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## Research Article

# Proximate, Physicochemical and Antimicrobial Analysis of Honey Produced by *Apis mellifera* and *Meliponula ferruginea*

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## Abstract

**Background and Objective:** Honey is a natural sweetener that is used for various applications and widely available for potential treatments and is beneficial to the agricultural sector. Samples of honey produced by *Apis mellifera* (honey bee) and *Meliponula ferruginea* (stingless bee) were collected from Kampani Dorowa farmlands, Niger State, Nigeria.

**Materials and Methods:** The properties of these samples were determined using standard analytical methods. Physicochemical parameter determined includes acid value, saponification value, iodine value, peroxide value, pH value and electrical conductivity.

**Results:** *Meliponula ferruginea* has higher acid, peroxide and saponification values than *A. mellifera*. However *A. mellifera* had higher pH and iodine values than *M. ferruginea*. Both *M. ferruginea* and *A. mellifera* had similar specific gravity and refractive indices. The proximate analysis of the samples revealed that the moisture, ash and protein contents of *M. ferruginea* were higher than those of *A. mellifera*. The phytochemical screening of the samples revealed the presence of alkaloids, flavonoids, glycosides, saponins, steroidal, phlobatannins, terpenes and anthraquinones while tannins were not detected in both samples. The antibacterial screening of honey samples showed that *M. ferruginea* was active against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *C. albican* and honey from *A. mellifera* showed activity against *P. aeruginosa*. **Conclusion:** Honey samples produced by *M. ferruginea* and *A. mellifera* varieties are rich sources of important nutritional, phytochemical of pharmacological significance.

**Key words:** Honey, physicochemical parameters, proximate analysis, phytochemical screening

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Food production for health, growth and the well being of people globally depends on different nutrients used to maintain body structure and supply energy for all essential activities. Thus, the sources of this food are from plants and animals. At present, the dependency of the human population on pollinators such as insect has increased and the fact remains that they are the largest group of species known<sup>1,2</sup>. One of these species is the honey bee, known for its valuable applications in Nigeria.

Several researchers have come up with validated definitions of honey. For instance, Silva *et al.*<sup>3</sup> defined honey as a viscous substance in solution which contains fructose, glucose, water, ash, proteins and amino acids, vitamins, enzymes and phytochemical compounds. The common types of honey bees are *Apis mellifera* (honey bee) and *Meliponula ferruginea* (stingless bee), belong to the family Apidae having different species, colonies and body sizes<sup>4,5</sup>. This natural product is extensively used for numerous applications and contains approximately 200 different chemical compounds<sup>6</sup>.

Honey has primarily contributed to food and medicinal formulations for human well-being and living. It has helped to build and maintain body structure and acts synergistically as a traditional cure for various diseases<sup>7,8</sup>. The populace in rural communities living in abject poverty in most African countries have no contemporary ways but use honey as a source of sustainable resource to address inadequate health care facilities and nutritional diet. Therefore, there is the need for utilization of *Apis mellifera* and *Meliponula ferruginea* in order to explore and utilize their potentials to the communities. The aim of the present study was to analyze honey samples produced by *Apis mellifera* and *Meliponula ferruginea* from Kampani Dorowa farmlands, Niger State, Nigeria for their proximate, physicochemical and antimicrobial potentials.

## MATERIALS AND METHODS

**Materials and equipment:** All the reagents/chemicals were analytical grade chemicals. Deionized water was used throughout the study. Some equipment used includes Abbe Refractometer (Bellingham and Stanley Ltd, 60/70, England), Gallenkamp muffle furnace (size 2, England), Gallenkamp Fenway dry oven (ov 880, England), Ohaus weigh balance (AR 223CN), Jenway meter (3510PH), Gallenkamp Flame Analyzer, Soxhlet extractor and Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer; Analyst 200).

**Sample collection and pre-treatment:** Samples of honey from *Apis mellifera* and *Meliponula ferruginea* were collected from farmlands in Kampani Dorowa along Minna-Suleja road, Niger State, Nigeria in March (honey bee), in June (stingless bee), 2015. The samples were authenticated by the Department of Biological Sciences, Federal University of Technology, Minna and was voucher specimens depended in the Herbarium were sieved (to remove particles) and kept on plastic bottles, labelled and stored in dark room at ambient temperature prior for analysis.

**Physicochemical properties:** Physicochemical analysis of the honey samples from *A. mellifera* and *M. ferruginea* were carried out by using AOAC<sup>9</sup>.

