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Jian Lateif Hussein
Department of Chemistry, College of Science, Salahaddin University- Erbil, Kurdistan Region, Iraq

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ORIGINAL STUDY

Comparison Between Honey Produced By Bees, Aphids and Wasps in Kurdistan Region - Iraq Based on Biochemical Parameters

Jian L. Hussein

Department of Chemistry, College of Science, Salahaddin University- Erbil, Kurdistan Region, Iraq

Abstract

Honey is a sweet substance that has been valued by humans for its taste and health benefits. It is produced by honey bees from the sugary secretions of plants. However, other insects, such as aphids and wasps, also produce honey-like substances. This study aimed to compare the physical and biochemical properties of honey produced by honey bees, aphids, and wasps in the Kurdistan Region of Iraq. To achieve this, samples of honey, bitter honey, honeydew, and wasp honey were collected and examined. The samples were analyzed for total sugar, sucrose, water, Hydroxymethylfurfural (HMF), acidity, total acidity, diastase activity, total phenolic content (TPC), antioxidant activity (DPPH), and elements present. The tests applied were based on the international standard methods for honey analysis, Folin−Ciocalteu test for total phenols, DPPH radical scavenging test, and elemental analysis by ICP-OES. The study found that the physical and biochemical properties of honey produced by aphids and wasps were similar to those of honey produced by bees, with differences in total sugar, sucrose, water, HMF, acidity, total acidity, and diastase activity. Significant differences were found in TPC and antioxidant activities for most of the samples. However, the amounts of different elements present in the samples varied, with wasp honey containing high concentrations of Al, Mg, Fe, Mn, V, Ti, and Sr and being the only sample to contain vanadium. Overall, the results of this study suggest that honey samples produced by aphids and wasps were close to the specifications of bee honey and within accepted standards. However, when compared to bee honey, a significant difference was found in the total TPC and DPPH values. Honey is regarded as a necessary economic component and a healthy food, and this study contributes to our understanding of the properties of different types of honey.

Keywords: Bitter and sweet honeybee, Honeydew, Wasp honey, Antioxidant, Trace and heavy metals

1. Introduction

Honey is a delicious substance made by honey bees from sugary plant secretions. Carbohydrates make up the majority of honey's chemical makeup. Fructose is the primary sugar found in all varieties of honey [1]. While the majority of honey has a sweet flavor, some species produce bitter honey, which is another variety of honey that can be found in many parts of the world and comes from a variety of plant sources [2]. In the Kurdistan Region of Iraq (KRI), bitter honey is a unique honey made from the wild dandelion (*Taraxacum officinale*) and yellow star thistle (*Centaurea solstitialis*) flowers that thrive in the region. Bitter honey is produced in the mountainous and hillside areas of Erbil Governorate. Hymenopterans other than bees, such as wasps and aphids, make a sweet honey-like substance [3,4]. Aphids produce delectable sugary syrup known as manna honey. These insects are most commonly known as garden and crop pests, and they can be identified as warty lumps on plant stems. Manna from the KRI is known locally as gazo. This sweet substance is collected from oak trees in Iraqi Kurdistan, particularly in Penjween, as manna ash. When these trees are attacked by insects, their leaves produce sticky secretions, and it is likely that the insects' exudates are also added to them and included in the mass that forms [5]. The predominant component in manna, accounting for 50% of all sugars, is mannitol. Additionally, fatty acids and phenol components were discovered by a recent phytochemical analysis [6,7]. Aphids, scale insects, and Psylloidea ingest plant sap by

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E-mail address: jian.hussen@su.edu.krd.

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puncturing a plant's leaves and tender shoots and sucking out the fluid, which, after being stripped of the protein that serves as these insects' diet, is discharged as drips, known as honeydew. Only a few wasp species can produce honey. One of which is the *Brachygastra mellifica*, also known as the Mexican honey wasp. However, compared to bees, its honey production is quite small. It tastes like bee honey but is much more viscous. Colonies of social insects have been observed to produce and store honey for use as nutrition during times of drought or cold weather [8]. According to Grüter (2020), social polistine wasps have been observed to store honey; however, this practice is still less well known [9]. A historical account of the early reports of this type of wasp and the honey produced by them is reported in detail by Hunt et al. (1998) [4].

