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Original Research Article

The Antioxidant Capacity of Monofloral Eucalyptus Flower Honey and Weed Honey Obtained from Jerash and Al-Zarqa Jordanian Governorates

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ABSTRACT

Honey contains natural antioxidants that can help to get rid of free radicals and improve our health. The antioxidant capacity of honey is determined by its chemical makeup. The antioxidant capacity of Monofloral honey, including Eucalyptus flower honey and Weed honey obtained from different Jordanian governorates (Jerash and al-Zarqa) were examined using the phenolic contents (GAE), DPPH free radical scavenging test (%), and Ferric reducing/antioxidant power assay (FRAP). The Folin–Ciocalteu assay was used to assess the total phenol components in terms of the Gallic acid equivalent (GAE) in mg/g. Results showed that the Eucalyptus flower honey, and weeds honey both obtained from Jerash governorate followed by weeds honey obtained from Al-Zarqa governorate have the highest phenolic contents among the honey samples with the values of (10.6734 ± 0.006) mg/g, (10.43889 ± 0.012) mg/g and (10.1345± 0.004) mg/g; respectively. The DPPH scavenging assay as compared to BHT scavenging (reference control) 59.61%, has demonstrated that the eucalyptus flower honey obtained from Al-Zarqa governorate and weeds honey obtained from Jerash governorate have exhibited the highest DPPH scavenging among honey samples with the values (12.8734± 0.008)% and (11.5635± 0.006)% respectively. Moreover, the reducing power tests of the reference compound ascorbic acid (0.751 mg/ml) have demonstrated that the weeds honey and eucalyptus honey both obtained from Jerash governorate have exhibited the highest reducing power with values of (15.8857± 0.002) mg/ml and (16.8787± 0.004) mg/ml respectively. It is important to note that honey has varying levels of antioxidant activity depending on its source and content.

***Keywords*:** Honey; antioxidant potential; phenolic contents; DPPH; Ferric reducing/ antioxidant power assay (FRAP)

Introduction

From ancient times, honey has been regarded as a very valued natural substance. It contains bioactive substances that have therapeutic impacts on human health; it also has antioxidant, antibacterial, and wound-healing qualities. The Apis mellifera is the bee species responsible for honey production. They feed on the nectar of flowers. People used honey in their lives about 8000 years ago, and what proves this is the Stone Age paintings. The ancient Greek philosopher Aristotle (384 – 322 BC) described honey which is characterized by it is pale color as a good source for the "treatment of wound and sore eyes".[1, 2] Honey can be categorized based on several considerations, such as the origin and the method which has been used in harvesting the honey.[2, 3]

The composition of honey is influenced by several factors. Nectar from different plants has different amounts of the main sugars and trace components.

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According to plant species, environmental factors, floral sexual stages, and flower position within inflorescences, the nectar's composition can vary significantly. The type of soil, the environment, and the plant's surroundings all affect how they are made. Consequently, the nature of the bacteria, as well as the concentration of honey, is two crucial things in the bactericidal effect of honey.[4-6] The honey can be categorized based on several considerations, such as it is the origin and the method which has been used in harvesting it. Honey is characterized by its health properties; it has antioxidant potential due to its contents of phenolic compounds, ascorbic acid, amino acids, carotenoid-like chemicals, and Millard reaction products.[3-4] The antioxidant potential of honey varies from one region to another, because of botanical and graphical dissimilitude. Flavonoids are the main polyphenols. The type of nectar determines the phenolic compounds. Coriander honey exhibited antitumor potential. It has polyphenols which confer the antioxidant property. [6] Many studies have conducted the role of the antioxidant of honey in reducing the risk of heart disease. The therapeutic properties of honey are not restricted to an antioxidant agent; it has antibacterial, anti-mutagenic, and many other properties. Honey's antibacterial potential is due to the high acidity of honey, the antibacterial action of hydrogen peroxide, the high osmolality, and the scavenging activity of the Methyl syringe. [5] Researchers have exhibited the anticancer effects of honey. Raw honey has exhibited chemo-preventive potential against different cancer cell lines by exerting its effect with different mechanisms; such as induction apoptosis, cell cycle arrest, immune modulation, and modulation of oxidative stress. Therefore, honey can be used as an alternative medical treatment for specific types of cancer.[6,7]

