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Comparison Study of Antioxidant and Antibacterial Activity Between Dates and Manuka Honeys

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ABSTRACT

Honey is long known having beneficial health benefits both internal and external of the human body. The current study was carried out to detect the antioxidant and antibacterial activity of Manuka and Date honey. The antioxidant activity was detected using DPPH assay to show the reduced free radical cells effect by honey samples, while antibacterial activity of honey samples against *Staphylococcus aureus* pathogenic bacteria was detected using Microtiter plate assay. The results varied with the highest antioxidant activity related to the concentration of 2000 ppm with 37.37 ± 2.66 % by Date honey, while 2000 ppm of Manuka Honey had the lowest antioxidant activity with 3.38 ± 18.78 %. Antibacterial activity results were also diverse, the highest was from Manuka honey with concentration of 20 % with 77.44 ± 6.72 % after 48h time of incubation. In addition, the lowest inhibition activity against *Staphylococcus aureus* was related to Date honey with concentration of 20 % with 18.17 ± 23.00 % after 12h incubation time. *Staphylococcus aureus* was resistant to 20 % Date honey after the 1st 6h of incubation time. This study concluded that both Manuka and Date honey have antioxidant and antibacterial activity, which was related to the source of honey, its content and concentrations of diluted honey samples. Overall, date honey is more effective than Manuka honey as an antioxidant, while Manuka honey has a higher antibacterial activity than date honey.

1. Introduction

Honey is a sweet liquid and gold product produced by honeybees; honey has a long history with humans, which was used in various food and beverages as a sweetener and flavouring agent. Moreover, various medicinal tradition applications to treat ailments. In addition, the health effects of honey has been noticed by humans since the ancient times, honey contains sugars, vitamins, minerals, amino acids, peptides, enzymes, proteins, flavonoids and phenolic compounds [1]. Honey

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is the oldest wound healing agent known to human because of its bioactivities such as antibacterial and anti-inflammatory [2], and it has a beneficial effects in treatment of diabetics mellitus, and ability to act in reducing asthma related symptoms and as a preventing agent in chronic bronchitis, honey improves coronary vasodilation, reduces the ability of platelets to transform to a clot and inhibiting low density lipoprotein (LDL) from oxidizing in coronary heart disease [3]. Honey also acts as nutraceutical antioxidant, exerts anxiolytic, antidepressant, anticonvulsant and antinociceptive effects. Honey ameliorates the oxidative content of the central nervous system [4]. Many previous studies described the analysis of honey and the study of its physical and chemical properties, which may explain its ability to work against various microorganisms and free radicals [5].

A previous study was also conducted to detect the ability of honeys to act as antioxidant agents as scientists have been drawn towards a new compounds that are less toxic and useful to treatment and do show less significant side effects compared to chemical and radio therapy treatments which are commonly used and well known for their side effects. A number of previous studies was conducted on honey in different countries of the world, and targeted its effectiveness as an antioxidant looking for substances with novel activity to prevent and treat cancer by the formation of free radical cells, which are the main cause of cell DNA damage and mutations. Cancer today represents a major health burden and a frequent cause of death. The number of deaths has been estimated according to statistics at approximately 8.2 million cases annually [6], and the World Health Organization estimates that this number will double in the coming years, especially in the third world countries, these countries are not focusing on upgrading the quality control departments to face the un expected import and local manufacturing. Therefore, efforts intensified to search for treatment methods, including the use of honey as an anticancer. Scientists have conducted several tests targeting honey to determine its effectiveness as an inhibitor of the growth of microbes of all kinds, specifically bacteria and their pathogenic strains, which in the current era have become resistant to chemical antibiotics due to its frequent use and the formation of bacteria to strains resistant to the antibiotic. Moreover, world health organization on 2010, stated that the war of upcoming time will be with the microorganisms, because of the huge resistant activity which clearly noticed even by the normal patients. In addition, studies on honey and its novel components and activity attracted attention by the scientists lately all over the world, but studies on Libyan honeys are still few, unless they are published in legal journals [7]. Therefore, the current study aimed to detect the ability of Libyan Natural date honey and New Zealand artificial Manuka honey to inhibit activity of free radical cells and pathogenic bacteria, specifically *Staphylococcus aureus*.

2. Methodology

2.1 Sample Collection

Two different samples of honey were applied in the current study; Manuka honey from New Zealand produced by Manuka Health Company and Date honey from the central region of Libya.

