	0
	Phenolic acid	0.18	0	0.03	0	0	0	0	0
	Phenolic derivative	0.03	0	0.02	0	0	0	0	0
	Carboxylic acid	0.21	0	0.01	0	0	0	0	0
	Alcohol	1.1	0.18	0.39	0.33	0.54	0.05	0	0
	Sterol	0.46	0	0	0	0	0	0	0
	Tetracyclic triterpenoid	0	0.65	0	0	0	0	0	0
	Vitamin A (Retino)		9	0	2.76	0	0	0	0

Table 3. The TPC values and FRSA of the propolis extracts of May - September 2014. (Means of triplicates ±SD) (P < 0.05).

Solvent	Parameters	Year 2014					Year 2015		
		May	June	July	August	Sep	July	August	Sep
DCM	TPC mg GA/g	93.5±13.4a[1]	102±24a	101±15.5a	38.5±9.1b	38±15.5b	52.5±36a	48.5±23.3a	108.5±29a
	FRSA (%)	9.1±3.5ba[2]	10.6±2.8ba	6.6±3.6b	7.2±2.2b	13.5±4.5a	4.8±2.7b	6.85±4.1ba	11.9±0.99a
DCM:MeOH	TPC mg GA/g	88±12.7cb	98±15.7b	96.5±19.1b	50±7.1c	168.5±23.3a	32±8.5a	53.5±20.5a	33.5±1a
	FRSA (%)	14.7±0.9ba	10±1.1bc	19.2±3.9a	8±1.7c	13.4±4.5b	7±2b	7.7±2b	12.9±1.9
MeOH	TPC mg GA/g	30.5±7.8a	51.5±19.1a	62±32.5a	33±17a	44.5±12a	40.5±14.8b	51.±16.3ba	93.5±10.6a
	FRSA (%)	10.2±5ba	10±4.8ba	17±9.6a	5.6±2.5b	6.2±2.1b	8±2.4a	8.2±1.9a	7.1±1.7a

1 = Means with same letters are not significant different at P <0.05.

2 = Comparisons significant at the 0.05 level are indicated by a. b.

Table 4. The ZOI (mm) measurements of different propolis extracts of May and June 2014 against four human pathogens. (Means with the same letter are not significantly different (P < 0.05), where significant at the 0.05 level are indicated by a. b.)

Pathogens	Solvent	Year 2014					Year 2015		
		May Mean± SD	June Mean± SD	July Mean± SD	August Mean± SD	Sep Mean± SD	July Mean± SD	August Mean± SD	Sep Mean± SD
E. coli	DCM	17.3 ± 2.1 a	11.3 ± 0.58 c	16.7 ± 0.58 a	14.7 ± 0.58 b	15.7 ± 0.58 a	14.3 ± 1.15 b	14.3 ± 0.58 a	14.0 ± 0.0 b
	DCM:MeOH	17.7 ± 0.58 a	13.3 ± 1.2 b	12.7± 0.58 b	14.7 ± 0.58 b	14.3 ± 1.2 a	16.7 ± 0.58 a	15.0 ± 0.0 a	14.0 ± 0.0 b
	MeOH	19.7 ± 0.58 a	19.3 ± 0.58 a	12.3 ± 0.58 b	17.7 ± 1.2 a	14.7 ± 0.58 a	16.3 ± 1.15 a	11.7 ± 0.58 b	14.7 ± 0.58 a
S. aureus	DCM	16.3 ± 0.58 a	14.0 ± 1.0 b	11.7 ± 0.58 b	13.3 ± 0.58 b	13.0 ± 1.0 b	17.0 ± 1.0 a	14.3 ± 1.2 ba	14.0 ± 1.0 b
	DCM:MeOH	16.0 ± 1.0 a	15.3 ± 0.58 b	15.3 ± 0.58 a	13.3 ± 0.58 b	16.3 ± 1.2 a	14.3 ± 1.2 b	15.0 ± 0.0 a	14.0 ± 0.0 b
	MeOH	17.7 ± 2.1 a	17.3 ± 1.2 a	14.3± 0.58 a	16.7 ± 0.57 a	13.0 ± 1.0 b	15.7 ± 0.58 ba	12.7 ± 1.15 b	16.3 ± 1.5 a
C. albicans	DCM	15.0 ± 1.0 a	15.7±1.15 a	15.3 ± 1.5 a	14.7 ± 0.58 ba	14.0 ± 1.0 a	13.7 ± 1.2 b	14.0 ± 1.0 b	15.0 ± 1.0 b
	DCM:MeOH	13.7 ± 0.58 a	16.0±1.0 a	16.3 ± 1.5 a	15.3 ± 0.58 a	13.7 ± 0.58 a	14.3 ± 0.58 b	14.7 ± 0.58 ba	17.7 ± 0.58 a
	MeOH	14.7 ± 0.58 a	14.0±1.0 a	17.7 ± 2.1 a	13.7 ± 0.58 b	13.3 ± 1.2 a	18.7 ± 2.1 a	16.3 ± 1.15 a	15.7 ± 0.58 b
A. niger	DCM	17.0 ± 0.0 ba	17.3 ± 0.58 a	10.7 ± 1.5 c	16.0 ± 1.0 a	16.0 ± 1.0 a	14.7 ± 0.58 c	15.0 ± 1.0 b	13.3 ± 0.58 b
	DCM:MeOH	15.7 ± 0.58 b	17.0 ± 1.0 a	14.0 ± 1.0 b	15.3 ± 0.58 a	17.0 ± 2.0 a	18.3 ± 0.58 a	17.0 ± 1.0 a	14.3 ± 1.2 b
	MeOH	17.7 ± 1.5 a	17.3 ± 1.2 a	20.7 ± 1.15 a	15.3 ± 0.58 a	16.0 ± 1.0 a	16.7 ± 1.2 b	15.3 ± 0.58 ba	16.7 ± 1.5 a