Jordanians obtain honey locally from several regions in Jordan. Jordan is one of the honey-exporting countries, as it exported approximately 320 tons of honey in 2019.[3] The topography of Jordan is highly variable. There are four biogeographic zones in Jordan: Mediterranean, Irano-Turania, Saharo-Arabian, and Sudania. The diversity in the geographical nature of Jordan leads to a richness in plant biodiversity. Approximately 20% of the recorded plants are listed as medicinal plants. Flowering plants are abundantly distributed, which makes Jordan a beekeeping country. This biodiversity could address the different phenolic content in different honey types produced in Jordan[3, 8-9] This paper aims to evaluate the phenolic contents (GAE), DPPH scavenging activity, and the reducing power of selected local honey samples marketed in Jordan.

Materials and Methods

Honey samples

Honey samples from various Monofloral sources were gathered from local beekeepers in Jordan's various regions including Jerash governorate, and Al-Zarqa governorate (Figure 1) in the Main Harvesting period (between June and July) in 2022, where the highest production is reached in those months. Two types of honey were obtained from each governorate, Eucalyptus flower honey and weed honey. The selected honey types were identified by Dr. Wesal Y. Alraei, Assistant Professor at the Department of Diet Therapy Technology and Dietetics, Zarqa University. Throughout the examination; honey samples were stored at room temperature (24C± 2) tell used in the assay.

Screening the antioxidant activity of the honey samples.

Determination of total phenolic content (TPC).

The total phenol contents of the honey samples were determined by using the Folin-Ciocalteu reagent with Gallic acid used as a standard. 0.5 mL of honey was added to 2 mL of sodium carbonate (75 g/L) and 2.5 mL of 10 % (v/v) Folin-Ciocalteu reagent (Sigma-Aldrich, Germany). The absorbance was measured at 765 nm (Biotek, USA) after 30 minutes of incubation at room temperature. The total phenolic compound contents (mg/g) were expressed as Gallic acid equivalent (GAE) determined according to the regression equation based on the calibration curve: $y = 0.3638x - 0.3029$, $R^2 = 0.899$. Where (Y) is the absorbance and (X) is the Gallic acid concentration in (mg/l). [10]

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of honey.

DPPH has been used for evaluation of the free radical scavenging activity of the antioxidants including honey. The honey-free radical scavenging activity was evaluated for the five honey samples by DPPH assay. DPPH compound in its radical form absorbs at 517nm, the absorption capacity decreases upon reduction by a radical species or an antioxidant. 4 ml of 0.1mM DPPH prepared in methanol were added to 1 ml (200µg/ml) of a honey sample, and BHT was used as a reference compound. After that, it was incubated for 30 mins at room temperature. Then, the absorbance of the mixture was spectrophotometrically measured at 517 (Biotek, MO, USA), and compared to the standard antioxidant BHT. Finally, the capacity to scavenge the DPPH radical was calculated based on the following equation as described by Ruiz-Ruiz, J et al. (2017): % DPPH scavenging activity = (A control – A sample/A control X 100%). Where A control: absorbance for the control sample, A sample: absorbance for tested honey samples. [11]

Ferric reducing/antioxidant power assay (FRAP)

The ferric reduction antioxidant power (FRAP) assay is a spectrophotometric technique for determining an antioxidant's ability to reduce Fe3+-tripyridyltriazine complexes to Fe2+-tripyridyltriazine forms (intense blue hue) that absorb at 593 nm. A technique for determining the ferric-reducing power of honey samples was established earlier. [12] The higher absorbance indicates an increase in the reducing power of the honey samples which occurs due to the reduction of the ferric 2,4,6-tripyridyl-s-triazine complex to its ferrous, colored form (Fe2+-TPTZ) in the presence of antioxidants. From each honey sample, 5 mg was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml (10 g/l) potassium ferricyanide, and incubated at 50°C for 30 minutes, followed by adding 2.5 ml trichloroacetic acid (100g/l) and centrifuged at 1650 x g for 10 minutes. The upper layer was mixed with 2.5 ml ferric chloride (1 g/l). The absorbance of the reagent combination was measured spectrophotometrically at 593 nm. Ascorbic acid (300 µg /ml) was used as a standard.[12]

Statistical analysis

To identify any statistical differences between the control group and the various treatment groups, Graph-Pad Prism ANOVA was used. Dennett's post hoc analysis was then used. The accepted cutoff for statistical significance in all analyses was a p-value of 0.05 or less.

