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Quality Assessment and Nutraceutical Potential of a Traditionally Harvested Honeydew Honey from the Wild in Ghana

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Authors' contributions

This work was a collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this research was to ascertain the quality of the honeydew honey harvested with wood smoke.
Methodology: The sample was stored in a container underlined with aluminium foil and then covered with a cork and kept for subsequent analyses. The sample was analyzed for its qualities like phytochemical properties, antiradical activity, physicochemical properties, antibacterial potency, heavy metal composition and pesticide residue content.
Results: The total phenolic and total flavonoid contents and antioxidant potential were comparatively higher than most honeys reported from other countries. The different concentrations of honey exhibited varying levels of antibacterial activity against the three different pathogenic indicator strains (*Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*). The inhibition of bactericidal activity was reduced with reduction in the honey concentration. The

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physicochemical parameters and pesticide residue studied were within the acceptable limit. Some heavy metals were present in the honey, but were within the permissible safe limits of Codex Alimentarius Commission and FAO/WHO. However, Cadmium, Manganese and Nickel levels were above the safe limit.

Conclusion: The inhibition of bacterial growth indicates the honey's health benefits in fighting bacterial infections. Generally, the honeydew honey harvested with wood smoke honey exhibited medicinal/nutraceutical properties.

Keywords: Honeydew honey; phytochemical; antibacterial; antiradical; physicochemical; pesticide residue.

1. INTRODUCTION

Honeydew is particularly common as a secretion in the Hemipteran insects and is often the basis for trophobiosis [1]. The quality of honey is mainly determined by its sensorial, chemical, physical and microbiological characteristics. The methods employed for harvesting are crucial to the quality of the product. There are different methods of harvesting honey of which smoking is one. Smoking the bee hives before harvesting is essential, but the smoke to be used should be rich, dense and cool but not hot smoke or flames which will damage the bees. Honey generally, has been scientifically tested and confirmed to possess functional and biological properties such as antioxidant, anti-inflammatory, antibacterial, antiviral, antiulcerative activities, antilipid and anticancer properties [2-7] due to the presence of polyphenols (flavonoids and phenols).

Honey may contain metals of which some may be beneficial or injurious if consumed. Some essential metals are involved in numerous biochemical processes and adequate intake of certain essential metals relates to the prevention of deficiency diseases. The essential metals may become toxic when the metal intake is excessively elevated [8]. Heavy metals such as lead (Pb) are toxic even at trace levels [9].

Honey may also be contaminated with pesticide residues which may include acaricides, organic acids, insecticides, fungicides, herbicides and bactericides. Many of these contaminants are banned because of their health hazards such as carcinogenic effect on humans. Over 150 different pesticides have been found in colony samples [10]. DDT and its derivatives are one of the commonest pesticide residual contaminants. *p, p*'-DDT (*p*, *p*'-dichlorodiphenyltrichloroethane) is a white crystalline solid, the technical mixture a white or cream coloured waxy solid or amorphous powder. When DDT is sprayed, any that fails to adhere to its target drifts away; vaporization from treated fields can be detected for more than 6 months after application. With rare exceptions, concentrations of DDT in air in non-agricultural areas have been in the range <1–2.36 ng/m^3. In agricultural communities, concentrations have ranged from 1 to 22 ng/m^3 [11]. In Ghana, many people use honey as a substitute for sugar without any idea about the mode of harvesting and the subsequent effect of the harvesting process on the quality. The quality of some honey harvested with modern techniques had been reported. On the contrary, honeys from the wild harvested using wood smoke are widely used in Ghana without any prior quality data base. The aim of this research was therefore to ascertain the quality of honey harvested with wood smoke.

2. MATERIALS AND METHODS

2.1 Sample Preparation

The honey sample was bought fresh from the farm gate after harvesting from the wild in a village in Ghana. The sample was stored in a container lined with aluminium foil and then covered with a cork and kept for subsequent analyses.

2.2 Methanol and Aqueous Extractions for Phytochemical Analysis

The samples were freeze dried and 0.05 g weighed and extracted in 15 ml deionized water and methanol at a temperature of 60°C for 3 hours and the supernatant recovered for further analyses.