2.2 Sample Preparation

Five different concentrations were prepared of each type of selected honey (20, 40, 60, 80 and 100 %) using distilled sterile water. Amounts of 2, 4, 6, 8 and 10 g of honey were weighted separately, after that, appropriate amount of distilled water was poured to each concentration as 8, 6, 4 and 2 ml, respectively. The tubes that contain the prepared concentrations were shacked well to obtain a homogeneous solution and that filter disk was used (filter disk, 0.4 μ, China) to avoid the presence of

large molecules, bacteria and fungi spores. Lastly, all diluted samples were stored at 4°C for the following experiments [8].

2.3 Collection and Preparation of Pathogenic Bacteria

The growth of the pathogenic bacteria varied depending on its type. During the preparation of the pathogenic bacteria, all bacteria samples were collected in a solid culture and sup-cultured in both Nutrient broth and agar. After that the growth of the overnight culture showed purity in bacteria colonies with specific colours and shapes [9].

2.4 DPPH Assay

Anti-oxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [8].

2.4.1 Preparation of DPPH solutions

The 100 µg/ml of DPPH solution was produced by weighing 5 mg of DPPH and dissolving it in 50 ml of methanol 96 % in a measuring flask [8].

2.4.2 Preparation of sample solutions

Stock solutions of the two types of honey of Manuka (40 µg) and Date (60 µg) was made by adding (96 %) methanol, then it was diluted and the series of concentration of 5000, 4000, 3000, 2000 and 1000 ppm were produced [8].

2.4.3 Measurement of antioxidant capacity

Measurement of blank antioxidant capacity was conducted by measuring 2 ml of DPPH mixed with 3 ml of methanol, incubated at 37 °C for 30 min. The maximum wavelength was then measured in the range of 510 to 520 nm using spectrophotometer (Riele, Germany) and Eliza reader (Biotek, Germany) [9]. The measurement of antioxidant capacity of the diluted honey samples were carried out by piping 1 ml of sample solution of all the concentrations separately. Afterwards, 2 ml of DPPH and 2 ml of methanol were added to concentrations. Then all samples were incubated at 37 °C for 30 min. Measurements were taken by Elisa reader at wavelength of 513 nm [8].

2.4.4 Calculation of the control sample

The positive control sample used was ascorbic acid, and then prepared in the same manner as honey samples [8].

2.4.5 Determination the antioxidant activity

The analysis of DPPH method was done by observing the colour changes of each sample. If all DPPH electrons were paired with electrons in the honeys sample, there would be a colour change in the sample from dark purple to bright yellow [8].

2.5 Antibacterial Activity Evaluation Using Microtiter Plates

Samples were tested against the selected pathogenic bacteria applying the Microtiter plate assay, following the method of Aween *et al.,* [10]. Briefly, 100 µL of nutrient broth containing 107 CFU mL^{-1} was placed in the 96-well plate and 100 µL of diluted samples (20 and 40 mg mL^{-1}) from two types of honey were poured into the wells. The plates were incubated at 37 °C for 24 h. Optical density of bacterial growth was measured at 600 nm using Elisa plate reader (BIOTEK, BioTek Instruments, Winooski, VT, USA). Sample with nutrient broth without bacteria was used as negative control, and nutrient broth with pathogenic bacteria was used as positive control. This experiment was done in triplicate and growth inhibition percentage was calculated as mentioned in the statistical analysis section [10].

2.6 Statistical Analysis

All experiments were conducted in triplicate and the results were analysed using the Minitab 18 system to calculate the average, standard division, percentage of inhibition and one way ANOVA test. The following Eq. (1) was applied to calculate the percentage of inhibition.

$$\text{Percentage of Inhibition} = \frac{(+\text{Control Absorbance Sample Absorbance})}{+\text{Control}} \times 100 \quad (1)$$

3. Results

3.1 Collection and Preparation of Pathogenic Bacteria

The growth of selected pathogenic bacteria *Staphylococcus aureus* was well sup-cultured. During the preparation, bacteria sample showed growth in the first broth sup-culture, and then the second streaked strains on the nutrient agar, *Staphylococcus aureus* showed purity in grown colonies specifically colour and shape.