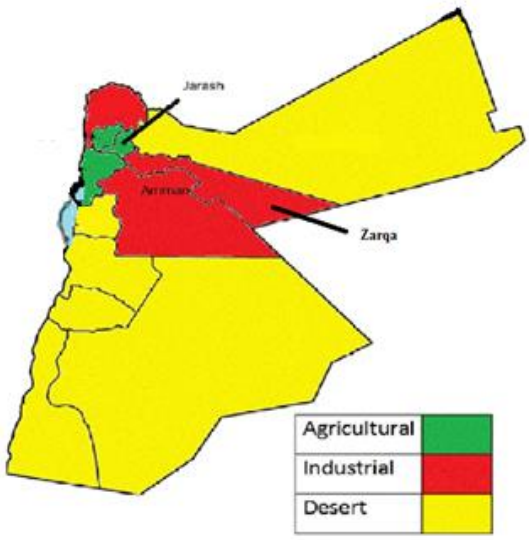

Figure 1: Jordan maps showings the honey obtained samples collected from different governorates across the kingdom.

Results and Discussion

Screening the antioxidant activity for the honey samples.

Results showed that the Eucalyptus flower honey obtained from Jerash governorate, weeds honey obtained from Jerash governorate, and weeds honey obtained from Al-Zarqa governorate have the highest phenolic contents among honey samples with the values of (10.6734 ± 0.006) mg/g, (10.43889 ± 0.012) mg/g and (10.1345 ± 0.004) mg/g; respectively. The DPPH scavenging assay as compared to BHT scavenging (reference control) 59.61%, has demonstrated that the eucalyptus flower honey obtained from Al-Zarqa governorate and weeds honey obtained from Jerash governorate have exhibited the highest DPPH scavenging among all honey samples with the values (12.8734± 0.008)% and (11.5635 ± 0.006)% respectively. Moreover, the reducing power tests of the reference compound ascorbic acid (0.751 mg/ml) have demonstrated that the weeds honey and eucalyptus honey both obtained from Jerash governorate have exhibited the highest reducing power with values of (15.8857± 0.002) mg/ml and (16.8787± 0.004) mg/ml respectively (Table 1, Figure 2).

When biomolecules are subjected to oxidative stress, it damages their structure and impairs the performance of key processes in the cells, leading to the development of various illnesses such as cancer. Regular consumption of antioxidants can lessen the unfavorable effects of the oxidant molecules such as free radicals. Honey is a natural chemical, which possesses antioxidant characteristics that are considered vital.

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Table1: The phenolic contents (GAE), The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity, and The Ferric reducing/antioxidant power assay (FRAP) of different Honey samples collected from different governorates across Jordan.

Tested honey samples	Location	Total Phenolic Compounds[1,2] (mg/g)	DPPH Scavenging[3] (%)	Reducing Power (mg/ml)
Eucalyptus flower	Al-Zarqa Governorate	6.2752^{c} ± 0.004	12.8734^{a} ± 0.008	5.7825^{b} ± 0.003
weeds Honey		10.1345^{a} ± 0.004	8.9329^{c} ± 0.002	9.5992^{b} ± 0.001
Eucalyptus flower	Jerash Governorate	10.6734^{bc} ± 0.006	10.6388^{b} ± 0.006	16.8787^{a} ± 0.004
weeds Honey		10.43889^{ab} ± 0.012	11.5635^{b} ± 0.006	15.8857^{a} ± 0.002

(1) Results are means ± SEM of three determinations.
(2) Means in the same row followed by different letters are significantly different (P<0.05).
(3) DPPH scavenging activity of 200 µg/ml of honey.