Honeydew represents a significant portion of the food consumed by many species in beech forests. Honey bees (*Apis mellifera* L.), German wasps (*Vespula germanica* F.), and common wasps (*V. vulgaris* L.) are some of the most notable and widespread consumers of honeydew [10]. Wasp honey is a traditional food called shametrenka in KRI. However, it is pricey compared to the best local rates for bee honey. However, not enough research is present to establish its nutritional value and quality. One aspect that determines the nutritional value and, at the same time, the toxicity of these natural sweet substances is their elemental content. More poisonous metals are found in plant leaves than in flowers or pollen [6,7]. Given that certain elements, especially hazardous ones, cannot decompose, they pose a serious threat to the ecosystem. Concentrations of Pb, Cd, Cu, and Zn in soil have increased because of industrial activity, coal combustion for cars, the melting and burning of municipal garbage, and atmospheric pollution deposition [11]. Because bees are influenced by a variety of environmental factors when producing honey, honey is a suitable matrix for incorporating contaminants. Given the numerous negative impacts that hazardous elements, such as As, Cd, Pb, Ni, and Cr, have on human health, particularly due to their detrimental biochemical, histological, cognitive, and cancer-causing effects, special emphasis should be given to their examination [12]. Elements such as Fe, Cu, and Zn play important structural and regulatory roles in a variety of metabolic processes required for the function of organisms [13]. However, at high concentrations, these metals can negatively impact the operation of cells and a number of organs, as well as contribute to the onset of Wilson's and Alzheimer's disease in the case of copper or anemia and neurological illnesses in the case of iron [14,15]. The aims of the present study were to investigate and compare the biochemical properties, phenolic content, and antioxidant activity of some naturally sourced, delectable foods in KRI, including honey, including honey and bitter honey from bees, wasp honey from wasps, and honeydew from aphids.

2. Material and methods

2.1. Collection of samples

The experimental material consisted of honeys of mixed floral origins, bitter honey, honeydew (locally known as *gazo*), and wasp honey (locally known as *shametrenka*), which were collected from mountainous regions in KRI.

2.2. Chemicals and instruments

All reagents and chemicals were of analytical grade. The honey samples were subjected to a number of biochemical test samples were subjected to a number of biochemical tests (TPC and antioxidant activity) as follows.

2.3. Biochemical tests

2.3.1. Water content and electrical conductivity

Water content was determined by measuring the refractive index (RI) of honey samples using an Abbe refractometer. Water content values corresponding to RI measurements were calculated from tables, and variations in temperature were corrected. For measuring electrical conductance, the conductivity of a 20% (w/v) honey solution in distilled water was measured using a DDS-2230 conductometer, and the results were expressed in S/cm. Both parameters were determined according to [16].

2.3.2. Hydroxymethylfurfural (HMF)

The analysis of HMF was based on the original method by (White Jr, 1979). Briefly, 10% solutions of honey samples containing 0.5 mL of each of Carrez I and II solutions were prepared and filtered. The basic principle of this method involves the determination of HMF by measuring the absorbance of honey solutions at 284 nm. However, other compounds in honey (primarily phenolics) may also absorb at this wavelength. Therefore, a correction is made by adding sodium bisulfite solution to the honey solution and measuring its absorbance at 330 nm. The difference between the two absorbance values corrects for the effect of interfering compounds [17].

2.3.3. PH and free acidity content

pH was measured for a solution of 10 g of honey in 75 mL of water, employing a magnetic stirrer and a pHS-550 pH meter.

Total free acidity was determined for the same solution by titration against a 0.1 M NaOH solution to pH 8.30, taking into account that the process would not take longer than 2 min to get to a final steady reading of pH [18].