3.2 DPPH Assay

All the results confirmed that all honey samples contained substances that had antioxidant capacity and activity against free radical cells from which changing of the colour of honey samples in the Microtiter plates from dark purple to bright yellow (Figures 1 and 2). All the different honey concentrations (1000, 2000, 3000, 4000 and 5000 ppm) showed varied levels of antioxidant activity and this activity were mostly comparable to the activity of the standard ascorbic acid (1.23 ± 69.70 %), which all diluted honey samples was lower than ascorbic acid in the antioxidant activity as shown in Figure 2. A clear change in the colour of all samples that were mixed with the DPPH after 30 min of incubation time at 37 °C, proved their ability to reduce the free radical cells activity.

The highest antioxidant activity in Manuka honey presented as a percentage of inhibition which was obtained from concentration of 1000 ppm as 0.115 ± 33.80 %, lower than the ascorbic acid (1.232 ± 72.5 %), and the lowest percentage of inhibition was related to the concentration of 2000 ppm as 3.38 ± 18.78 %, lower than the ascorbic acid (1.93 ± 68.94 %). A concentration of 5000 ppm as 2.17 ± 29.73 %, followed by 3000 ppm as 0.474 ± 27.69 %, after that, 4000 ppm as 1.176 ± 20.06 %, thus, the antioxidant activity of these concentrations (5000, 3000 and 4000) also was lower than the ascorbic acid (1.178 ± 68.94 %, 0.514 ± 73.52 % and 1.245 ± 68.17 %, respectively). In all honey concentrations, there was a significant difference between the antioxidant activity of Manuka honey

and ascorbic acid ($p < 0.05$) as shown in Figure 1. In contrast, over all Manuka honey tested dilutions, the scavenging activity was less than that obtained from Dates honey, while the activity of all Dates honey concentrations was lower than the ascorbic acid.

The antioxidant activity of Dates honey was 2.66 ± 37.37 % from concentration of 2000 ppm, which was lower than ascorbic acid (1.93 ± 68.94 %) at the same concentration, followed by 1000 ppm as 0.502 ± 32.28 %, then 5000 ppm as 1.726 ± 30.24 %, the activity of 4000 ppm was as 1.410 ± 29.989 % and lastly 3000 ppm as 1.92 ± 26.68 %. Over all, ascorbic acid showed higher antioxidant effect over all tested concentrations, (1.232 ± 72.5 %, 0.514 ± 73.52 %, 1.245 ± 68.17 % and 1.178 ± 68.94 %, respectively). Although, there was a significant difference between Dates honey and the used standard solution ascorbic acid in the effect of antioxidant of all tested concentrations (p value < 0.05), also there was a significant difference in the antioxidant activity between Date honey and Manuka honey, Dates was more effective than Manuka ($p < 0.05$) (Figure 2).

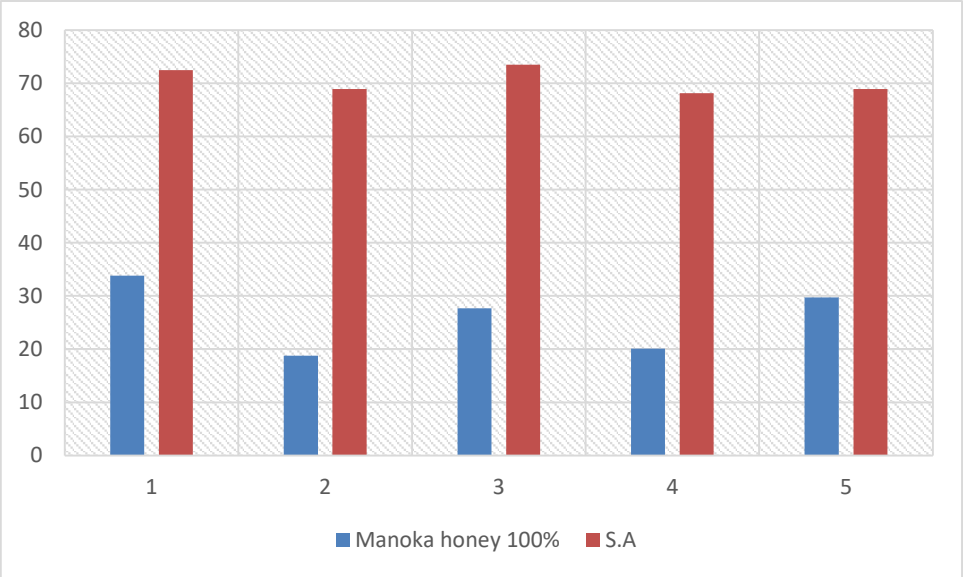

Fig. 1. Antioxidant activity of Manuka honey samples by DPPH assay. S.A = *Staphylococcus aureus*