**Proximate analysis:** Proximate analysis of honey samples for moisture, ash, proteins was carried out in triplicate using the method described by Oloyede<sup>10</sup>. The nitrogen was determined by the micro Kjeldahl method described by Oloyede<sup>10</sup> and the nitrogen content will be converted to protein by multiplying by a factor of 6.25.

**Mineral analysis:** Each sample (1.0 g) was digested into digestion tube with 20 cm<sup>3</sup> of a mixture of HNO<sub>3</sub> and HCl at 3:1. This was allowed to stand overnight to allow the initial reaction to subside. The mixture was heated on a hot plate at about 100°C for 2 hrs until the colour became clear. The warm digests were filtered into 100 cm<sup>3</sup> volumetric flasks and diluted to mark with distilled water. The digested samples were stored in plastic sample bottles for metal analysis. Metal concentrations were determined using flame atomic absorption spectrophotometer and atomic absorption spectrophotometer (AAS).

**Phytochemical screening of the honey samples:** Phytochemical screening procedures as reported by Oloyede<sup>10</sup> was adopted to determine the presence or absence of tannins, phlobatannins, flavonoids, terpenoids, glycosides, saponins, alkaloids and anthraquinones.

**Antimicrobial activity of honey samples:** The antimicrobial activity was determined using the agar well diffusion method<sup>11</sup>. Nutrient agar was used to study antimicrobial susceptibility while Sabouraud dextrose agar was used for antifungal susceptibility test.

**Standardization of organism:** The organisms used in the test were inoculated into the nutrient broth (bacteria) and yeast extract broth (fungi) for 24 and 72 hrs, respectively. The culture was equally sub-cultured into the fresh broth and incubated for 2-3 hrs.

**Reconstitution:** Each honey sample was reconstituted in sterile distilled water to give concentrations ranging from 40-100% (v/v).

**Susceptibility test:** The Agar well-diffusion method was used. The wells were bored using a cork borer on the agar and swab stick was used to inoculate the 3 hrs old culture on the agar. Wells were filled up with a different concentration of honey and incubated at 37°C for 24 hrs (bacteria) and 72 hrs (fungi) and zones of inhibition were recorded using a transparent plastic ruler.

**Minimum inhibition concentration (MIC):** The minimum inhibitory concentrations from the honey samples that displayed activity were determined by recording the lowest concentration that inhibited the activity of the organisms using tube diffusion method.

**Minimum bactericidal concentration (MBC):** This is the lowest concentration of the honey that was able to kill the bacteria and fungi after incubation. The minimum bactericidal concentration was determined by streaking out the tubes from minimum inhibition concentration (MIC) and a tube shows no visible growth is regarded as bactericidal concentration (MBC).

## RESULTS AND DISCUSSION

**Physicochemical properties:** The results obtained from the physicochemical properties of the honey samples from *M. ferruginea* and *A. melliponula*, are shown in Table 1. The samples were light yellow and dark brown in colour for *A. mellifera* and *M. ferruginea*, respectively. Both were in a liquid state has a sweet and aromatic odour. The honey samples were liquid at ambient temperature.

The physicochemical properties of the samples of honey were determined and the results are given in Table 1.

The acid value which measures the amount of free fatty acids present in honey depends on the degree of its rancidity. Therefore, total acid value and free fatty acids are important parameters used to indicate the quality, edibility and suitability of honey for a specific resolution. The acid values

Table 1: Physicochemical properties of the honey produced by *Apis mellifera* and *Meliponula ferruginea*

Parameters	Samples	
	<i>Meliponula ferruginea</i>	<i>Apis mellifera</i>
Acid value (mg NaOH g <sup>-1</sup> )	77.42	62.73
Saponification value (mg KOH g <sup>-1</sup> )	353.43	207.54
Peroxide value (meq O <sub>2</sub> kg <sup>-1</sup> )	0.38	0.20
Iodine value (mg I <sub>2</sub> /100 g)	16.12	21.17
pH value	3.64	4.62
Specific gravity (g cm <sup>-3</sup> )	1.01	0.79
Colour	Dark brown	Light yellow
Odour	Aromatic	Aromatic
Refractive index (25°C)	1.49	1.46
Electrical conductivity (μS cm <sup>-3</sup> )	16.01	17.23
State	Liquid	Liquid

obtained were 77.42 and 62.73 mg KOH g<sup>-1</sup> for *M. ferruginea* and *A. mellifera* samples respectively. The acidity value for *A. mellifera* honey was below the maximum limits of 78.54 meq kg<sup>-1</sup> set internationally for honey<sup>12</sup> and both samples were within the range of 57.36-79.42 mg KOH g<sup>-1</sup> reported for Nigerian honey<sup>13</sup>. This important parameter of these honey samples will help to contribute to their stabilities against microorganism, enzymes and other physical factors such as light and heat