2.3 Phytochemical Analysis

2.3.1 Total phenolic

The total phenolic contents were measured by the Folin- Ciocalteau method using Gallic Acid as standard [12]. The sample (50 µl) plus 3ml of distilled water, 250 µl of Folin- Ciocalteau (fc

1/10) and 750 µl 20% of Na_2CO_3. The mixture was vortexed to mix, incubated for 30 min in the dark and the absorbance measured at 760 nm.

2.3.2 Total flavonoid

The aluminium chloride colorimetric assay method [13] was employed for the total flavonoid content. Quercetin was used as standard. Total flavonoid content was determined as microgram (µg) Quercetin equivalent using the calibration linear regression equation. 2800 µl distilled water, 1500 µl ethanol, 500 µl samples, 100 µl potassium acetate (1M) and 100 µl of 10% aluminium chloride were mixed and incubated in the dark for 30 min. The absorbance was read at 415 nm.

2.3.3 Antiradical scavenging activity

The DPPH free radical scavenging activity was determined using 2, 2-diphenyl-1-picylhydrazyl (DPPH) free radical. 200 µl of extracts was added to 3800 µl of 0.004% DPPH in an ethanol solution. After 30 min of incubation at room temperature in the dark, the absorbance was measured at 517 nm. Radical scavenging was calculated as follows; 1% = [(Abs_0 –Abs_1)/ Abs_0], where Abs_0 was the absorbance of 0.004% DPPH without analyte and Abs_1 the absorbance of 0.004% DPPH plus the test compound.

2.4 Antibacterial Activity

The test organisms used in this research consisted of two Gram-negative and one Gram-positive isolates (*Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*) obtained from the Food and Microbiology laboratory of the Radiation Technology Centre of the Ghana Atomic Energy Commission. The test organisms were cultured on agar slants and stored in the refrigerator at 4°C. Subcultures were made at two-week intervals. The inhibitory potential of honey was investigated using the agar well diffusion method as described by [14,15]. Nutrient agar was poured into Petri dishes and allowed to solidify and dry for 1-2 days. Circular wells were made in the agar using sterile cork borers. A volume of 0.1 ml of the test inoculum was transferred into wells and left to diffuse into the agar for approximately 4-5 h. The wells were overlaid with about 10 ml of soft Nutrient agar (0.7% agar) containing the indicator strains. The indicator lawns were prepared by adding 0.25 m1 of 10^{-1} dilution of an overnight culture of the indicator organism to 10 ml of Nutrient agar. The plates were incubated at 37°C for 24 hours and the diametre of the zone of inhibitions were measured.

2.5 Physicochemical Analysis

2.5.1 Ph determination

The pH was determined by measuring out 10 ml of the sample into a clean beaker and then measured directly with a pH meter (Radiometer PHM 92 Radiometer Analytical A/S, Bagsvaerd, Denmark) after calibration using standard buffers.

2.5.2 Determination of total titratable acidity (TTA)

The TTA was determined by taking 25 ml of the sample (diluted) which was titrated against 0.1N NaOH using 0.25 ml phenolphthalein as an indicator. The relevant amount of lactic acid was determined using the mathematical formula [15]:

$$\text{Lactic acid (\%)} = \frac{\text{Titre Value x Normality x 9}}{\text{Volume of Sample}}$$

2.5.3 Determination of moisture

The moisture content of the sample was determined by measuring 5 g of the sample into a pre-weighed aluminium drying dish. The sample was dried to a constant weight in an oven at 105°C for 4 hours under vacuum [16].

2.5.4 Determination of total solids

The percentage total solids were determined [16] using the equation:

Total Solids (%) = 100 – Moisture content.

2.5.5 Determination of total soluble solids (TSS)

TSS was determined using the hand refractometer. Few drops of the sample were mounted on the lip of the refractometer and the level of clearness was taken and recorded as the degree brix for the sugar concentration.

