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Antibacterial and Antioxidant Activities of Some Saudi Arabia Honey Products

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ABSTRACT

Background: The current study was aimed to evaluate the antibacterial and antioxidant activities of some Saudi Arabia honey products.

Methods: For this investigation, sixty Saudi Arabia honey products were tested to determine the antimicrobial activity against highly antibiotic-resistant pathogens as well as antioxidant activity in comparison with Manuka honey as a standard.

Results: Testing Saudi Arabia honeys, different levels of growth suppression were observed against five bacterial strains. The pathogenic strains were *Staphylococcus aureusas, Escherichia coli, Proteus vulgaris, Citrobacter diversus* and *Salmonella enterica*. These suppression levels depended on the type of honey. The comparative study of Saudi Arabia honeys revealed a strong correlation between total polyphenol and flavonoid contents and significant radical scavenging activities.

Conclusion: It was concluded that Saudi Arabia honey products have the capacity to suppress the growth of pathogenic bacteria and perform significant radical scavenging activities.

Keywords: Antibacterial activity, Antioxidant activity, Saudi Arabia honey

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Introduction

The widespread, excessive and unnecessary use of antibiotics has made the bacterial infections treatment difficult as it contributes to the development of resistance to the harmful pathogens (1,2). Bacterial resistance against antibiotics has led to the serious public health (3,4).

Since ancient times, honey has been used as an alternative medicine, eldest sweetener and nutritive agent (5). It is an effective remedy (6), and bactericidal (7-11) combination. Honey was also used to be applied topically in the management of wounds and burns (12), and also for the liver problems (13).

Physicochemical properties of honey depend mainly on several factors as floral source (14), environmental climatic conditions and the type of flowers utilized by the bees (15). The chemical composition and physical properties of the honey products from different sources have been studied in different researches (16-22).

Phenolic acids, flavonoids, vitamins, enzymes, mineral contents particularly copper and iron were

determined to be the main honey constituents, which were responsible for the redox properties of the natural dietary antioxidants **(21-26)**. Great variation in the biological properties of the honeys was related to the geographical and botanical origin of the product. The storage condition and processing of honeys also affect the biological proprieties **(21,25)**.

In Saudi Arabia, there are many types of honeys either monofloral or polyfloral with great variations in their botanical origin as well as geographic features. A comparison has been made previously between Egyptian and Saudi Arabia honey products **(8)**. Honey has been available in the Saudi Arabia markets either local or imported from other countries with variable prices and qualities **(27,28)**. The present investigation evaluated the antibacterial activity of sixty Saudi Arabia honey samples against some resistant bacterial strains of medical importance as well as their antioxidant activity and physicochemical properties as compared to the global standard honey; Manuka honey from New Zealand.

Materials and Methods

Honey Samples

Sixty Saudi Arabia honey samples either monofloral (30 samples) or polyfloral (30 samples) from different geographical and botanical origins were tested. The monofloral honeys (10 each) were Sidr, Somir, and Thym while polyfloral honeys (10 each) were Gezan Mountain, Acacia, and Talh. The samples were provided from Alnahal Aljwal apiary farm, Saudi Arabia, from six different geographical regions during the harvesting and flowering season period in 2019. Manuka honey (monofloral honey) was used as a standard authorized honey type from New Zealand. The monofloral honey was selected according to the previous study **(29)**. Of Louveaux et al. ten grams of honey was dissolved in 20 ml of warm distilled water (40 °C). They were centrifuged for 10 min at 2500g. The entire sediment was putted on a slid and spread out over an area about 20 X 20 mm, after drying by slight heating at 40 °C. The sediment was mounted with gelatine, liquefied by heating in water bath at 40°C. Melissopalynology was used as a reference. **(29)**. The honey samples were sent immediately to the laboratory in the dark glass containers kept at 4ºC until analysis.

Determination of Honey Physiochemical Constituents

The physicochemical constituents of the honey samples were determined moisture, glucose, fructose, sucrose, hydroxymethylfurfural, acidity and diastase enzyme contents according to EL-Metwally *et al.* study **(30)**. The pollens were identified by the sedimentation technique as described by Louveaux *et al.* who used sedimentation of pollen analysis **(29)**. The analysis of moisture **(31,32)**, hydroxymethylfurfural **(33)**, diastase activity (31), acidity **(31)** sugar composition (Official Methods of Analysis) **(34)** were also determined.