The saponification value was used to check adulteration of samples. The saponification value recorded was 353.43 and 207.54 mg KOH g<sup>-1</sup> for *M. ferruginea* and *A. mellifera* honey, respectively. The obtained results were similar to the finding of Shahidi<sup>14</sup> who worked on honey bee from the North Central Area in Nigeria. This value to 376.25 mg KOH g<sup>-1</sup> for North Central bee honey<sup>14</sup>. This suggested that the honey could be used industrially either for soap making or as a blend for other industrial formulations.

The peroxide value measures the occurrence of rancidity reactions in samples. The recorded peroxide value was 0.38 meq O<sub>2</sub> kg<sup>-1</sup> for *M. ferruginea* and 0.47 meq O<sub>2</sub> kg<sup>-1</sup> for *Apis mellifera*. The recorded low peroxide values indicated that the honey samples have resistance to lipolytic hydrolysis oxidative deterioration<sup>15</sup>. Hence, this confirms the stability of the honey samples.

The iodine value of honey measures the presence of unsaturation compounds and also give its insight into oxidative stability. *Meliponula ferruginea* had iodine values 16.12 mg I<sub>2</sub> g<sup>-1</sup> and *A. mellifera* had 21.17 mg I<sub>2</sub> g<sup>-1</sup>. This work is comparable to those reported as 19.53-26.10 mg I<sub>2</sub> g<sup>-1</sup> by Shahidi<sup>14</sup>.

The values of specific gravity for *M. ferruginea* and *A. mellifera* honey were similar to *Trigonas carbonaria* recorded by Schmidt<sup>16</sup>. The refractive index of honey indicates the level of optical clarity of the sample relative to

Table 2: Proximate composition of the honey sample produced by *Apis mellifera* and *Meliponula ferruginea*

Parameters	Samples (mg/100 g)	
	<i>Meliponula ferruginea</i>	<i>Apis mellifera</i>
Moisture	23.17	12.05
Ash	2.38	0.28
Protein	29.06	3.09
Free fatty acid (FFA)	38.70	3.39
Lipids	0.39	0.38
Carbohydrate	17.96	80.83

Table 3: Mineral composition of the honey sample produced by honeybee (*Apis mellifera*) and stingless bee (*Meliponula ferruginea*) (mg/100 g)

Parameters	Honey sample		
	<i>Meliponula ferruginea</i>	<i>Apis mellifera</i>	FAO/WHO
Sodium	0.06	0.03	0-4 mg
Potassium	0.20	0.10	1-52
Calcium	0.01	0.01	0-6
Manganese	0.21	0.02	0-2
Iron	0.20	0.02	0-3
Copper	0.01	0.10	0-2

FAO: Food and agricultural organization, WHO: World health organization<sup>18</sup>, Source: Codex alimentarius commission<sup>27</sup>

water. The refractive indices of 1.49 and 1.46 were obtained for *M. ferruginea* and *A. mellifera*, respectively. Similar index values were obtained to be within the values of US set standard, 1.89 at ambient temperature<sup>12</sup>. Electrical conductivity measures organic and inorganic substance present in honey. The electrical conductivity values of the honey sample were 16.01  $\mu\text{S cm}^{-1}$  for *M. ferruginea* and 17.23  $\mu\text{S cm}^{-1}$  for *A. mellifera*. This study showed values similar to those reported on Nigeria honey from South-West<sup>17</sup> and were found to be within the standard values of 17.42-18.25  $23 \mu\text{S cm}^{-1}$  of WHO<sup>18</sup>. The low pH values of the honey samples will help to inhibit the presence and growth of microorganisms which could influence their stability, texture and shelf life<sup>19</sup>.

**Proximate compositions:** The results of the proximate compositions of honey samples *Meliponula ferruginea* and *Apis mellifera* are presented in Table 2. The moisture content, ash, protein, free fatty acids and carbohydrates were found to be 23.17, 2.38, 29.06, 38.70 and 17.96 mg/100 g for *Meliponula ferruginea* while it was (12.05, 0.28, 3.09) for *Apis mellifera*.

