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 aureus, 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

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).
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 Talih. 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 slide 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 4°C 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 McFarland scale standard. The antimicrobial activity of honeys was detected by well diffusion method according to Katirciolo 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 25°C. 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 (AlCl₃) for determination of total flavonoid content (TFC). The mixture was then incubated for 10
min at 25°C. 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 by Molyneux 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).

| 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 Mountain    | +           | +                  |                 |                        |                     |               |               |                   |                   |                 |
| 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%.
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 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.
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 *Blépharis 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 samples 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, Sdir, 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), inhibit (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 g 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 IC50 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 (41.10%) (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 samples 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.

**References**

1. Ayukekong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: causes and control strategies. Antimicrob Resist Infect Control. 2017;6(1):47. [DOI:10.1186/s13756-017-0208-x] [PMID] [PMCID]

2. Patel A, Chauhan BP. Antimicrobial effect of Honey on MRSA isolated from pus samples. Int J Drug Res Tech. 2016;6(2):58-63.

3. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med. 2004;10(12):S122-9. [DOI:10.1038/nm1145] [PMID]

4. Mandal S, Pal NK, Chowdhury IH, Debmandal M. Antibacterial activity of ciprofloxacin and trimethoprim, alone and in combination, against Vibrio cholerae O 1 Biotype El Tor serotype Ogawa isolates. Pol J Microbiol. 2009;58(1):57-60. [PMID]

5. Alvarez-Suarez J. M, Tulipani S, Romandini S, Bertoli E, Battino M.: Contribution of honey in nutrition and human health: A review. Med J Nutr Metab. 2010;3:15-23. [DOI:10.3233/s12349-009-0051-6]

6. Hegazi Ag. Medical importance of bee products. Uludağ Arıcılık Dergisi. 2012;12(4):136-46.

7. Chute RK, Deogade NG, Kawale M. Antimicrobial activity of Indian honey against clinical isolates. Asiatic J Biotech Res. 2010;1:35-8.

8. Hegazi AG. Antimicrobial Activity of Different Egyptian Honeys as Comparison of Saudi Arabia Hone, Res J Microb. 2011; 6(5); 488-495. [DOI:10.3923/jm.2011.488.495]

9. Hegazi A. G, Abd Allah F. M. Antimicrobial activity of different Saudi Arabia honeys. Glob. Vet. 2012; 9(1):53-59.

10. Pimentel, R.B.dQ., da Costa C.A., Albuquerque and Duvoisin Junior S. Antimicrobial activity and rutin identification of honey produced by the stingless bee Meliponacompressipesmanaosensis and commercial honey. BMC Comp. and Alter. Med. 2013; 13: 151. [DOI:10.1186/1472-6882-13-151] [PMID] [PMCID]

11. Hegazi A. G., Al Guthami, F. M, Al Guthami AF. M., Abd Allah F M., Saleh A A. and Fouad E A. Potential antibacterial activity of some Saudi Arabia honey, Vet. World, 2017; 10 (2): 233-237. [DOI:10.14202/vetworld.2017.233-237] [PMID] [PMCID]

12. Subrahmanyam M, Hemmady A, Pawar S. G. Antibacterial activity of honey on bacteria isolated from wounds. Ann. Burns Fire Disasters. 2001; 14:198-201.

13. Ajibola A, Chamunorwa JP, Erlwanger KH. Nutraceutical values of natural honey and its contribution to human health and wealth. Nutr Metab (Lond). 2012;9:61. [DOI:10.1186/1743-7075-9-61] [PMID] [PMCID]

14. Kahraman T., Buyukunal S K., Vural A., Altunatmaz SS. (2010): Physico-chemical properties in honey from different regions of Turkey. Food Chemistry 2010; 123 (1): 41-44. [DOI:10.1016/j.foodchem.2010.03.123]
15. Abu-Tarboush, H. M., Al-Kahtani, H. A., & El-Sarrage, M. S. Floral type identification and quality evaluation of some honey types. Food Chemistry 1993; 46:13-17. [DOI:10.1016/0308-8146(93)90068-Q]