2.3.4. Total sugars

Reducing and total sugar content were determined following the Lane-Eynon method [18]. Where sucrose and reducing sugars (represented by glucose and fructose) were determined titrimetrically using a modified Fehling method that measures reducing sugar content before and after acid hydrolysis of honey. Modified Fehling solution was titrated at the boiling point against honey solution (0.5%) using methylene blue as an indicator. Honey samples were treated with HCl at 65 °C in order to convert sucrose to glucose and fructose; the excess acid was neutralized with NaOH, and the resulting solution was used for titration as before. The increase in reducing sugar value after hydrolyses reflects the amount of sucrose present in the sample.

2.3.5. Diastase activity

Diastase activity was determined by applying a modified version of Schade's method. Ten milliliters of a 20 percent honey solution containing NaCl and acetate buffer was brought to equilibrium with an equal volume of a 2 percent aqueous starch solution in a 40 °C water bath for 15 min. After which, 5 mL of the starch solution was added to the honey solution, mixed well, and kept in the same temperature water bath. Aliquots of 0.5 mL were periodically removed from this solution and added to 5 mL of iodine solution and 20 mL of distilled water (the exact water volume was determined from the calibration of the starch solution). The resulting solution was mixed well, and its absorbance was recorded at 660 nm using water as a blank. Subsequent aliquots were removed at different time intervals, covering a range of absorbance values from 0.770 to below 0.200. The value of tx (time at absorbance value 0.235) was determined by applying the linear regression equation to the data plot of time versus absorbance. Diastase number was calculated as (300/tx) [19].

2.3.6. Folin–Ciocalteu test for phenols

TPC analysis was performed according to the method modified by Piljac-egarac et al. (2009) [20].

2.3.7. Radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Antiradical activity of honey samples was determined according to the method described by Khalil et al. (2011) [21].

2.4. Elemental analysis

Aliquots of 1 g samples were digested using a 9:1 mixture of HNO_3 (69%) and H_2O_2 (30%). Samples were heated to 200 °C for 20 min and held at this temperature for another 20 min. The digested mixture was filtered, and the filtrate was made up to 50 mL with deionized water prior to analysis. An ICAP 7600 ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) Thermo Fisher instrument was used, employing a high-performance solid-state CID 86 chip detector. Elements analyzed included: Pb, Sb, As, Zn, Cu, Ni, Co, Fe, Mn, Cr, V, Ti, Tl, Mg, Al, Sr, Ba, Cd, Se, and Mo. The emission lines for the elements and their measuring modes were selected based on exhibiting the highest signal-to-noise ratios and the lowest interference. Table 1 shows the list of standard parameters and conditions for analysis.

2.5. Statistical analysis

The assay findings were examined with the use of the program GraphPad Prism 8.0. The ANOVA test was used to compare several varieties of honey. Data presented as mean ± SE.

3. Results and discussion

While most plants produce honey with a sweet flavor, others also produce bitter honey. Bitter honey is typically harvested in the fall, and its composition and use are influenced by various plant species, weather, and environmental factors. The chemical composition of the samples varies depending on pollen sources, climate, environmental conditions, and processing [22]. With the exception of the

Table 1. Instrumental parameter for ICP-OES an.

Method parameter	Value
UV exposure time	15 ms
UV RF power	1150 W
UV Nebuliser gas flow rate	0.5 L min^{-1}
Vis exposure time	5 ms
Vis RF power	1150 W
Vis Nebuliser gas flow	0.5 L min^{-1}
Cool gas flow rate	12 L min^{-1}
Auxiliary gas flow rate	0.5 L min^{-1}