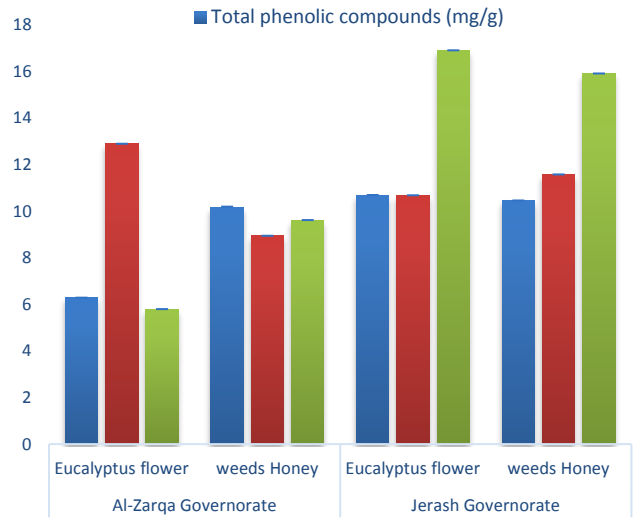

Figure 2: The phenolic contents (GAE), The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity, and The Ferric reducing/antioxidant power assay (FRAP) of different Honey samples collected from different governorates across Jordan.

The total phenolic compound contents (mg/g) were expressed as Gallic acid equivalent (GAE) determined according to the regression equation based on the calibration curve: y = 0.3638x - 0.3029, R^2 = 0.899. Where (Y) is the absorbance and (X) is the Gallic acid concentration in (mg/l). The capacity to scavenge the DPPH radical was calculated based on the following equation: % DPPH scavenging activity = (A control – A sample/A control X 100%). Where A control: absorbance for the control sample, A sample: absorbance for tested honey samples. Ascorbic acid (300 µg /ml) was used as a standard in the FRAP assay.

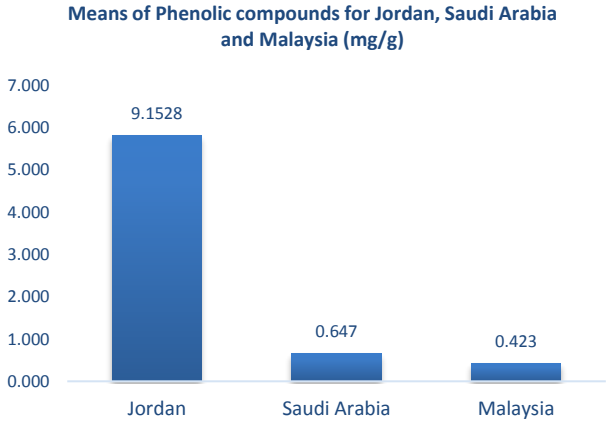

Figure 3: illustrates the means of phenolic compounds of honey samples from Jordan (selected honey in this study), in comparison to Saudi Arabia and Malaysia.

The antioxidant properties of honey are due mainly to its polyphenols content, such as flavonoids. Honey consists of chemicals that vary among different types of honey. The chemical composition of honey is related to its botanical origin, processing, and environmental conditions. Honey consists mainly of sugars and water, in addition to several vitamins such as B complex and vitamin C, as well as a lot of minerals, and enzymes. Honey has been used for its healing properties, nutritional and therapeutic properties since early times. Moreover, honey is known for its anti-bacterial, anti-inflammatory, and antioxidant properties that could be beneficial against multi-drug-resistant bacteria such as MRSA. It also prevents chronic inflammatory conditions, such as atherosclerosis. [13-15]