16. Mendes, E., Brojo, P. E., Ferreira, I. M. P. L. V. O., & Ferreira, M. A. Quality evaluation of Portuguese honey. Carbohydrate Polymers 1998; 37(3):219-223. [DOI:10.1016/S0144-8617(98)00063-0]

17. Yilmaz, H., & Yavuz, O. Content of some trace metals in honey from southeastern Anatolia. Food Chemistry1999; 65, 475-476. [DOI:10.1016/S0308-8146(98)00205-2]

18. Przybylowsk, P., & Wilczynska, A. Honey as an environmental marker. Food Chemistry 2001; 74:289-291. [DOI:10.1016/S0308-8146(01)00153-4]

19. Umal, C., & Kuplulu, O. Chemical quality of strained honey consumed in Ankara. Ankara Universitesi Veteriner Fakultesi Dergisi 2006;53: 1-4. [DOI:10.1501/Vetfak_0000000058]

20. Ouchemoukh, S., Louailiche, H., & Schweitzer, P. Physicochemical characteristics and pollen spectrum of some Algerian honeys. Food Control 2007; 18: 52-58 [DOI:10.1016/j.foodcont.2005.08.007]

21. Kamboj R. Bera MB. and Nanda V. Evaluation of physicochemical properties, trace metal content and antioxidant activity of Indian honeys. International Journal of Food Sci, and Tech. 2013; 48, (3): 578-587 [DOI:10.1111/jfs.12002]

22. Kulkarni SS., Patil SB., Mishra S., Math AK., Panda SK. and Rao KL. Physicochemical characteristics, Heavy Metal Analysis and Antioxidant Potential of Jamun Honey (Syzygium Cumini L.) from Western Ghats, India. Indian J. of Pub.Heal. Research & Devel.2020;11, (2), DOI Number: 10.37506/v11i2/2020/jiphrd/194744 17 [DOI:10.37506/v11i2/2020/jiphrd/194744]

23. Meda A., Lamien C. E., Romito M., Millogo J., and Nacoulma O. G. "Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity," Food Chemistry 2005; 91 (3):571-577. [DOI:10.1016/j.foodchem.2004.10.006]

24. Hegazi A.G.; Nagia Z. Moharm; Fyrouz Abd Allah; M.S. Nour and A.M. Khair Antibacterial activity of different Egyptian honeys in relation to some bee products. Egypt. J. Vet. Sci. 2002; 36: 31-42.

25. Chua LS., Rahaman NLA., Adnan NA. and Tan TTE. Antioxidant Activity of Three Honey Samples in relation with Their Biochemical Components. Jf Anal. Meth.Chem. 2013; Article ID 313798, 8 pages. [DOI:10.1155/2013/313798] [PMID] [PMCID]

26. Gheldof N., Wang X., and Engeseth N. J. “Identification and quantification of antioxidant components of honeys from various floral sources,” Journal of Agricultural and Food Chemistry 2002; 50, (21):5870-5877. [DOI:10.1021/jf0256135] [PMID]

27. Halawani E. M. and Shohayeb M.M. Shaoka and Sidr Hones Surpass in Their Antibacterial Activity Local and Imported Honeys Available in Saudi Markets Against Pathogenic and Food Spoilage Bacteria Australian Journal of Basic and Applied Sciences 2011- a; 5(4): 187-191.

28. Halawani E. M. A, Shohayeb M. M. Survey of the antibacterial activity of Saudi and some international honeys. Jpn. Meteorol. Agency. 2011-b;3(4):94-101.

29. Louveaux, J., Maurizio, A., Vorwhol, G. Methods of melissopalynology. Bee World 1978; 59:139-157. [DOI:10.1080/0005772X.1978.11097714]

30. EL-Metwally, A.A.E., Factors Affecting the Physical and Chemical Characteristics of Egyptian Beehoney. Ph. D. Thesis,Fac. Agric. Cairo Univ. 2015; 320p.

31. AOAC. Official Methods of Analysis (15th edn), ed. K. Helrich. Association of Otticial Analytical Chemists, Inc., Arlington, VA, USA, 1990.