sucrose level, which was higher than the bee honey (9.1, 1.7), the bitter honey in Tablele 2 had an acceptable value within the normal range for all parameters. This finding is within acceptable limits, as reported by Otmani et al. (2019) [23].The diastase enzyme activity in bitter honey was lower than that of sweet bee honey (17 and 21, respectively). This may indicate that honey has been heated or stored, which reduces the activity of these enzymes. In some countries, the diastase enzyme activity is used as an indicator of honey's purity and freshness [24].When compared to bee honey, some of the parameters for honeydew were within the normal range. While levels of reducing sugar (47, 76) and diastase (0, 21) were lower than the bee honey, in agreement with Halouzka et al. (2016) [25]. The honeydew sample may have been heated during processing or storage, which could have directly affected its quality. However, more information is still needed to specify precisely which elements and how much they influence the diastase activity of honeydew hives. On the other hand, the sucrose level of the honeydew sample was higher than that of the bee honey (36, 1.7), and this result was lower than that of Nicknejad (1976) [26], who observed that manna contained about 40% sucrose in a gazo sample taken from desert trees. Additionally, its water content level was higher than that of the bee honey (25, 15.4) in Table 2. In most cases, the nectar or honeydew that insects collect has up to 80% more water than honey. The insects in the colony evaporate water from the liquid, adding glandular secretions that contain several enzymes in the process. One enzyme, invertase, converts sucrose, the primary sugar in many types of nectar, into fructose and glucose. A very high final sugar concentration is reached due to the relative solubilities of sucrose, fructose, and glucose at temperatures around 35 °C, as cited in [3]. Honey is likely to be protected from fermentation and subsequent spoilage if the water content is less than 18.6%. The individual values of the water content of 1063 European *A. mellifera* honeys produced in four different countries ranged between 13.4% and 26.6%. Wasp honey primarily contains sucrose and glucose without fructose and therefore consists of concentrated nectar without alteration by invertase. Wasp honey also contains higher levels of HMF (15.3, 0.9), pH (6.7, 4.0), and sucrose (28, 1.7) than bee honey [4]. Wasp honey scored lower than bee honey in terms of water content (9% vs. 15.4%), reducing sugar (30% vs. 76%), and diastase (9 vs. 21), respectively. Wasp honey has a lower water content than bee honey because it is stickier, as was noted in this study. The electrical conductance of all samples-bitter honey, honeydew, and wasp honey-showed acceptable values (567, 542, and 245) within the normal range when compared to bee honey (436.6) Table 2. Despite having special nutritional and medicinal qualities that make honey and other bee products frequently consumed as food, contamination from anthropogenic activities could pose health risks [27]. The precise composition of honey is influenced by a number of complex factors, including geographic location, botanical sources, season, storage, and processing conditions. Standards for honey quality are provided by the widely accepted Codex standard (chemical and physical requirements) [18], whereas the basic characteristics of honey are described in the FSANZ standard [28]. Since honey has a wide range of minerals and trace elements, it can be a good source of these nutrients for humans [29]. Table 3 shows the concentrations of 20 elements in honey produced by bees, aphids, and wasps in KRI along with their Tolerable Upper Intake Levels (TUIL). Bitter honey's elemental concentration is very similar to honey, with the exception of Al, which is lower (24.48 vs. 41.35 ppm). The bitter honey is almost identical to the elemental concentration of bee honey. Concentrations of Ni, Mn, and Mg in honeydew were higher than those in bee honey (0.33, 0.13; 1.11, 14.13; and 163.96, 60.37 ppm), respectively. The accumulation of Mn was found to be responsible for the unique smell in honey from the genus Rubus [13,30]. The other elements were found in nearly the same concentrations as in bee honey. According to Yazdanparats et al. (2014) [31], the amount of iron in four different types of manna was as follows: Gaz-Alafi (oak manna) (1730.0396 ppm) of iron, Taranjebin (camel's thorn manna) (781.5932 ppm), Bid-Khesht (willow manna) (138.7188 ppm), and Shir-

Table 2. Biochemical parameters for different honey samples.