Antibiotic-Resistant Pathogenic Strains

Five antibiotic-resistant bacterial strains (Gram-positive and Gram-negative) used in this investigation included *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 35218), *Proteus Vulgaris* (ATCC 13315), *Citrobacter diversus* (ATCC 13315) and *Salmonella enterica* (ATCC 700931). These organisms were provide and maintained by Department of Zoonotic Diseases, National Research Centre, Egypt.

Antibacterial Assays

Each bacterial strain suspension was freshly prepared by inoculating fresh stock culture into the broth tube containing 10 mL Muller Hinton Broth (Company brand). The inoculated tubes were incubated aerobically at 37°C for 24 hr. Serial dilutions were then prepared for each strain and matched with a 0.5 Mc-Farland scale standard. The antimicrobial activity of honeys was detected by well diffusion method according to Katirciolu *et al.* **(35)**. Each honey sample was added to individual tube (50 µl) and left for 1 hr incubation time at 25°C to allow homogenous diffusion and minimize the effect of variation between the applications of different solutions. After that, the plates were aerobically re-incubated at 37°C for 24 hr to allow the bacterial growth. After incubation, the inhibition zones were measured to evaluate the antimicrobial activity of each tested honey sample. The experiments were performed in triplicates for the statistical relevance and mean±SE data was used for calculation.

Detection of Total Phenolic Content (TPC)

Honey total phenolic content (TPC) was detected using Folin Ciocalteu reagent **(36)** with the method described by Chua *et al.* and Bertoncelj *et al.* **(25,37)**. Honey solution (0.5 mL) was mixed with 2.5 mL Folin Ciocalteu reagent (2N) and incubated for 5 min. Subsequently, 2 mL sodium carbonate solution (75 gr/L) was added and incubated for another 2 hr at 25ºC. The absorbance of the solution was measured at 765 nm after incubation using a UV-Visible spectrophotometer (Perkin-Elmer Lambda 25, Waltham, MA, USA). Gallic acid (0–1000 mg/L) was used as a standard for the calibration curve preparation. The mean value of triplicate assays of TPC was reported and expressed as milligram of gallic acid equivalent (GAE) in the gram of honey **(38)**.

Determination of Total Flavonoid Content (TFC)

The volume of 5 mL honey solution with 0.1 gr/mL concentration was mixed with 5 mL 2% aluminum chloride ($AlCl_3$) for determination of total flavonoid content (TFC). The mixture was then incubated for 10

min at 25ºC. The absorbance of the formed complex was measured at 415 nm using a UV-Visible spectrophotometer. The standard chemical for the calibration curve preparation was Rutin with concentration 0–100 mg/L. The mean value of triplicate assays of TFC was reported and expressed as milligram of rutin equivalent (RE) in the gram of honey **(25,38)**.

Antioxidant Assay to Determine DPPH Scavenging Activity

This test is based on the change in the absorbance by reducing the purple DPPH radical using an oxidizing antioxidant. The scavenging effect of vitamin C and caffeic acid as well as honey samples were corresponded to the quenching intensity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) as carried out byMolyneux *et al.* **(39)**. The absorbance by reducing the purple DPPH radical by an oxidizing antioxidant was measured at 520 nm.

Statistical Analysis

The tests were conducted in triplicate then subjected to SPSS Ver. 21 (IBM, New York, US) software for the statistical analysis. One-way ANOVA was applied for comparison between and within the tested groups. The mean±standard deviation (SD) or SE? was given to all data and the P value less than 0.05 was taken as significant.

Results

No appearance deformation was detected in the honey samples neither undesirable flavors nor any fermentation. Table 1 shows the variability in the melissopalynological analysis of the honey samples from different geographical regions. The pollens from some other plants and flowers were found in each sample which indicates the presence of known and unknown sources of nectars (Table 1). These results showed that honey samples were rich in different pollen types. The pollen content of Manuka honey was of *Kunzea ericoides.* The pollen contents of sidr, somir and thymus honey samples showed *Ziziphus nummularia* and *Ziziphus spina-christi, Blepharis ciliaris*, and *Thymus serpyllum*, respectively. The acacia had the same sediment as *Acacia asak, Anisotes trisulcus, Acacia negrii* and *Acacia senegal (L.)*. Gezan Mountain honey contained the main sediment of pollen; *Acacia asak, Anisotes trisulcus and Ziziphus spina-christi* while Talh honey contained *Acacia asak, Acacia origena* and *Acacia negrii* (Table 1).