32. Crane, E.. Bees and Beekeeping. Science, Practice and Worm Resources. International Bee Research Association,Comstock Publishing Associates, Ithaca, New York, USA., 1990.

33. AOAC, Association of official analytical chemists. Sugars and sugar products. In: Horwitz, W. (Ed.), Official Methods of Analysis of AOAC International, 2000; 2(44):22-33.

34. AOAC, Association of Official Analytical Chemists. Official Methods of Analysis of AOAC International, 18th ed. Official Methods of Analysis, Maryland, USA., 2005.

35. Katircioulu, H, N Mercan. Antimicrobial activity and chemical compositions of Turkish propolis from different regions. African J Biotech 2006.nolo; 5 (11):1151-1153.

36. Beretta G, Granata P, Ferrero M, Orioli M, Facino RM. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Anal Chim Acta. 2005; 533(2):185-91 [DOI:10.1016/j.aca.2004.11.010]

37. Bertoncelj J., Dobër”sek U., Jamnik M., and Golob T. “Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey,” Food Chemistry 2007; 105, (2): 822-828. [DOI:10.1016/j.foodchem.2007.01.060]

38. Isla MI, Craig A, Ordoñez R, et al. Physico chemical and bioactive properties of honeys from...
Northwestern Argentina. Lebensmittel-Wissenschaft & Technologie. 2011;44(9):1922-1930. [DOI:10.1016/j.lwt.2011.04.003]

39. Molyneux, P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. sci. technol 2004; 26(2): 211-219.

40. SASO Saudi Organization for standardization and quality control. Bee Honey and Methods of Analysis, 1978.

41. Vorwhol, G. Die beziehungen zwischen derelektrischen litfalingkiet der Honig und ihrer trachmissen herkunft. Ann. Abeille., 1984; 7 (4):301-309. [DOI:10.1051/abpid:19640403]

42. Abd Alla, A.E., Nour, M.E., Ewies, M.A., Parameters of sugar feeding honey produced from honeybees, Apis mellifera L. colonies in Egypt. Bull. Ent. Soc. Egypt 2014; 91, 43-53.

43. Can, Z., Yıldız, O., Sahin, H., Turumtay, E. A., Silici, S., & Kolaylı, S. An investigation of Turkish honeys: their physico-chemical properties, antioxidant capacities and phenolic profiles. Food Chemistry 2015;180: 133-141 [DOI:10.1016/j.foodchem.2015.02.024] [PMID]

44. Malkoç, M., Çakır, H., Yakup, K. A. R. A., Żehra, C. A. N., & Kolaylı, S. Phenolic composition and antioxidant properties of Anzer honey from black sea region of Turkey. Uludağ Arıcılık Dergisi 2019; 19(2): 1-13. [DOI:10.31467/uluaricilik.602906]

45. Singh, N., Bath, P.K. Quality evaluation of different types of Indian honey. Food Chem. 1997; 58: 129-133. [DOI:10.1016/S0308-8146(96)00231-2]

46. Terrab A., MJ. Diez and Heredia FJ. Palynological, physicochemical and colour characterization of Moroccan honeys: I. River red gum (Eucalyptus camaldulensis Dehn) honey. jsfa. 2003; 38 (4): 379-386 [DOI:10.1016/j.jsfa.2003.00715.x]

47. Alimentarius C. Draft revised standard for standard of honey (at step 10 of the Codex procedure). Alinorm. 2001;1(25):19-26.; 01 (25): 19-26.

48. Council EU. Council Directive 2001/110/EC of 20 December 2001 relating to honey. Official Journal of the European Communities L. 2002;10:47-52.

49. Crane, E. Honey: A Comprehensive Survey. Heinemann, London, 608, 1979.

50. Buba, F., Gidado, A., Shugaba, A. Analysis of biochemical composition of honey samples from North-East Nigeria. Biochem. Anal. Biochem 2013; 2 (3), 139. http://dx.doi.org/10.4172/2161-1009.10000139. [DOI:10.4172/2161-1009.10000139]

51. Manzoor, M., Shah, G.H.N., Mathivanan, V., Mir, G.M., Shahnawaz, A.D. Chemical analysis of honey of Apis cerana F. and Apis mellifera from Plains of Jammu and Kashmiri and Tamil Nadu. Int. J. Agri. Sci. Res. 2013; 3 (4): 139-146.