2.6 Elemental Analysis Using Atomic Absorption Spectrophotometry (AAS)

The honey sample was weighed (0.5 g) into a labelled 100 mL polytetrafluoroethylene Teflon bombs. 6 mL of conc. HNO_3 (65 %) and 1 mL of H_2O_2 (30%) was added to the samples in a fume chamber. The samples were then loaded on a microwave carousel. The vessel caps were

secured tightly. The complete assembly was microwave-irradiated for 20 min in a milestone microwave laboratory station ETHOS 900 D model using the following parameters; 250 W for 02 min, 0 W for 02 min, 250 W for 06 min, 400 W for 05 min, 600 W for 05 min with 100 pressure, 400°C and 500°C. Five minutes was allowed for venting (Milestone Cook Book, 1996). After digestion, the Teflon bombs mounted on the microwave carousel were cooled in a water bath to reduce internal pressure and allow volatilized materials to re-solubilize. The digest was made up to 20 mL with distilled water and assayed for the presence of iron, copper, zinc, manganese, cadmium, magnesium, selenium in an acetylene-air flame. Reference standards for the elements of interest, blanks and repeats of the samples were digested the same way as the actual samples. These served as internal positive controls. The digested samples were then aspirated using Varian AA240FS fast sequential Atomic Absorption Spectrophotometer. The instrument was initially calibrated before the reading of any element with a standard solution of the element. A linearity of the calibration curve was always checked before the samples were aspirated. Calculation was obtained as stated below:

$$\text{Final concentration (ppm)} = \frac{(\text{Concentration} \times \text{nominal volume})}{\text{Weight of sample in grams}}$$

Concentration recorded = given on the monitor attached to the instrument
Nominal volume = final volume after reagent and water were added
Weight of sample = 0.5.

2.7 Pesticide Residue Analysis

Pesticide residues in the honey sample were determined [17] with modifications. Honey (20 g) was added to 20 ml of water and 100 ml of acetonitrile was added. The mixture was homogenized and centrifuged at 2,500 rpm for 5 min. The upper layer was taken and 10 g of NaCl added and shaken for 3 min. it was allowed to stand and the separating water layer excluded. The acetonitrile layer was taken and concentrated. To eliminate possible water, 20 ml of ethyl acetate and anhydrous Na_2SO_2 was added, extracted with sonication, filtered and concentrated. The residue was dissolved to the concentration of 2.5 g of sample per ml with 1:1 acetone: n-hexane to obtain the sample extract. The sample extract was loaded onto PSA column [Bond Elut PSA (500 mg)]. The column was eluted with 18 ml of 1:1 acetone: n-hexane to collect the eluate. The eluate was concentrated and the residue dissolved with 1 ml of 1:1 acetone: n-hexane. The test solution was then taken through GC analysis. The residue was analyzed by Shimadzu gas chromatograph GC-2010 equipped with ^{63}Ni electron capture detector that allowed the detection of contaminants even at trace level concentrations from the matrix to which other detector do not respond. The GC conditions and the detector response were adjusted so as to match the relative retention times and response.

The conditions used for the analysis were: capillary column coated with ZB-5ms (30 m × 0.25 mm, 0.25 µm film thickness). Carrier gas and make-up gas was nitrogen at a flow rate of 1.0 ml/min and 29 mL/min, respectively. The injector and detector temperatures were set at 280°C and 300°C respectively. The oven temperature was programmed as follows: 60°C held for 1 min, ramp at 30°C min-1 to 180°C, held for 3 min, ramp at 3°C min-1 to 220°C, held for 3 min, ramp at 10°C min-1 to 300°C. The injection volume of the GC was 1.0 µL. The residue detected by the GC analysis was further confirmed by the analysis of the extract on two other columns of different polarities. The first column was coated with ZB-1 (methylpolysiloxane) connected to ECD and the second column was coated with ZB-17 (50% phenyl, methyl polysiloxane) and ECD was also used as detector. The conditions used for these columns were the same.

2.8 Statistical Analysis

Statgraphics centurion (version 16) statistical tool was used for the analysis of variance and mean separations. Data obtained for the phytochemical, antibacterial and physicochemical analysis were subjected to one-way ANOVA. Values were represented as mean ± S.D of triplicate data.