Table 1. Pollen content of different honey

Honey type/Pollens	*Acacia asak*	*Anisotes trisulcus*	*Thymus serpyllum*	*Ziziphus spina-christi*	*Acacia senegal (L.)*	*Acacia origena*	*Acacia negrii*	*Ziziphus nummularia*	*Blepharis ciliaris*	*Kunzea ericoides*
Manuka										+
Sidr				+				+		
Somir									+	
Thymus			+							
Acacia	+	+			+		+			
Gezan Mountan	+	+		+						
Talh	+					+	+			

The physicochemical properties of honey samples indicated that all Saudi Arabia and Manuka honey samples were comparable in moisture, glucose, fructose, sucrose, and diastase enzyme contents, but some significant differences were observed in the hydroxymethylfurfural (HMF) and acidity (Figure 1). The mean moisture content of different types of honey samples was ranged from 11 to 15 gr/100 gr (Figure 1). The honey samples' mean moisture content was similar to or less than that of the Manuka honey, being 14%.

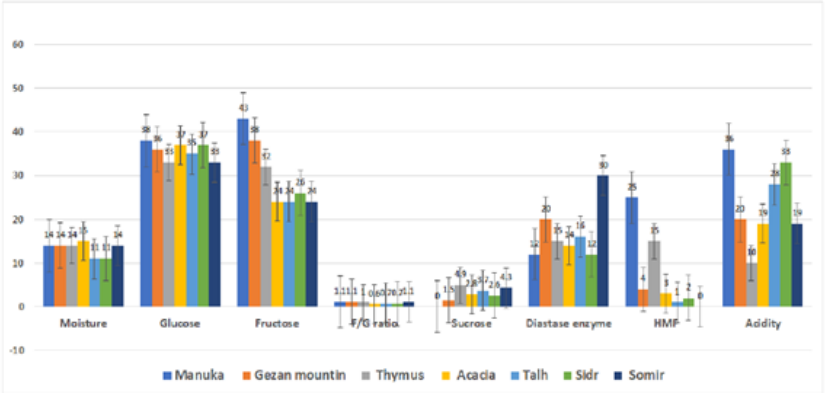

Figure 1. Physiochemical properties of some Saudi honeys tested compared with Manuka honey

The sugar contents analysis of the sixty Saudi Arabia honey samples and Manuka honey was shown in Figure 1. The mean fructose contents of the examined Saudi Arabia honey samples and Manuka honey were 24 and 43 gr/100 gr, respectively. No significant difference was observed in the glucose content of all types of honey samples. The sucrose contents were 1 to 5 gr/100 gr. Figure 1 shows that mean diastase number varied from 12 to 30°G, and the average content of HMF means ranged from 0.58 to 25 mg/kg.

The antimicrobial activity of different types of honey samples against the tested bacterial strains (*S. aureus, E. coli, P. vulgaris, C. diversus* and *S. enterica)* was shown in Figure 2. The antimicrobial activity of all honey samples was comparable. All types of honey samples (at a concentration of 20.30%) exhibited relatively higher antibacterial activity against tested bacterial strains compared to clindamycin. The growth inhibition of different drug-resistant bacterial strains was dependent to the origin and the type of honey.