In this study, the antioxidant activities of different honey types collected from two different areas in Jordan were determined (Figure 1). To assess the antioxidant capacity of honey samples; the total phenolic contents, free radicals scavenging activity, and reducing power were evaluated. Because of its high diversity as well as complex composition, honey samples from the same botanical origin can show different antioxidant activity. The total phenol contents of honey were determined using the Folin-Ciocalteu assay and expressed in (mg/g) as Gallic acid equivalent (GAE). Results showed that the weeds honey and eucalyptus flower honey (obtained from Jerash governorate), and weeds honey (obtained from Al-Zarqa governate) have exhibited the highest phenolic compounds among honey samples (10.6388± 0.006 mg/g, 10.43889± 0.012 mg/g and 10.1345± 0.004 mg/g respectively) (Figure 1). A recent study in Jordan investigated the total phenolic content of royal jelly, multiflora honey, and citrus honey and demonstrated that the total phenolic contents of royal jelly were high compared with multiflora honey and citrus honey. Phenolic compounds provide antioxidant potential; this was studied in Malaysian honey, which proved the correlation between the total phenolic contents and the antioxidant activity. [16] Another study has conducted that dark multi-floral honey obtained from Jordan showed the highest total phenolic and flavonoids (15.84 mgGAE/100g, 5.74 mg QE/ 100g respectively), while light multi-floral honey demonstrated the lowest in total phenolics and flavonoids content.[17] The means of the phenolic compounds for the samples in this study is approximately 9.1528mg/g, which indicated a high composition of phenolic compounds compared with the means of the phenolic compounds for honey samples selected from Malaysia and Saudi Arabia (Figure 3). [18-20]

The scavenging activity of DPPH as a free radical was used to evaluate the antioxidant activity of different honey types; the results, as compared to BHT scavenging effect (61.59%), the eucalyptus flower honey obtained from Al-Zarqa governorate and weeds honey obtained from Jerash governorate have exhibited the highest DPPH scavenging activities of all honey types (12.8734± 0.008 % and 11.5635± 0.006 %, respectively). Although eucalyptus flower honey obtained from Jerash governorate exhibited a higher value of scavenging activity than weeds honey obtained from Al-Zarqa governorate (10.6388± 0.006 % and 8.9329± 0.002 %, respectively). The mean of DPPH scavenging for honey samples in this study is 10.906%, this is considered low compared with the DPPH scavenging activities of honey samples from Turkey and Kosovo (Figure 4).[21-22]

In terms of reducing power as compared to ascorbic acid (0.7652 mg/ml), the weeds honey and eucalyptus honey both obtained from Jerash governorate showed significantly the highest reducing power

(15.8857± 0.002 mg/ml and 16.8787± 0.004 mg/ml, respectively). It is recognized that phenolic acids, flavonoids, and certain enzymes are particularly important for honey's antioxidant action and that this has a strong association with other qualities including color.[12] Phenolic compounds like phenolic acids and flavonoids present in the weeds honey and eucalyptus honey could be responsible for their antioxidant activity.[11] The mean of reducing power (mg/ml) in this study is 11.215 mg/ml, which means a high value compared with the means of the reducing power of selected honey samples from Mexico and India (Figure 5). [11-12]

Several studies had showed that different types of honey that are light in color (eucalyptus and rosemary) showed lower values of both Total phenolic content and DPPH scavenging ability (6.4689[i]± 0.0007, 9.895± 0.001) and (4.639± 0.003, 4.919± 0.003) respectively than other types of darker color honey which were directly correlated with their higher reducing power. [21, 23-25] Total phenolic content of almonds and avocado honey, as well as their DPPH scavenging ability, were determined as the following; 11.082± 0.003, 12.248± 0.009, and 12.723± 0.022, 29.195± 0.000, respectively. Other studies suggested that the increase of browning during heat treatments of honey is correlated very well with its antioxidant activities. [24, 25]

In terms of reducing power, results demonstrated variable reducing power as compared to ascorbic acid. Reducing power is an important indicator of antioxidant activity. The value of reducing power indicates that compounds act as electron donors, reducing the oxidized intermediates in the peroxidation process of lipids.[26-29] Free radical scavenging activity of honey samples had been measured by using the DPPH method, where ascorbic acid was used as a positive control. The unpaired electron of DPPH forms a pair with hydrogen donated by the antioxidant from honey and thus converting the purple-colored electron DPPH to its reduced form in yellow. To determine the scavenging activity of honey, the degree of de-colorization can be measured by a UV-Visible spectrophotometer. It was found that the Free radical scavenging activity of ascorbic acid was lower than that of all honey samples. The lower the Free radical scavenging activity value the higher the scavenging capacity of honey, because it requires a lesser amount of radical scavenger from the honey to reduce DPPH.[22-23, 31-32]