The moisture contents of *M. ferruginea* and *A. mellifera* were generally low as 23.17 and 12.05 mg/100 g, respectively. The result obtained agreed with the range of 12.50-25.22 mg/100 g reported for honey bee from North-South of Nigeria<sup>20</sup>. The relatively higher moisture contents from *M. ferruginea* in this work is an indication that

both honey sample could be preserved for a useable period of time without the risk of microbial deterioration and spoilage. Long shelf life stressed as an added advantage over other sources of protein example, beef, fish and eggs which are prone to spoilage where proper care is not administered.

The ash content provides a measure of the total amount of minerals in food substance. Ash contents of *M. ferruginea* and *A. mellifera* were 2.38 and 0.28 mg/100 g, respectively. Similar ash content was reported for *M. mellifera* (0.140 and 0.78 mg/100 g) by Ayansola and Banjo<sup>21</sup>. The ash content obtained from *M. ferruginea* was higher than the 0.55-0.79 mg/100 g reported for *Trigonas carbonaria* by Odeyemi *et al.*<sup>22</sup>. The ash contents of *M. ferruginea* was also higher than 0.1-0.50 mg/100 g reported for *A. mellifera* and also reported by Buba *et al.*<sup>23</sup> from North East of Nigeria. Thus, honey in this study could serve as a good dietary source of supplements which will be useful particularly for children and pregnant women.

The crude proteins values of 29.06 and 3.09 mg/100 g were recorded for *M. ferruginea* and *A. mellifera*, respectively hereby value obtained from *M. ferruginea* was higher than 1.43-2.72 mg/100 g reported for *A. mellifera* from three states in Nigeria by Abu-Tarboush *et al.*<sup>1</sup>. Thus, the high level of proteins in *M. ferruginea* showed a clear indication that it can contribute significantly to the daily proteins requirements from 30-56 g for humans<sup>24</sup>. This could serve as a good source of protein for growing children, nursing mothers as well as those at risk of protein deficiency diseases.

Fats are vital in the structural and functioning of the cells and help in the transport of nutritionally essential fat-soluble vitamins<sup>25</sup>. *Meliponula ferruginea* had 0.39 mg/100 g while *A. mellifera* had 0.38 mg/100 g which is conformity with set standard between 0.4 and 0.32 mg/100 g<sup>26</sup>.

Carbohydrate absorbed in the body system raised the level of blood glucose which is essential in the conversion of food energy. The amounts of carbohydrates obtained from the honey samples in this study were 17.96 and 80.83 mg/100 g for *M. ferruginea* and *A. mellifera*, respectively. The value for *M. ferruginea* however, was lower than the value reported for *A. mellifera* by Buba *et al.*<sup>23</sup>.

**Mineral composition:** The mineral composition of the honey sample was as presented in Table 3. The sodium, potassium, calcium, manganese, iron and copper were found to be 0.06, 0.20, 0.01, 0.21, 0.20 and 0.01 mg/100 g for *Meliponula ferruginea* and 0.03, 0.10, 0.01, 0.02, 0.02 and 0.10 mg/100 g for *A. mellifera*, respectively.

Table 4: Phytochemical screening of honey produced by *Apis mellifera* and *Meliponula ferruginea*

Constituents	Honey samples	
	<i>Apis mellifera</i>	<i>Meliponula ferruginea</i>
Alkaloids	+	++
Flavonoids	+++	+++
Phlobatannins	+++	+++
Saponins	+++	+++
Phenols	+	++
Glycosides	+	+
Terpenes	+++	+++
Tannins	-	-
Steroidal compound	+++	+++
Anthraquinones	-	+++

+++ : Highly present, ++ : Moderately present, + : Slightly present, - : Nil

Sodium is very active and chemically combines with many substances for the regulation of osmolarity and in balancing body fluids within the body cells<sup>28,29</sup>. The sodium contents for *M. ferruginea* and *A. mellifera* were 0.6 and 0.03 mg/100 g, respectively. These value obtained were lower than 1.02 mg/100 g reported for *A. mellifera* by Ibrahim<sup>24</sup> and higher than 0.01 mg/100 g for *Eucalyptus* honey by Cotton *et al.*<sup>29</sup>. Potassium is needed in the conversion of glucose into glucogen and maintaining normal blood pressure<sup>28</sup>. The potassium contents were 0.20 and 0.10 mg/100 g for *M. ferruginea* and *A. mellifera* respectively. These value obtained were within the range of 0.30-0.34 mg/100 g for *A. mellifera* reported by Ibrahim<sup>24</sup>. The presence of this mineral could enhance protection of the body system against diabetes, obesity, heart diseases and kidney dysfunction. Calcium is an essential ingredient for bone development. The calcium contents of *M. ferruginea* and *A. mellifera* were 0.01 and 0.01 mg/100 g, respectively. The similar result was obtained for *A. mellifera* 0.01 and 0.01 mg/100 g reported by Buba *et al.*<sup>23</sup>.