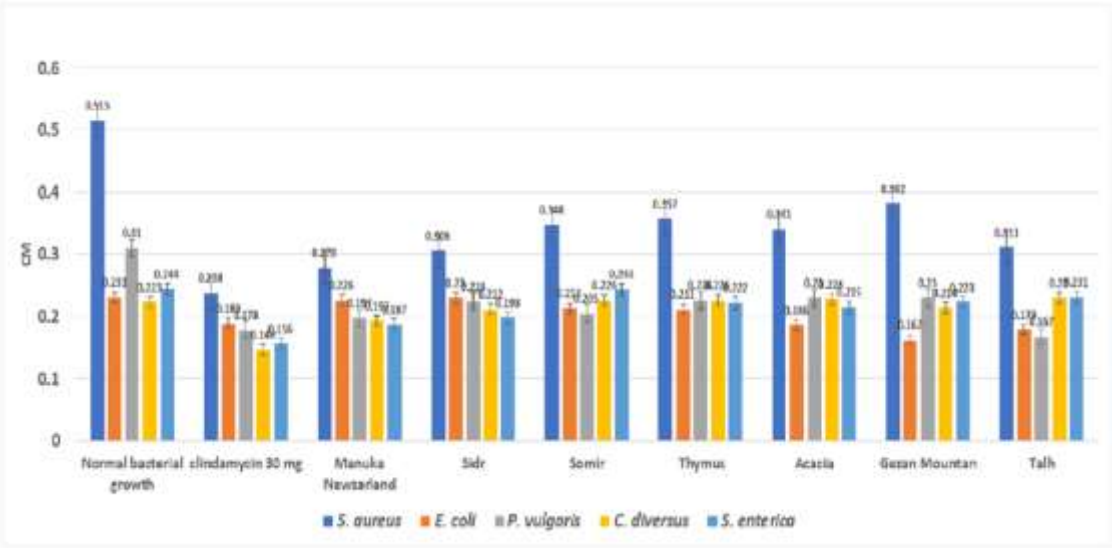

Figure 2. Antibacterial activity of Saudi honeys compared with Manuka honey

Figure 3, shows the concentration of the total phenolic content (TPC) of the tested honey samples ranging from 25 to 50 mg GAE/100 gr honey. The total flavonoid content (TFC) of honey samples was detected based on the method of aluminum chloride. The TFC in the honey samples exhibited values ranging from 44 to 26.511 mg RE/100 gr honey (Figure 3). Radical scavenging DPPH activity in the honey samples was shown ranging from 174 to 118 mg/mL.

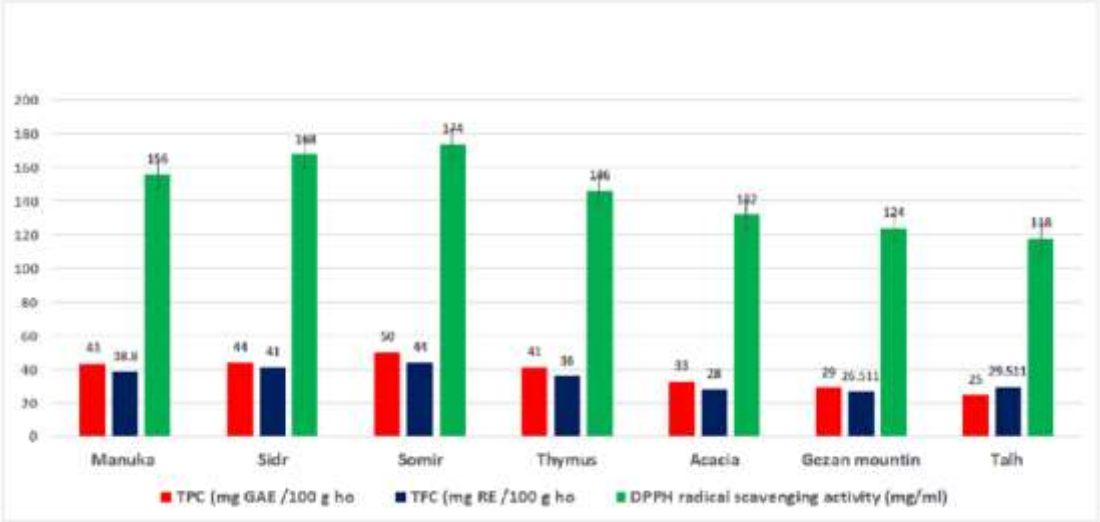

Figure 3. Total phenols, total flavonoids and DPPH Saudi Aeabia honeys samples in comparsion with Manuka honey

Discussion

The quality of all honey samples in this investigation was free from any visible mould growth, undesirable flavors or any fermentation, insect fragments, and sand particles. The findings obtained in this study were in agreement with the general requirements **(40)**. The honey samples from different geographical origins showed variability in their melissopalynological analysis. The pollen contents in different Saudi Arabia honey samples were from different sources as sidr honey showed *Ziziphus nummularia* and *Ziziphus spina-christi*, and somir honey showed *Blepharis ciliaris*. Thymus honey contained *Thymus serpyllum*, Acacia contained the same sediment as *Acacia asak, Anisotes trisulcus, Acacia negrii* and *Acacia senegal (L.),* and Gezan Mountain honey contained the main sediment of pollen of *Acacia asak, Anisotes trisulcus,* and *Ziziphus spina-christi.* The talh honey contained *Acacia asak, Acacia origena* and *Acacia negrii* (Table 1). The results showed that honey samples were rich in different pollen types. This indicated that honey samples were produced from different types of pollen and nectar plant sources existed in the geographic area. These types of honey were produced from pressing the honeycombs as previously mentioned by some authors **(29,41)**. The obtained findings of the melissopalynological analysis of the investigated samples revealed that the examined honey samples were considered as natural bee honey. Also, the results of pollen analysis indicated that Saudi Arabia honey products are produced from bee colonies fed with nectar from different flowers but no sugar syrup. Our results were confirmed by other investigators who found that Kashmiri honey as a collection of medicinal plants such as thymus sp., eucalyptus spp., rhamnus sp., and papaver sp. **(21,22,42)**.