Sample	HMF	pH	Total acidity	Water %	Reducing sugars %	Sucrose %	Electrical conductance	Diastase number
Bee honey	0.9	4.0	10.0	15.4	76	1.7	436.0	21.0
Bitter honey	6.0	4.1	4.0	13.0	74	9.7	567.0	17.0
Honeydew (*gazo*)	0.0	4.8	5.0	25.0	47	36.0	542.0	0.0
Wasp honey (*shamatrenka*)	15.3	6.7	2.0	9.0	30	28.0	245.0	9.0
Accepted value	$\leq$80 mg kg^{-1}	3.5–6.1	<50 mmol kg^{-1}	$\leq$23%	<83%	<5%	<800 μS cm^{-1}	>80 U

Table 3. Concertation of 20 elements (ppm) for sample honey produced by bees, Aphids and wasps in KRI.

Elements	Bee honey (ppm)	Bitter honey (ppm)	honeydew (*gazo*) (ppm)	Wasphoney (*shametrenka*) (ppm)	TUIL mg.day^{-1}
Pb	0.012	0.024	LDL	0.263	0.18
Sb	LDL	LDL	0.087	0.017	0.006
As	0	LDL	LDL	LDL	ND
Zn	0.974	0.692	0.517	2.783	40
Cu	0.137	0.272	0.01	1.198	10
Co	0.062	0.049	0.053	0.16	ND
Fe	3.663	4.31	2.434	240.818	45
Mn	1.119	0.841	14.139	9.357	11
Cr	2.289	1.885	2.154	3.076	ND
V	1.268	1.252	1.572	3.751	1.8
Ni	0.137	0.14	0.33	0.507	1
Ti	0	0	0	7.702	ND
Tl	LDL	LDL	LDL	LDL	0.01
Mg	60.375	80.377	163.965	322.871	400
Al	41.355	24.483	23.218	1543.351	70
Sr	0.935	0.916	1.122	2.301	0.13
Ba	0.394	0.01	0.116	0.972	0.21
Cd	0.004	LDL	0.05	0.011	0.062
Se	0.449	0.371	0.418	0.291	0.4
Mo	0.04	0.019	0.044	0.045	2

*LDL = lower detectable limit.
TUIL and RDA values are based on: for antimony [51], aluminum [52], cadmium [53], Sb, Sr, Ba, Tl [54] and for the rest of the elements [55]. RDA are minimum and maximum values recommended for adult female and males and are based on [54,55]. ND: not determined, when the element's TUIL is not clearly set due to unavailability of sufficient data and/or toxicity-related testing limitation.