The manganese contents of *M. ferruginea* and *A. mellifera* were 0.21 and 0.02 mg/100 g, respectively. The concentration of Mn recorded in this work for *M. ferruginea* was higher than reported for *A. mellifera* from Northwest of Nigeria by Buba *et al.*<sup>23</sup>. *Meliponula ferruginea* had to value of 0.20 mg/100 g and *A. mellifera* had 0.02 mg/100 g respectively. The level of iron in *M. ferruginea* and *A. mellifera* were 0.20 and 0.02 mg/100 g, respectively. The contents of iron in honey from *M. ferruginea* and *A. mellifera* are comparable to those reported by Adebisi *et al.*<sup>17</sup>. The concentration levels of copper in *M. ferruginea* and *A. mellifera* were 0.01 and 0.10 mg/100 g, respectively, the values were lower than values of 0.31 and 0.20 mg/100 g reported by Buba *et al.*<sup>23</sup>. The adequate presence of these minerals would stimulate the body immune system.

**Phytochemical constituents:** Phytochemical screening of honey from *A. mellifera* and *M. ferruginea* presented in Table 4 revealed the presence of alkaloids, flavonoids, saponins, steroids, cardiac glycosides, anthraquinones and terpenes in the honey samples. The alkaloids and phenols were moderately present in *M. ferruginea*, whereas they were only slightly present in *A. mellifera*. The presence of other phytochemicals such as flavonoids, saponins, terpenes and steroids were significantly present in both honey sample except glycosides that were fairly present while anthraquinones were present in greater amount in *M. ferruginea*, but completely absent in *A. mellifera*. These bioactive compounds of the honey samples have nutritional and medicinal properties, if traditionally been consumed directly could be used as anti-analgesic anti-cancer, anti-malarial, anti-inflammatory, anti-diuretic, anti-bacterial, anti-viral and anti-fungal.

**Antimicrobial activity:** The results of the antimicrobial study of honey samples from *A. mellifera* and *M. ferruginea* honey are presented in Table 5. The samples showed inhibitory activity against (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Candida albicans*). Honey from *M. ferruginea* was more effective against *S. aureus*, *E. coli* and *S. typhi* at higher concentration (50-100% v/v) with zone diameter of inhibition ranging from (11-26 mm) comparing to *A. mellifera* whose activity were much higher at 60-100% v/v with diameter of zone ranging from 13-26 mm. The results in this study showed *M. ferruginea* and *A. mellifera* were reactive to *K. pneumoniae* at concentration of 80% v/v with diameter ranging from 14-18 mm and the standard drug used (Bechan ampiclox) had activity against pathogens at 100% v/v concentration with zone diameter of inhibition ranging from 26-39 mm.

The good diffusion studied shows honey samples can be used for the treatment of diseases caused by *S. aureus*, *E. coli*, *K. pneumoniae*, *S. styphi*, *P. aeuruginosa* and *C. albicans*. A similar work had shown that honey has been used to heal disruptive wounds whereby, it was observed to be effective *in vitro* against a wide range of multi-resistant organism such as methicillin resistant *Staphylococcus aureus* (MRSA) and multi-resistant *Pseudomonas aeruginosa* by George and Cutting<sup>30</sup>. The present study is also in agreement with the work by Nzeako and Hamdi<sup>31</sup>. The report by Ashenafi<sup>32</sup> revealed that honey produced by *Apis mellifera* was effective against some food burn pathogens in human.

Table 5: Antimicrobial activity of honey samples from *Apis mellifera* and *Meliponula ferruginea* (mg cm<sup>-3</sup>)

Test organisms	<i>Meliponula ferruginea</i>							<i>Apis mellifera</i>							
	40	50	60	70	80	90	100	40	50	60	70	80	90	100	AMPX
<i>Pseudomonas aeruginosa</i>	-	-	-	16	21	21	28	-	-	13	17	18	23	26	36
<i>Klebsiella pneumoniae</i>	-	-	-	-	14	16	20	-	-	-	-	18	17	21	31
<i>Escherichia coli</i>	-	-	11	13	13	18	22	-	-	-	14	17	19	24	27
<i>Salmonella typhi</i>	-	-	-	-	-	16	19	-	-	-	-	17	18	20	36
<i>Staphylococcus aureus</i>	-	10	12	16	16	18	27	-	-	-	18	16	19	26	29
<i>Candida albicans</i>	-	-	-	-	18	21	26	-	-	-	-	-	16	20	29