The moisture content of different types of honeys in this study was ranged from 11 to 15 gr/100 gr (Figure 1). The moisture content of honey is important for the honey quality. According to the Saudi Organization for Standardization and Quality Control, it was revealed that the moisture content of honey must not exceed 23% for heather and clover while for other honeys could be 21% **(40)**. Thus, our investigation finding showed that none of the honey samples reached such high moisture content. The Saudi Arabia honeys had the moisture content similar to or less than that of the Manuka, which was detected at 14%. The moisture values were found similar in the blossom honey types and they were explained as acceptable limits of the honey codex **(43,44)**. Meanwhile, the quality determination of the honey is a limiting factor for the moisture content which reflects the stability and spoilage resistance against fermentation by yeast **(45)**. The higher moisture content increases the probability of honey fermentation during storage. On the other hand, the elongation of honey shelf-life is related to the lower moisture (<20%) limits **(46)**. These findings for the honey quality were acceptable by the international regulations (47,48). The temperature and relative humidity in the geographical origin during honey production process in the honey colonies play important roles on the honey moisture content **(49)**.

Sugar analysis was determined in honey samples as shown in Figure 1. The content of fructose was ranged from 24 to 43 gr/100 gr in all the examined honey samples either Saudi Arabia or Manuka honeys. Furthermore, the highest glucose content was recorded at 37 gr/100 gr in Acacia and Sidr honey samlpes followed by 36 gr/100 gr in Gezan Mountain honey. The lowest glucose content (33gr/100 gr) was detected in somir honey. The results were in accordance with the findings obtained previously by several studies on different honey types (30, 50,51). The value of reducing sugars ranged

from 61.3 to 75.5 gr/100 gr in Saudi Arabia honey samples compared to the Manuka honey (81 gr/100 gr). All the reducing sugars values were in line with previously obtained results by Council and Alimentarius *et al.* **(47,48)**. Fructose and glucose were the most dominant sugars in the honey samples which was in agreement with previous studies **(21,22,52)**, who found no limits for their individual values as calculated by the sum of fructose + glucose which have the values corresponding to the limits not less than 60 gr/100 gr as the international standard **(48)**. The results showed that sucrose content varied from 0 to 4.9 gr/100 gr (Table 2). In this study all tested honey samples did not have more than 5 gr/100 gr sucrose, which were accepted by the national and international regulations **(48,53)**.

The fructose/glucose (F/G) ratio was listed in Figure 1. The F/G ratio was 1.1, 1.1, 1, 0.6, 0.7, 0.7 and 1.1 for Manuka, Gezan Mountain**,** Thymus, Acacia, Talh, Sidr, and Somir honey samples, respectively. Glucose is less soluble in water than fructose and the F/G ratio shows the ability of honey to crystallize **(54)**.

The freshness of honey is widely recognized by the parameters hydroxymethylfurfural (HMF) and diastase activity **(15,42)**. The HMF is a Maillard reaction product, responsible for the freshness of honey and whether it is subjected to the heat treatment. The low HMF value indicates that honey is raw and/or fresh **(56)**. Briefly, the high diastase and invertase activities also imply that honey is raw with no heat treatment **(57)**. Figure 1 shows diastase number ranging from 12 to 30°G, and HMF content averaged from 0.58 to 25 mg/kg in all honey samples tested in this study. These results fell within the legal regulations for diastase number and HMF content **(55)**. They reported the mean diastase activity at 22.4%, 19.7%, 17.9%, and 39.1%, respectively.

Unlike invertase and diastase activities, low glucose oxidase activity indicates the high-quality of raw honey **(58,59)**. The set minimum value for diastase activity of eight on Gothe's scale, and a maximum HMF content of 40 mg/kg were mentioned as legal regulations set in Spain. Low enzymatic content of diastase number on Gothe's scale is permissible as long as HMF content does not exceed 15 mg/kg **(60,61)**. They showed lower HMF values and their results were in agreement with those reported by Kamboj *et al.*, 2013 and Kulkarni *et al.*,2020 **(21,22)** from India., France (3.28 mg/kg) **(55)**, Italy (7.80 mg/kg) (62), and Turkey (25.9 mg/kg) (63) , **(16)** Mendes *et al.*, 1998 reported the HMF level in their study in the range of 1.7–471 mg/kg.