Khesht (pocke manna) (99.7218 ppm). These variations could be attributed to the kind and number of additional materials present with manna as well as the method of cleaning the manna samples that were purchased from the local market. The recommended daily amount (RDA) and TUIL for iron are 8 mg and 45 mg, respectively [32]. This identifies manna as a superlative source of iron [33]. The magnesium content in honeydew was found to be 163.965 ppm. This content is quite high in comparison to bee honey. According to Edori (2017) [32], the RDA and TUIL for magnesium are 310 and 400 mg, respectively. Wasp honey revealed a different outcome when compared to bee honey. All the elements were present in higher concentrations than bee honey, with the highest levels being found in Al (1543.4, 41.35 ppm), Mg (322.87, 60.37 ppm), Fe (240.81, 3.66 ppm), Mn (9.35, 1.11 ppm), V (3.75, 1.26 ppm), Ti (7.7, 0.0 ppm), Ba (0.97, 0.39 ppm), and Sr (2.30, 0.93 ppm), Wasp honey's Tl content was below detection limits, and Se was of lower concentration than the bee honey sample. Alkaline-earth metals Ba and Sr were also detected. These elements are naturally present in soil, rocks, and groundwater, which is likely why they are found in the samples [34]. Bees are sometimes forced to live and forage close to human activity, where they may accumulate certain contaminants and trace elements that raise questions about the safety of honey consumption [35]. Widely distributed in nature, vanadium is a transitional element whose oral administration has been shown to reduce the symptoms of diabetes mellitus in both humans and lab animals [36,37]. For example, the increase of insulin sensitivity in the liver, kidney, and other tissues has been proposed as one of the many mechanisms by which vanadium improved diabetes [38,39]. The wasp honey sample, which was found to contain vanadium in higher concentrations compared to the other samples, needs to be subjected to further study and research to determine its potential in lowering blood sugar levels in people with type 2 diabetes, as reported by [40]. In contrast, wasp honey showed a high level of aluminum, which is toxic and considered to be a heavy metal. This may be because the wasp honey was collected from pits in the mountains of the Qandil region of Iraqi Kurdistan, where many heavy metals were found. These findings, presented in Table 3, show that the minerals and heavy metals present in the honey produced by wasps are similar to those reported [41]. Iron concentration was found to be much higher than the other study samples and can be attributed to the variability of the vegetation and the different types of soil [42]. Botanical, environmental, and geographic factors can be held responsible for this variation in mineral content [43,44].Moneim et al. (2013) included accounts of previous reports of elements in honey that were in agreement with results from this work regarding Mn (1.019 mg/kg) and Fe (2.05 mg/kg) [1]. However, they disagreed on the level of Zn (9.61 mg/kg). According to the results of the current study, Pb and Cd were found in bee honey at concentrations of 0.012 and 0.004 ppm, respectively; this finding is in agreement with what was reported by Al-Khalifa and Al-Arify (1999) [45]. Statistical analysis did not indicate any significant differences between the elements present in these samples compared to bee honey, despite the large difference in their total elemental content (Fig. 1). To ensure the protection of human health, element composition control and monitoring of toxic metal contents in honey are required. According to the results for total phenolic content (TPC) shown in Fig. 2, bitter honey contained 522.5 ± 10.5 mg.100g −1 of the gallic acid equivalent of phenolic compounds, which was significantly different than bee honey at (141.2 ± 0.2) mg.100g-1 at $P \leq 0.001$. Additionally, TPC of honeydew (882.1 ± 5.9) and wasp honey (946.7 ± 48.7) were highly significantly different at $P \leq 0.0001$ from bee honey (141.2 ± 0.2) mg.100 g^{-1}. Between the groups, the comparison between bitter honey with honeydew and wasp honey was highly significant at $P \leq 0.0001$, whereas there was no significant difference between honeydew and wasp honey. A darker honey color is frequently interpreted as indicating higher TPC levels, which are also related to higher quality, better nutritional value, and more proactive health effects [46]. Phenolic compounds impart a significant biological activity to honey due to their anti-oxidant power that can counteract the damage caused by free radicals in the body [47]. The total amount of polyphenolic compounds in honey is therefore directly proportional to its antioxidant potential. Antioxidant activity on the DPPH radical was found to be (73.0 ± 0.4), (6.0 ± 1.5), (71.2 ± 0.1), and (16.1 ± 0.8) for bee honey, bitter honey, honeydew, and wasp honey, respectively. It is worth noticing however that antioxidant activity may not reflect the total phenolic content in the different samples. Comparing the data in Figs. 2 and 3, it can be seen that bitter honey has higher TPC than honey, but its antioxidant activity is lower. Additionally, wasp honey which has higher TPC than honeydew honey also shows lower antioxidant potential. This might be explained by the fact that antioxidant activity is affected by the presence of other compounds as well. Such compounds include: vitamins (e.g, ascorbic acid), carotenes, absence or presence of certain metal elements, age of the samples and their exposure to heat or light [48,49]. Also, the presence of sugars that can interact with phenolic compounds

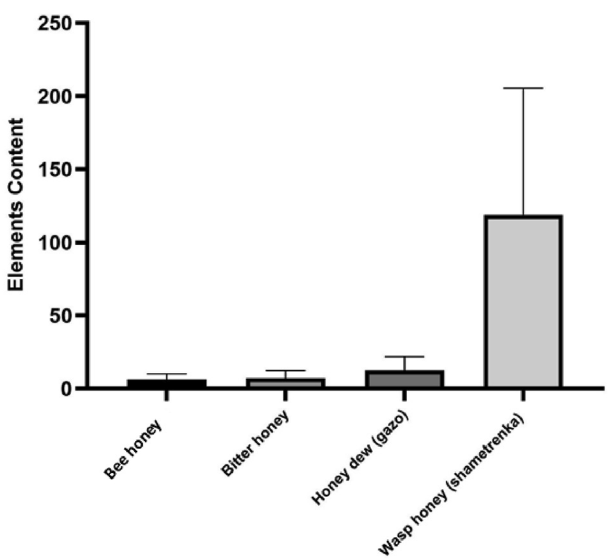

Fig. 1. Comparison of elemental content for different honey samples. No significant differences were found between the samples.