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التركيب الكيميائي, مجموع الفينولات, والنشاط المضاد للأكسدة والميكروبات للبروبوليس المنتج بواسطة سلالة نحل العسل اليمني من نبات التين البري في منطقة الباحة- المملكة العربية السعودية

نوفل إبراهيم محمد بايعقوب[1]، أحمد إبراهيم رشدي[3] و أحمد عبدالله الغامدي[2]
مركز عسل النحل، جامعة حضرموت[1]
كلية علوم الأغذية والزراعة، جامعة الملك سعود، المملكة العربية السعودية[2]
[3]ETAL Consulting, 2951 SE Midvale Dr., Corvallis, Oregon 97333, USA.
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الملخص

خلايا نحل العسل التي خضعت للدراسة تم وضعها في وادي فيق بمنطقة الباحة جنوب المملكة العربية السعودية حيث أشجار التين البري منتشرة في غابات أشجار العرعر. تم جمع عيّنات البروبوليس من الخلايا لأكثر من سنة. ثلاثة مذيبات عضوية أُستخِدمت في عملية الاستخلاص (ثنائي كلورو ميثان, مزيج من دراي كلوروميثان والميثانول بنسبة 2:1 و الميثانول). جهاز التحليل الكرموتوجرافي وطيف الكتلة أُستخِدم في تعريف المركبات الكيميائية لكل مستخلصات البروبوليس. مجموع المركبات الفينولية تم تقديره باستخدام طريقة (Folin-Ciocalteu) في كل مستخلصات البروبوليس في حين أنَّ نشاط الجذور الحرة لجميع المستخلصات قدُر بطريقة (DPPH) 1,1-Diphenyl-2-picrylhydrazyl . أظهر التحليل الكيميائي لمستخلصات البروبوليس احتوائها على المركبات الفينولية Diterpenoids, Triterpenoids, Fatty acids, n-alkane, n-alkene. مجموع المركبات الفينولية في مستخلص ثنائي كلورو ميثان من 30.5±7.8 مليجرام لكل جرام Galic acid في حين أنَّ مستخلص مزيج الداي كلوروميثان والميثانول تراوح من 23.3± 168.5 مليجرام لكل جرام Galic acid. نشاط الجذور الحرة تراوح من 6.56-19.22% لمستخلص البروبوليس (مزيج الثنائي كلورو ميثان والميثانول) لعينة شهر يوليو 2014.م ومستخلص البروبوليس الميثانولي سمية عالية ضد البكتيريا السالبة لصبغة جرام *Escherichia coli* والموجبة لصبغة جرام *Staphylococcus aureus*, في حين أظهر مستخلص البروبوليس (ثنائي كلورو ميثان + ميثانول) سمية عالية ضد الخميرة *Candida albicans* والفطر *Aspergillus niger*

الكلمات المفتاحية: الفينولات، النشاط المضاد للأكسدة، سلالة نحل العسل اليمني، التين البري، المملكة العربية السعودي.