Table 6: Minimum inhibition concentration (MIC) of honey from *Apis mellifera* and *Meliponula ferruginea* on the test organisms (mg cm<sup>-3</sup>)

Test organisms	Samples	
	<i>Meliponula ferruginea</i>	<i>Apis mellifera</i>
<i>Pseudomonas aeruginosa</i>	70	60
<i>Klebsiella pneumoniae</i>	80	80
<i>Escherichia coli</i>	60	70
<i>Salmonella typhi</i>	90	80
<i>Staphylococcus aureus</i>	50	70
<i>Candida albicans</i>	80	90

Table 7: Minimum bactericidal concentration (MIC) of honey produced by *Apis mellifera* and *Meliponula ferruginea* (mg cm<sup>-3</sup>)

Test organisms	Samples	
	<i>Meliponula ferruginea</i>	<i>Apis mellifera</i>
<i>Pseudomonas aeruginosa</i>	80	70
<i>Klebsiella pneumoniae</i>	80	80
<i>Escherichia coli</i>	60	80
<i>Salmonella typhi</i>	90	90
<i>Staphylococcus aureus</i>	60	80
<i>Candida albicans</i>	90	100

Minimum inhibitory concentration presented in Table 6 showed honey from *M. ferruginea* was active against *Staphylococcus aureus* (50%, 10 mm), *Escherichia coli* (60%, 11 mm) and *Candida albicans* (80%, 18 mm), while honey from *Apis mellifera* was active against *Pseudomonas aeruginosa* (60%, 13 mm), *Salmonella typhi* (80%, 17 mm), *Klebsiella pneumoniae* (80%, 14 and 18 mm) for both *M. ferruginea* and *A. mellifera* respectively. Similarly, *Bechan ampiclox* as controlled standard showed growth of bacteria and fungi at 100%, 39 mm.

Minimum bactericidal concentration (MBC) of honey from *M. ferruginea* and *A. mellifera* were presented in Table 7, where *M. ferruginea* showed bactericidal activity against *Staphylococcus aureus* (60%, 12 mm), *Escherichia coli* (60%, 11 mm) while *Pseudomonas pneumoniae* (70%, 17 mm) for *A. mellifera* compare to standard drug used at 100%, 36 mm. The study is in conformity with the report by Molan<sup>33</sup>. Thus, the variation in the antimicrobial potential of honey samples used in the present work as compared to previous studies disclosed that source of the nectar may have contributed to the differences in the antimicrobial activity of the honey

samples produced from *M. ferruginea* and *A. mellifera*. The variation may also be due to differences in the nutritional requirement, temperature and inoculum sizes<sup>34</sup>. The presence of antimicrobial substances as demonstrated by zones of inhibition showed comparative efficacy of *Meliponula ferruginea* and *Apis mellifera* as a medicinal source against various types of bacteria, fungi and viruses.

## CONCLUSION

The phytochemical study has shown the presence of chemical constituents, including alkaloids, flavonoids, steroids, glycosides, saponins, phlobatannins, anthraquinone and terpenes. The presence of bio-constituent support the traditional use of honey plays in preventing various diseases. The honey could be a source of phytochemical constituents which are useful as an antibiotic substitute for synthetic antibiotics. The phytochemical analysis of samples from *A. mellifera* and *M. ferruginea* is important and has a commercial interest both in research institutes and pharmaceutical for the manufacturing of the new drugs for the treatment of different infections. The proximate composition showed high contents of proteins, moisture, total acidity and carbohydrate. Honey samples exhibit good physicochemical properties and therefore have prospective to be developed either for food, chemical and pharmaceutical industries. The study also provides essential minerals such as sodium, iron, copper, potassium, manganese and calcium. The information provided in this study makes a clear evaluation that the honey samples are good dietary sources for human and animal feeds formulations.

## SIGNIFICANCE STATEMENT

This study discovers the honey that can be beneficial for human and animal consumption. This study will help the researcher to uncover the critical areas of phytochemical, proximate and antimicrobial studies of *A. mellifera* and *M. ferruginea* that many researchers were not able to explore. This study discovers the possible synergistic effect responsible

for their nutritional properties, which supports the relevance of these types of honey being important dietary sources of phytochemical compounds and its traditional use as a medicinal product.

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