52. White, J.W., Doner, L.W. Honey Composition and Properties: Beekeeping in the United States. Agriculture Handbook No. 335, 82-91., 1980.

53. EOSC. Egyptian Organization for standardization and quality control, EOSC, 2005. Bee Honey and Methods of Analysis. Part 1, p. 10, 2005.

54. Amir, Y., Yesli, A., Bengana, M., Sadoudi, R., Amrouche, T. Physico-chemical and microbiological assessment of honey from Algeria. Electron. J. Environ. Agric. Food Chem. 2010; 9 (9): 1485-1494.

55. Devillers, J., Morlot, M., Pham-Delegue, M. H., & Dore, J. C. Classification of monofloral honeys based on their quality control data. Food Chemistry 2004; 86: 305-312 [DOI:10.1016/j.foodchem.2003.09.029]

56. Turkut, G. M., Degirmenci, A., Yıldız, O., Can, Z., Cavrav, S., Karahalil, F. Y., & Kolaylı, S. Investigating 5-hydroxymethylfurural formation kinetic and antioxidant activity in heat treated honey from different floral sources. J Food Measur an Characte. 2018; 12(4): 2358-2365. [DOI:10.1007/s11694-018-9852-y]

57. Sahin H., Kolaylı S. and Beykaya M. Investigation of Variations of Invertase and Glucose Oxidase Degrees against Heating and Timing Options in Raw Honeys Hindawi, J Chem Volume 2020, Article ID 5398062, 7 pages [DOI:10.1155/2020/5398062]

58. Bankar SB., Bule MV., Singhal RS., and Ananthanarayan L "Glucose oxidase-an overview," Biotechnology Advances, 2009; 27, (4): 489-501. [DOI:10.1016/j.biotechadv.2009.04.003] [PMID]

59. Kamboj R., Sandhu RS., Kaler MSS., and Nanda V. "Optimization of process parameters on hydroxymethylfurural content, diastase and invertase activity of coriander honey," JFST. 2019; 56, (7): 3205-3214. [DOI:10.1007/s13197-019-03774-x] [PMID] [PMCID]

60. Perez-Arquillu C, Conchello P., Arifio A., Juan T. and Herrera A. Quality evaluation of Spanish rosemary (Rosmarinus officinalis) honey. Food Chem 1994; 51: 207-210 [DOI:10.1016/0308-8146(94)90258-5]

61. Sancho, M. T., Muniategui, S., Huidobro, J. F. & Simal, Variations of Invertase and Glucose Oxidase Degrees against Heating and Timing Options in Raw Honeys Hindawi, J Chem Volume 2020, Article ID 5398062, 7 pages [DOI:10.1155/2020/5398062]

62. Esti, M., Panfili, G., Marconi, E., & Trivisonno, M. C. Valorization of the honeys from the Molise region through physico-chemical, organoleptic and nutritional assessment. Food Chem 1997; 58(1-2): 125-128. [DOI:10.1016/S0308-8146(96)00228-2]
63. Akyuz, N., Bakirci, I., Ayar, A., & Tunceturk, Y. Van piyasasında satıls̄ a sunulan balların bazı fiziksel ve kimyasal özellikleri ve bunların ilgili standarda uygunluğu üzerinde bir araçtırma. Gida 1995;20(5): 321-326.

64. Al-Khalifa, A. S., & Al-Arify, I. A. Physicochemical characteristics and pollen spectrum of some Saudi honeys. Food Chem 1999; 67: 21-25. [DOI:10.1016/S0308-8146(99)00096-5]

65. Yilmaz, H., & Kufrevioglu, I. Composition of honeys collected from eastern and south-eastern Anatolia and effect of storage on hydroxymethylfurfural content and diastase activity. Turk. J. Agri.and Forest 2000; 25: 347-349.