3. RESULTS AND DISCUSSION

3.1 The Phytochemical and Free Radical Scavenging Activity of the Honey

There were no significant differences between the solvent used for the extraction in terms of total phenolic content of the honey (Table 1). The total phenolic content of the studied honey ranged from 79.40-84.50 mg GAE/100 g. The

total phenolic content of the studied honey was relatively high compared to other reports made on the total phenolic content of honey from different countries. The higher total phenolic content could be attributed to the plant source from which the pollen grains were taken from for the production of the honey. The plant source might have possessed large deposit of polyphenols in them. It has been reported that the total phenolic content of fifty sunflower honey from Turkey studied ranged from 6.89-23.20 mg GAE/100 g [18]. This implies that the total phenolic content of the honey under study from Ghana is far higher than that from Turkey unlike those reported from India, Yemen and Romania. Indian honeys had been reported [19] to have total phenolic content of 47-98 mg GAE/100 g, Yemeni honey [20] had been reported to range from 56.32-246.21 mg/100 g and Romanian honeydew honeys had total phenolic content ranging from 23.0-125.0 mg/100 g [21]. Research has found that honeys which are dark in colour have a higher amount of total phenolic compounds [6]. Although the colour of the studied honey was not determined, it looked very dark which could be due to the high content of the phenolics or the smoked used in harvesting. Phenolics or polyphenols are one of the most important classes of compounds found in honey. The total concentration of phenols in honey is highly dependent on its plant source. The determination of total phenolic content of honey is a good parameter for the assessment of its quality and possible therapeutic potential.

There was significantly higher amount of total flavonoid in the water extract than the methanol extract (Table 1). The amount of total flavonoid was higher than that of the phenolics which ranged from 99.7-119.7 mg/kg. The Ghanaian honey studied had total flavonoid content which were higher than some reported from other countries. The higher total flavonoid content could also be attributed to the plant source from which the pollen grains were taken from for the production of the honey. The plant source might have possessed large deposit of polyphenols in them. Some Algerian honeys had been reported to have total flavonoid content between 27.07-71.78 mg/kg [22], also report made on Turkish honey had total flavonoid content ranged from 4.80 to 22.80 mg/kg [23] and also report on Malaysian honeys indicated total flavonoid content ranging from 11.52–25.31 mg/kg [24]. This is an indication that the studied Ghanaian honey had comparatively higher content of total flavonoid. The high flavonoid content could be a contribution from the wood smoke which also contains some percentage of flavonoids used for the harvesting. Flavonoids are low molecular weight phenolic compounds that are vital components for the aroma and antioxidant properties of honey.

The antioxidant potential of honey has been shown to be directly associated with its phenolic and flavonoid contents [25]. The percentage free scavenging activity of the honey using the DPPH was significantly higher in the methanol extract than in the water extract, thus, 36.3 and 49.3% respectively (Table 1). The antioxidant activity of natural honeys depends largely on their chemical composition, such as phenolics, flavonoids, enzymes, organic acids, amino acids, Maillard reaction products, ascorbic acid, carotenoids, as well as their origins [26,27]. Although the scavenging activity of the honey (36.3-49.3%) was below 50%, this honey still has antioxidant potential greater than honey from most part of the world. The highest percentage of inhibition of the Algerian honey was 44.57% [22] which was lower than the Ghanaian one. The sunflower honey from Turkey had free scavenging activity of 24.647-65.437% [18], the Indian honeys had free radical scavenging activity of 44-71% [19] and pine honey from Turkey recorded 25.65-50.78% [28].

There have been earlier reports about a strong relationship between antiradical capacity and the total phenolic content of the honey [28-31,25]. The relatively high antiradical activity of this honey could be as a result of the presence of higher amounts of total phenolic and flavonoids which were probably might have been obtained from plant sources with higher phytochemicals.

3.2 Antibacterial Activity of the Honey

The different concentrations of honey exhibited varying levels of antibacterial activity against the three different pathogenic indicator strains as shown by the different zones of inhibition (Table 2). The antibacterial potentials of the different concentrations of the honey against the three pathogens increased with increasing concentrations. Pure honey contains alkaloids, glycosides, flavonoids and reducing compounds. The antibacterial properties of honey depend on the release of low lives of hydrogen peroxide and the possession of additional phytochemical antibacterial compounds. The antibacterial potency of honey is also due to its osmotic effect as a result of the high sugar content which inhibit