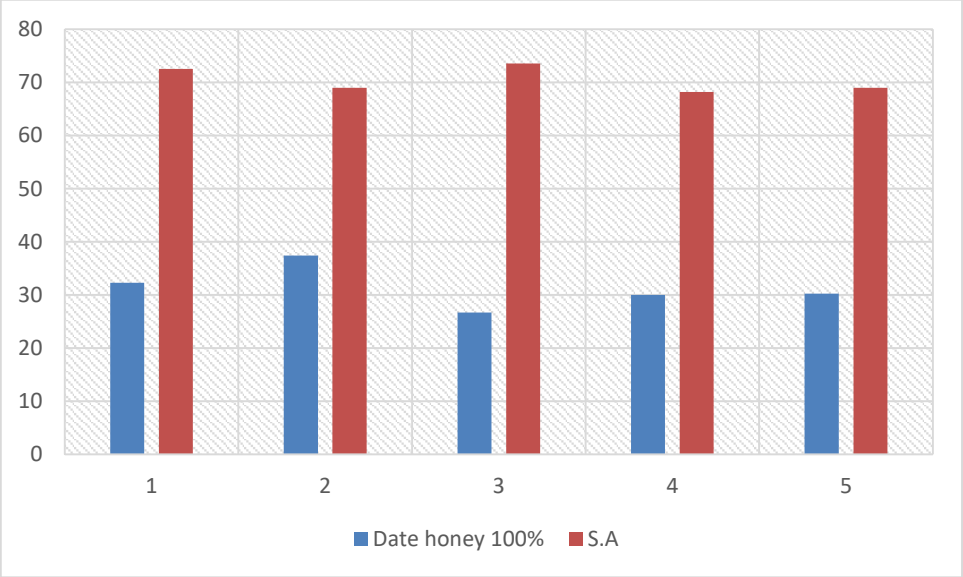

Fig. 2. Antioxidant activity of Dates honey samples by DPPH assay, S.A = *Staphylococcus aureus*

The results of the current study were comparable to the results that was published by Bazaid *et al*., [11], from out in Saudi Arabia, which focused on the antioxidant activity of Manuka honey against free radical cells, but the percentage of inhibition was not calculated. In addition, a study by Cianciosi *et al.,* [12] in Italy, presented that Manuka honey has a high antioxidant activity using DPPH assay. On the other hand, Kaźmierczak-Barańska and Karwowski [13] carried out a study in Poland on antioxidant activity of Manuka honey and the results were higher than the current study and the used standard was Trolox, which showed activity of 50.36 %. Thus, that contrast might be due to the difference in MGO concentration in both studies (Current: 115 and previous: 550) [13]. Venugopal and Devarajan [14] conducted a study in India about comparing the antioxidant activity of local and New Zealand Manuka honey. The results showed that Manuka honey had the highest antioxidant activity as 0.68 ± 50.7 % compared to other local honeys, but was lower than the ascorbic acid (0.39 ± 64 %), in which the five diluted concentrations of Manuka honey were lower in the antioxidant activity than the standard solutions ascorbic acid. Similarly, the present study all dilutions of Manuka honey showed activity of antioxidant lower than that obtained from ascorbic acid, 33.80 ± 3.38 %, 73.52 ± 1.178 %, respectively [14].

3.3 Antibacterial Activity of Honey Samples Using Microtiter Plates

All honey samples was effective against selected pathogenic bacteria *Staphylococcus aureus*, that the activity varied dependent on the type of honey, its concentration and incubation time. The highest activity was by Manuka honey as 6.72 ± 77.44 % by 20 % concentration, after incubation time of 48 h. While the lowest activity was obtained from Dates honey as 26.2 ± 33.05 % by 20 % concentration, after incubation time of 24 h. The concentration of 20 % of Dates honey did not show any antibacterial activity against *Staphylococcus aureus* after 6 h of incubation (00.00 %), as shown in Tables 1 and 2. The antibacterial activity of diluted Manuka honey samples was decreased after increasing the concentration of honey from 20 to 40 %, so the activity of Manuka honey is not related to the concentration of general honey sample, but is the opposite, that could be due to the active compound/s that are dynamic in diluted samples which might be due of the high osmolality of honey by sugars or other presented components as well. In contrast, the antibacterial activity of diluted Dates honey samples statistically significantly increased after increasing the concentration of honey, so the activity of Dates honey would be related to the concentration (Tables 1 and 2).