66. Ozcan, M., Arslan, D., & Ceylan, D. A. Effect of inverted saccharose on some properties of honey. Food Chem 2006; 99: 24-29. [DOI:10.1016/j.foodchem.2005.07.009]

67. Finola, M. S., Lasagno, M. C., & Marioli, J. M. Microbiological and chemical characterization of honeys from central Argentina. Food Chem 2007; 100:1649-1653. [DOI:10.1016/j.foodchem.2005.12.046]

68. Molan, P. Not all honeys are the same for wound healing. Bull. Eur. Tissue Rep. Soc. 2002; 9: 5-6

69. Halawani EM A study on Salmonella typhimurium causing food poisoning in Al-Taif city and antibacterial effect of Nigella sativa Honey and Camels urine. Ph.D Thesis. Taif University, Saudi Arabia, 2006.

70. Kwakman PH, Te Velde AA, De Boer L, Speijer DV, Embroucke-Grauls CM, Zaat SA How honey kills bacteria. FASEB J. 2010; 24: 2576- 2582. [DOI:10.1096/fj.09-150789] [PMID]

71. Nassar HM, Li M, Gregory RL. Effect of Honey on Streptococcus mutans Growth and Biofilm Formation. Applied and Environmental Microbiology. 2012;78(2):536-540. doi:10.1128/AEM.05538-11 [DOI:10.1128/AEM.05538-11] [PMID] [PMCID]

72. Brudzynski K Effect of hydrogen peroxide on antibacterial activities of Canadian honeys. Can. J. Microbiol. 2006; 52: 1228-1237. [DOI:10.1139/w06-086] [PMID]

73. Mercan N, Guvensen A, Celik A, Katiccioglu H. Antimicrobial activity and pollen composition of honey samples collected from different provinces in Turkey. Nat Prod Res. 2007; 21(3):187-95. [DOI:10.1080/14786410600906277] [PMID]

74. Nour, M. E. Some factors affecting quality of Egyptian honeys. Ph. D. Thesis. Fac. Agri. Cairo University, 1988.

75. Irish J, Blair S, Carter DA. The Antibacterial Activity of Honey Derived from Australian Flora. Otto M, ed. PLoS ONE. 2011; 6(3): e18229. [DOI:10.1371/journal.pone.0018229] [PMID] [PMCID]

76. Watt BE, Proudfoot AT, Vale JA. Hydrogen peroxide poisoning. Toxicol. Rev. 2004; 23: 51-57. [DOI:10.2165/00139709-200423010-00006] [PMID]

77. Zainol MI, Mohd Yusoff K, Mohd Yusof MY. Antibacterial activity of selected Malaysian honey. BMC Complementary and Alternative Medicine.2013;13:129. doi:10.1186/1472-6882-13-129. [DOI:10.1186/1472-6882-13-129] [PMID] [PMCID]

78. Mandal MD, Mandal S. Honey: its medicinal property and antibacterial activity. Asian Pac J Tropic Biomed. 2011;1(2):154-160. [DOI:10.1016/S2221-1691(11)60016-6]

79. Sufya N, Matar N, Kaddura R, Zorgani A. Evaluation of bactericidal activity of Hannon honey on slowly growing bacteria in the chemostat. Drug, Healthcare and Patient Safety 2014; 6:139-144. [DOI:10.2147/DHPS.S66496] [PMID] [PMCID]

80. Irish J, Blair S, Carter DA. The Antibacterial Activity of Honey Derived from Australian Flora. Otto M, ed. PLoS ONE. 2011; 6(3): e18229. [DOI:10.1371/journal.pone.0018229] [PMID] [PMCID]

81. Moniruzzaman M, Sulaiman SA, Khalil MI, Gan SH. Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honeys: a comparison with Manuka honey. Chemistry Central Journal. 2013; 7:138. [DOI:10.1186/1752-153X-7-138] [PMID] [PMCID]

82. Mullai V, and Menon T. Bactericidal Activity of Different Types of Honey against Clinical and Environmental Isolates of Pseudomonas aeruginosa. J Altern Complement Med. 2007;13(4):439-42 [DOI:10.1089/acm.2007.6366] [PMID]