the bacterial growth [32-34]. The naturally low pH levels obtained, thus, 3.51, 3.59 and 3.85 for the 50%, 75% and 100% honey concentrations respectively were similar to earlier reports on honey [35]. Values between 3.2 and 4.5 as the pH of honey [36,37] had also been reported. The low pH levels could have contributed to the antibacterial potency of the honey against bacteria pathogens [38]. There was antibacterial activity against all the enteric pathogens used. It was clear that the zone of inhibition reduced with dilution of the honey. Honey at 100% concentration produced the maximum activity (13.2 mm) against *Staphylococcus aureus* whilst the minimum activity was exhibited by honey at 50% concentration (2.0 mm) against *Escherichia coli*. The inhibition of bactericidal activity was reduced with reduction in the honey concentration as obtained in other similar studies. It has been reported that the antibacterial analysis of honey at 100% concentration revealed a significant activity against *Escherichia coli* (25 mm) and *Staphylococcus aureus* (20 mm) [38] which were higher than the results obtained in this study. The bactericidal activity slightly reduced with reduction in the honey concentrations (75% and 50%). It has also been reported that honey inhibited the growth of *S. aureus* even at 50% dilution [39]. Honey diluted to concentrations from 75 to 1% (w/v) of full-strength honey showed total antibacterial activity [40]. Similar results have been obtained in other studies on the antibacterial activity of honey against enteric pathogens [36,41-43].

3.3 Physicochemical Properties of the Honey

The pH value of the honey sample was within the acidic range (Table 3) as also reported by [44]. The determination of pH is important in honey in relation to darkening. As the pH value decreases the darkening of honey also increases. They also prevent the honey samples from constant infection by various species of micro-organism and thus help to ensure constant shelf-life. There has been earlier reports of pH values ranging from 0.36 to 3.4 [37] and this study agreed with that. Acidic pH of honey is desirable since acidification has been shown to promote healing by causing oxygen release from hemoglobin [45]. Moisture is one of the most important characteristics of honey, having profound influence on its keeping quality and granulation. The moisture content of the honey sample was 21.01% (Table 3) which conforms to that of the Codex Alimentarius. Honey had been stated not to have moisture content more than 21 percent which causes rapid aerobic growth of honey-fermenting yeasts occurs [46]. Varying reports have been made by researchers on the moisture contents of honey from different parts of the world [47-53].

Total solid is a measure of dissolved solids in the honey samples. A reduction or absence of the total solids in honey (Table 3) is an indicator that further processing has been done on the honey sample. The total solids results obtained are within the acceptable limit [54].

The total soluble solids are a measure of the sugar content present in the honey. The total soluble solids was generally, more than 80% (Table 3) and considered to be of high grade and highly stable upon storage. Similar report was made on honey from Algeria [55]. Thus, the honey investigated in this study is considered to be of high grade and stable with regard to fermentation upon storage. Similar report has been made earlier on some honey samples [53].

The Total Titratable Acidity (TTA) of the honey sample was expressed as percentage lactic acid (Table 3).

3.4 Heavy Metal Content of the Honey

Bee honey can be a good source of major and trace elements needed by humans, where it contains metals up to 0.17%. Metals such as Cr, Co, Cu, Fe, Mn and Zn are essential for humans, and they may play an important role in a number of biochemical processes [56,57]. Some of them are present at the trace level and may become toxic if they exceed safe limits [58].

Iron (Fe) content was the highest followed by Nickel and manganese (Table 4). Even though Fe is essential the level determined in the honey far exceeds the 15 mg/kg limit set by WHO [59].

Nickel (Ni) content was very high which is worrying because large quantity of Ni in the body poses a health threat. The Nickel level in the honey is so far higher compared to other foods documented. Nickel levels in food are generally in the range 0.01-0.1 mg/kg, but there are large variations [60-63]. Markedly higher levels had been reported in beans, seeds, nuts and wheat bran [64,61] and in cacao [64]. The high content of nickel in the honey could be from the plant sources from which the pollen were taken from or

the contamination of the air around the area of honey production. In that case the soil, water and air sources to the plant and bee could be a contribution. The smoke used in the harvesting could cause an increase in the level of the Nickel since wood smoke contains some substantial amount of Nickel.