The inhibition activity of 20 % concentration of Manuka honey was increased with incubation time starting from 6 to 48 h, except after 24 and 30 h, which showed a slight decrease in the activity as 65.53 to 65.15 %, respectively. The starting incubation time of 6 h did not show any antibacterial activity that could be because of the pathogenic bacteria defense activity against honey components by producing enzymes but after a time the honey active ingredients showed high activity against the bacteria. On the other hand, while the activity of Dates honey was increased gradually with incubation time starting from 12 to 30 h, it then started to decrease after 36 to 48 h. Similarly, the inhibition activity of 40 % concentration of Manuka honey was increased with incubation time starting from 6 to 48 h, except after 24 and 30 h there was a slight decrease in activity from 65.04 to 63.85 %, respectively. Moreover, 40 % concentration of Dates honey showed an antibacterial activity after 6 h of incubation 48.03 % and there was an increased after 24 h to 63.35 %, while, after 30 h the results showed a slight decrease in antibacterial activity as 62.47 %, and then started to increase again to 72.27 % after 36 h, then, after 48 h the activity slightly decreased to 72.21 %. The variety in the percentage of inhibition that achieved by concentration of 40 % in both honey samples would be related to the concentration of the active antibacterial compounds in honey samples which consumed by time or because of the resistant activity of tested pathogenic bacteria which produce

some enzymes or other components by its metabolism trying to defense the inhibition or killing effect of honey. Statistically, there was no significant difference in the antibacterial activity between Manuka and Dates honey 36 and 48 hours of and also between 6 and 12 hours of incubation ($p > 0.05$), while there was a significant difference in the antibacterial activity between Manuka and Dates honey between 6, 36 and 48 hours of incubation ($p < 0.05$).

Table 1
Showed the inhibition percentage of antibacterial activity of Manuka honey samples against *Staphylococus aureus*

Inhibition percentage ± StDev		
Incubating time / hour	40 %	20 %
6	48.4 ± 5.14	53.61 ± 11.58
12	64.50 ± 3.16	65.53 ± 2.31
24	65.04 ± 6.52	65.58 ± 3.18
30	63.85 ± 7.29	65.15 ± 4.63
36	75.28 ± 5.41	77.09 ± 3.58

Table 2
Showed the percentage of inhibition of antibacterial activity of Dates honey against *Staphylococus aureus*

Inhibition percentage ± StDev		
Incubating time / hour	40 %	20 %
6	48.03 ± 7.67	0.00 ± 0.382
12	61.77 ± 1.713	18.17 ± 23.00
24	63.35 ± 4.93	33.05 ± 26.20
30	62.47 ± 6.29	41.19 ± 29.20
36	72.27 ± 5.24	40.95 ± 36.00
48	72.21 ± 7.33	40.38 ± 24.4

The results of the current study were comparable to the outcome that published by Bazaid *et al*., [11] in Saudi Arabia, which reported that the antibacterial activity of Manuka and Dates Honey against *Staphylococcus aureus* pathogenic bacteria. Moreover, a study was done in Germany by Henriques *et al.,* [15] reported that honey has a high antibacterial activity against *Staphylococcus aureus*. On other hand, Aween *et al.,* [9] carried out a study in Libya, on the antibacterial activity of Manuka honey samples against *Staphylococcus aureus* pathogenic bacteria by Microtiter plate assay, and the results were higher than the current study and with percentage of inhibition of 99.33 %, while the highest percentage from the current study was 78.84 ± 6.72 %. In contrast to the current study, which showed higher antibacterial effects ranged from 18.17 ± 23.00 of 20 % concentration to 72.27 ± 5.24 % of 40 % concentration. From Dates honey, which is considered higher than the stated study, moreover the possibility of connecting some of the antibacterial activity of Dates honey to the protein source components is there based on the previous study above.

4. Conclusions

All honey samples proved to have antioxidant and antibacterial activity, dates honey has higher activity against free radical cells than Manuka honey, while the highest antibacterial activity was from Manuka honey. Researchers suggest for the upcoming studies to focus on detecting and extracting the active substances that are related to the antioxidant and antibacterial activities.

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