83. Mavric E, Wittmann S, Barth G, Henle T. Identification and quantification of methylglyoxal as the dominant Antibacterial constituent of manuka (Leptospermum scoparium) honeys from New Zealand. Mol. Nutr. Food Res. 2008; 52(4): 483-489. [DOI:10.1002/mnfr.200700282] [PMID]

84. Ayaad, T. H.; Shaker G. H. and Almuhnaa A. M. Isolation of antibacterial peptides from Saudi Arabian honeybees and investigating the antimicrobial properties of natural honey samples. Egypt. Acad. J. biol. Sci. 2009; 2 (2): 23 - 34 [DOI:10.21608/eaibsa.2009.15426]
85. Lu J., Carter D A., Turnbull L., Rosendale D., Hedderley D., Stephens J., Gannabathula S., Steinhorn G., Schlothauer R C., Whitchurch C B., and Harry E J. The Effect of New Zealand Kanuka, Manuka and Clover Honeys on Bacterial Growth Dynamics and Cellular Morphology Varies According to the Species. Manganelli R ed. PLoS ONE. 2013; 8(2):e55898. doi: 10.1371/journal.pone.0055898. [DOI:10.1371/journal.pone.0055898] [PMID] [PMCID]

86. Hegazi A. G, AbdEl-Moez S. I., Abdou A. M. and Abd Allah F. Synergistic antibacterial activity of Egyptian honey and common antibiotics against Clostridium Reference strains, Int.J.Curr.Microbiol.App.Sci. 2014a;3(8): 312-325.

87. Hegazi A.G, Abd El-Moez S. I., Abdou A. M. and Abd Allah F. Antibacterial activity of some types of monofloral honey against Clostridium acetobutylicum and Clostridium perfringens. Int.J.CurMicobl.ApScI 2014b; 3(9) 552-565.

88. Hussain M.B., Hannan A., Akhtar N., Fayyaz Q.G., Imran M., Saleem S., and Qureshi I.A. Evaluation of the antibacterial activity of selected Pakistani honeys against multi-drug resistant Salmonella typhi. BMC Complement Altern Med. 2015;15: 32. [DOI:10.1186/s12906-015-0549-z] [PMID] [PMCID]

89. Kucuk, M., Kolayli, S., Karaog˘lu, S., Ulusoy, E., Baltaci, C. & Candan, F. Biological activities and chemical composition of three honeys of different types from Anatolia. Food WHO, 2007. [DOI:10.1016/j.foodchem.2005.10.010]

90. Al-Mamary M, Al-Meeri A Al-Habori M, Antioxidant activities and total phenolics of different types of honeys. Nutr Res 2002; 22: 1041-1047. [DOI:10.1016/S0271-5317(02)00406-2]

91. Aljadi AM Kamaruddin MY, Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chem 2004; 85: 513-518. [DOI:10.1016/S0308-8146(02)00596-4]

92. Heidari, T., Roozbahani, N., Farahani, L. A., Attarha, M., Torkestani, N. A., Jamilian, M., & Bekhradi, R. Does Iranian Astragalus gossypinus honey assist in healing caesarean wounds and scars? EuroJ. Integr. Med. 2013; 5(3): 226-233. [DOI:10.1016/j.eujim.2013.01.005]

93. Frankel S, Robinson GE Berenbaum MR, Antioxidant content and correlated characteristics of 14 monofloral honeys. J Apic Res 37: 27-31 (1998). [DOI:10.1080/00218839.1998.11100951]

94. Khalil MI, Moniruzzaman M, Boukraâ L, Benhanifia M, Islam MA, Islam MN, et al. Physicochemical and antioxidant properties of algerian honey. Molecules. 2012;17(9):11199-215. [DOI:10.3390/molecules17091199] [PMID] [PMCID]

95. Al, M.L., Daniel, D., Moise, A., Bobis, O., Laslo, L. & Bogdanov, S. Physico-chemical and bioactive properties of different floral origin honeys from Romania. Food Chem. 2009;112: 863-867 [DOI:10.1016/j.foodchem.2008.06.055]

96. Saxena S, Gautam S, Sharma A. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem. 2010;118(2):391-7. [DOI:10.1016/j.foodchem.2009.05.001]