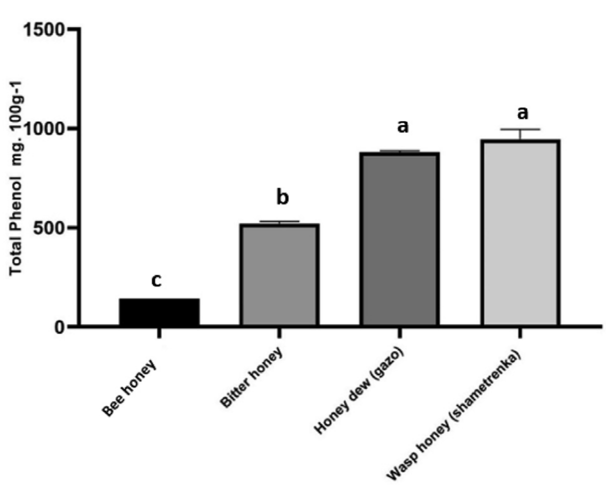

Fig. 2. Comparison of total phenolic content for diferent honey samples. Different letters on data bars indicate significant differences at P < 0.001 for (b and c); at P < 0.0001 for (a and b) and (a and c).

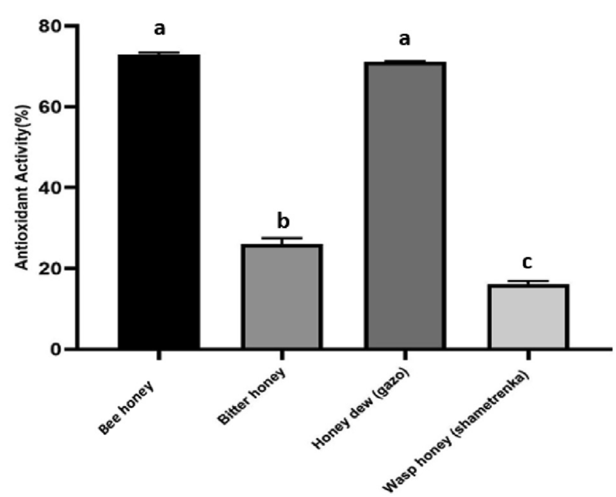

Fig. 3. Comparison of DPPH radical scavenging activity for the samples. Different letters on data bars indicate significant differences at P < 0.001 for (b and c); at P < 0.0001 for (a and b) and (a and c).

and alter the antioxidant power of the various samples [50]. Further investigation would be required to establish the contribution of the different compounds and effect of external factors on this parameter. There were highly significant differences ($P \leq 0.001$) between bee honey with bitter honey and wasp honey, while there was no significant difference between bee honey and honeydew. There were highly significant differences ($P \leq 0.001$) between bitter honey and honeydew. In addition, there were significant differences ($P \leq 0.001$) between the bitter honey and the wasp honey. Also, there were higher significant differences ($P \leq 0.001$) between the honeydew and wasp honey (Fig. 3).

4. Conclusion

In conclusion, the results of this study showed that all values from the biochemical analyses of the samples of bitter honey, honeydew, and wasp honey were close to the values of bee honey and within accepted standards. Significant differences were found in TPC and antioxidant activity between the study samples. Wasp honey was found to contain high concentrations of Al, Mg, Fe, Mn, V, Ti, and Sr. Among all the samples, only wasp honey contained vanadium, which studies have linked to benefits such as reducing blood sugar levels. More research is required to ascertain how the presence of vanadium in wasp honey can lower blood sugar levels in people with type 2 diabetes.

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