Cadmium is easily taken up from the environment by plants which could pass through the food chain into the process of honey production by bees and also wood smoke contains some amount of Cadmium. These two reasons could be the reason for the increase content of Cadmium in the honey. Therefore the intake of this honey with the high cadmium content is dangerous to human health and therefore moderate amount of it should be taken in as food. Cadmium (Cd) content in the honey was 0.56 mg/kg which is above the 0.2 mg/kg limit set by CODEX (Table 5). Mercury and lead are known to be toxic to humans even at trace levels. Pb level determined was below detection limit which is below the 0.3 mg/kg set by WHO. The in-take of these heavy metals (Cd, Zn, Cu) in large quantities could pose health risk [65,66].

3.5 Pesticide Residual Content of the Honey

General pesticide contaminants were run for the honey sample. Only *p*, *p*'-DDT was found to be present at insignificantly low level of 1.575 ppb.

Table 1. Total Phenolic, total flavonoid and free radical scavenging activity of honey

Phytochemicals/Antioxidant properties	Water	Methanol
Total phenolic content (mg GAE/g DW)	79.4±2.80[a]	84.5±1.90[a]
Total flavonoid content (mg GAE/kg DW)	119.7±2.10[a]	99.7±1.0[b]
Free scavenging Activity (%)	36.3±0.45[b]	49.3±0.60[a]

Means ± standard deviations in the same row with different superscripts are significantly different ($p < 0.05$)

Table 2. Antibacterial activity between different concentrations of honey samples and pathogenic indicator strains

Concentration (%)	Diameter of clear zone of inhibition in mm		
	Escherichia coli	***Staphylococcus aureus***	***Salmonella typhimurium***
50.00	2.05 ± 0.05[c]	6.00 ± 1.00[b]	3.55 ± 0.45[b]
75.00	7.20 ± 2.20[b]	10.00 ± 2.00[ab]	5.00 ± 0.50[b]
100.00	11.00 ± 2.00[a]	13.20 ± 2.80[a]	9.45 ± 1.45[a]

Means ± standard deviations in the same column with different superscripts are significantly different ($p < 0.05$)

Table 3. Physicochemical properties of the honey

pH	Moisture (%)	Total solids (%)	Totalsoluble solids (% Brix)	Total titratable acidity (Lactic acid %)
3.86±0.005	21.01±0.19	78.99±0.19	10.67±0.47	0.39±0.02

Mean ± standard deviation

Table 4. Heavy metal content of honey

Element	Cu	Fe	Mn	Zn	Pb	Cd	Ni	As	Hg
Quantity (mg/kg dry weight)	0.12	39.20	6.80	0.04	ND	0.52	19.60	ND	ND

ND: Not Detected; the limit of AAS detection is < 0.0001

Table 5. Safe values of some trace elements by Codex Alimentarius Commission

Element	Maximum allowable limits of elements in fruits and vegetables (mg/kg dry weight)
Cd	0.2
Cu	40
Zn	60

The presence of the *p*, *p*'-DDT in the honey could be as a result of the transfer of pollination and/or transfer of nectar from some contaminated plant source to the honey production site. Since the honey is from a wild source the contaminant could have been from the direct application of the chemical pesticides. There has been report of no measurable residues of insecticides in honey [67]. Several investigations conducted on different types of honey and through various analytical methods [68-71] showed, instead, the presence of pyrethrins and pesticides in honey from India [72] and Spain [73] respectively. In another study on honey from Spain and Portugal, residues of 42 different pesticides were examined [74,75] and most of the compounds found were organo chlorines, but most of them were below 0.5 mg/kg. The honey samples studied is safe for consumption in respect to chemical pesticide residue contamination and therefore could be used without fear as food or food supplement.

4. CONCLUSION

The high levels of the total phenolic and total flavonoid contents and higher percentage antiradical activity suggested that the sample has antioxidant potentials. The observed inhibition of the bacterial growth indicates the honey's health benefits in fighting bacterial infections in the body. The physicochemical parameters and pesticide residue studied were within the acceptable limit and therefore the sample has been established to be very safe for consumption. Although there are some heavy metals present in the honey most were within the permissible limits of Codex Alimentarius Commission and FAO/WHO safe limit. Cadmium, Manganese and Nickel levels were above the safe limit and therefore the honey needs to be screened to reduce their level before using it in medicinal and food formulations. Generally, the honeydew honey harvested with woodsmoke has exhibited medicinal/ nutraceutical properties.

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CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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