The presence of gluconic acid gives the acidity to honey by equilibrium with lactones or esters and inorganic ions such as phosphate and chloride **(64)**. In our study the total acidity mean value range was 10-33 meq/kg while it was 66 meq/kg in Manuka honey. These findings were similar to those results previously detected by Yilmaz & Kufrevioglu 2000; Ozcan, *et al.*, 2006 and Finola *et al.*, 2007 **(65,66,67)**. The geographic condition, harvesting procedure, and storage condition were different in this investigation. These differences were confirmed with the findings obtained from several studies **(21,22)**.

The antibacterial activity of the honey samples was recorded against *Staphylococcus aureus, Escherichia coli, P. vulgaris, C. diversus and S. enterica* shown in Figure 2. A concentration of 20.30% of all honey types caused growth suppression on different tested pathogens. The growth inhibition depended on the honey type and origin. The efficiency of clindamycin 30 mg was shown for the growth inhibition of different bacterial species. The biological properties of honey depend mainly on floral source which was confirmed previously **(68)**. There are several factors which could be attributed to the honey antibacterial activity **(11)** such as osmotic ability of honey **(68,69,70)**, acidity concentration **(11)**, t production of hydrogen peroxide **(71)**, endogenous hydrogen peroxide content **(72,73)**, inhibin **(73, 74)**, hydrogen peroxide **(73)**, non-peroxide substances **(76,77)**, presence of phytochemical factors **(78,79)**, and phytochemical components **(80,81)**. The results of the antibacterial activity of different Saudi Arabia honey samples were in agreement with those previously studied by many authors as (**6,7,8.11,17,20,24,26, 79,80,82,83,84, 85,86, 87,88)**.

The total phenolic content (TPC) of the tested honey samples was ranging from 25 to 50 mg GAE/100 gr honey. Such findings were observed previously by Kucuk *et al.*, 2007 and Kamboj *et al.*, 2013 **(21,89)** who observed that total phenolic content was low in honey. These findings depend mainly on the nectar of predominant plants composition which plays a significant role in the honey composition. Several earlier studies have reported for the polyphenol content in different honey samples such as Tualang (251.7±7.9 mg GAE/Kg) **(20)**. The Saudi Arabia honey samples studied in the current study were dark in color. Several studies have shown that honey becomes darker when the polyphenol content is increased. Chestnut, heather, and oak honeys are dark honey products which their total polyphenol contents are approximately 100 mg GAE/100 gr and their Hunter L values are below 50 **(90,91)**. Compared to the dark-colored flower honeys, Astragalus honey is a light color honey which is low in polyphenol and flavonoid contents **(43, 92)**. A wide range of antioxidant activities of honey samples showed their dependence on the botanical origin. A high correlation has been described between the antioxidant activity and honey color **(93)**. Previous research has also presented that polyphenol and flavonoid contents depend on the floral source and their geographical origin **(26)**.

Determination of the honey samples total flavonoid content (TFC) was performed based on the method of aluminum chloride. The TFC values in the honey samples

were ranging from 44 to 26.511 mg RE/100 gr honey (Figure 3). The honey samples TFC were statistically different (P<0.05). The TFC values ranged between 25.84±7.83 and 51.20±16.35 mg of QE/100 gr which is comparable to Algerian honey (54.23±0.62 mg catechin/kg). The TFC range was the same as reported in honey from different plant sources such as Acacia, lime, and sunflower honey **(95)**. Based on the previous research the polyphenol and flavonoid contents depend on the floral source and their geographical origin **(26)**.

DPPH assay measures the hydrogen/electron-donating capacity of the samples and is reduced in the presence of an antioxidant molecule. The current study represents DPPH IC_{50} values ranging from 174 to 118 mg/mL (Figure 3). The Saudi Arabia honeys studied in the current research displayed the significant highest-level antioxidant potential at lower concentration compared to the other honey samples determined by other investigators (P<0.05) **(22)**. They found it comparable to DPPH activity of Tualang honey (41.30%) **(22,94)**, Algerian honey (44.55%) **(22)**, and Indian honey samples **(96)**.

Conclusion

Differentiation in the physicochemical and enzyme inhibition properties, and antibacterial and antioxidant capacities of honey samples depend on the flora types. Our study gives information about the Saudi Arabia honey products obtained from different localities which showed the effects of different geographical regions on the features of the honey products having different phenolic and flavonoid contents, and antibacterial activities.

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Conflict of Interest

Authors declared no conflict of interests.

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