This study has demonstrated that the antioxidant activity is high for almost all honey types from different plant origins, geographical regions, and provinces which were presented by measuring their radical scavenging activity and reducing power. The high concentration of polyphenols (flavonoids and phenolic acids) found in honey is attributed to honey's antioxidant activity.[32-33] The phenolic content and chemical structures of honey samples are thought to use various radical scavenging routes, which accounts for the discrepancies. In reality, experts have said that the changes in phenolic substances' chemical structures (the number of OH and CH3O groups added to the aromatic molecule, location, and side chain structure) have a significant impact on the antioxidant activity of phenolic acids. [34, 45] Bioflavonoids have been shown to have a protective effect against hydroxyl radical-induced DNA damage and prevent brain damage due to diabetes mellitus.[46] Several studies suggested that the differences in the antioxidant activities of honey samples depend on both the floral sources and the sources of collection.[36-37] Other factors contribute to variation in the antioxidant activity in honey, including seasonal factors, and environmental factors. [38-41] In addition, the processing, handling, and storage of honey could also be attributed to the different contents of honey.[42-47]

Conclusion

Honey is a natural chemical, which possesses antioxidant characteristics that are considered vital. The chemical composition of honey is related to its botanical origin, processing, and environmental conditions. Honey samples from the same botanical origin can have varying antioxidant activity. Various floral sources of honey, including Eucalyptus flower honey and Weed honey from different Jordanian governorates (Jerash and al-Zarqa) scored a high antioxidant capacity.

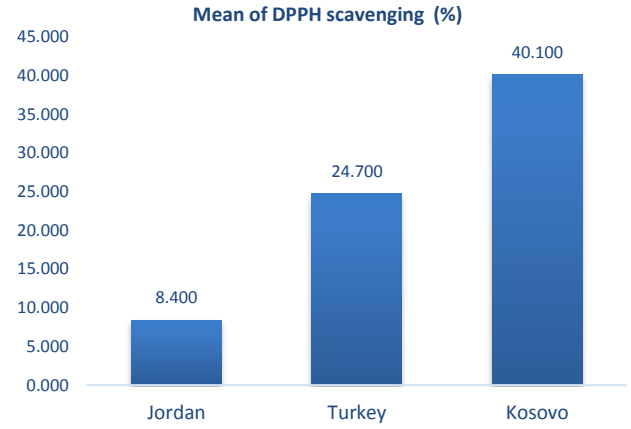

Figure 4: illustrates the means of DPPH scavenging activities of honey samples from Jordan (selected honey in this study), in comparison to Turkey and Kosovo.

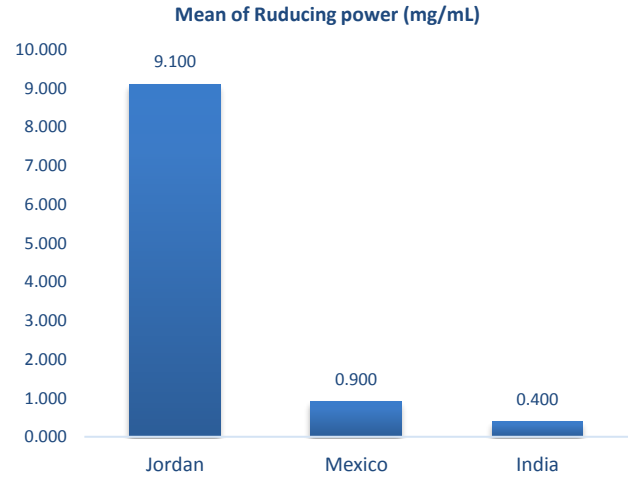

Figure 5: illustrates the means of reducing power (mg/ml) of honey samples from Jordan (selected honey in this study), in comparison to Mexico and India.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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