Physico-Chemical, Microbiological and Antibacterial Properties of *Apis mellipodae* and *Trigona spp.* Honey Against Bacterial Pathogens

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**Abstract:** In Ethiopia, honey of *Trigona* sp. is widely used for traditional treatment for various diseases. Therefore, there is a need to study the antimicrobial activity, physico-chemical and microbiological characteristics of this honey. The main objective was to investigate the antibacterial, microbiological and physico-chemical properties of *Trigona* sp. honey. The antimicrobial activity, minimum inhibitory concentration and minimum bactericidal concentration of samples were determined using standard methods. Determination of microbiological and physico-chemical characteristics of samples was carried out. Antimicrobial activity of tenegn honey (16±2.12 mm) against pathogenic bacteria was significantly (P < 0.05) greater than artificial honey (11.11±2.31 mm) and honeybee honey (11.11±2.31). MIC of tenegn honey was 6.25% against all test organisms, while honeybee honey and artificial honey were ranged from 6.25-12.5% and 12.5-25%, respectively. A minimum bactericidal concentration of tenegn against all tested pathogenic bacteria was low concentration than other samples. The pH of tenegn honey (2.87±0.01) was statistically (P < 0.05) less (3.8±0.02) than honeybee honey but the reverse is true in terms of moisture content. The electrical conductivity values of the tenegn honey (3.27±0.01 mS/cm) was significantly (P < 0.05) greater than honeybee honey (0.488±0.01 mS/cm). The values of free acidity, lactone and total acid of all samples were within the permissible limit in the literature. Low total aerobic mesophilic bacteria counts (1.54 x 10^5 cfu/g) were found in tenegn honey than honey from honeybee (excessive colony counts). Antimicrobial activity of tenegn honey against tested pathogens was greater than other honeys. This was may be due to chemical composition, production process and low pH of tenegn honey than honeybee honey. This might be the reason that local society preferably uses tenegn honey than other honeys for disease treatment.

**Kew words:** Antimicrobial activity • *Apis mellipodae* • Microbiological characteristics • Physico-chemical characteristics • *Trigona sp.*

**INTRODUCTION**

Honey from honeybees (*Apis mellifera*) has been used as a traditional medicine widely used for treatment of several human respiratory ailments, gastrointestinal infection and various other diseases starting the origin of mankind [1]. The broad-spectrum antibacterial activity of honey against pathogenic bacteria was recently reported [2, 3]. Their antibacterial activity varies with geographical origin, botanical source, harvesting, processes and storage conditions [4]. Moreover the antimicrobial activity of honey is largely associated with pH, hydrogen peroxide production, osmolarity and the availability of different phytochemical compounds like methylglyoxal [5].

The average composition of natural honey produced by honeybee (*A. mellifera*) was reported in percentage from 490 honey samples collected in United State of America [6]. In this report, the amount of moisture content, fructose, glucose, sucrose, disaccharides calculated as maltose, higher sugars, free acid as gluconic, lactose as glucono lactone, total acid as gluconic, ash and nitrogen were 17.2%, 38.19%, 31.2%, 1.31%, 7.31%, 1.5%, 0.43%, 0.14%, 0.57%, 0.169% and 0.041%, respectively (White, et al. 1962). Artificial honey can be prepared from...
sucre, maltose, fructose and glucose by dissolving with distilled water with respective of their standard composition in honey [7]. Artificial honey is important to compare and contrast the antimicrobial activity of honey produced by Apis mellifera and Trigona sp.

Trigona sp. is well known members of stingless bees. All African and Asian species of stingless bees are member of the tribe Trigonini [8]. Trigona is well known most widely distributed and largest genus, which accounts for 130 species and further grouped into ten subgenera. They are highly social insects and the smallest (in size) of the honey producing bees that living in permanent colonies inside termite mounds, nesting in old walls, logs, crevices, hollows in tree trunks and other places [8]. The cavity diameter varied with the type of nesting sites. Most of colonies of Trigona construct a resinous entrance tube. Inside the colony, brood cells and food pots were arranged separately. Food pots size were larger than the size of brood cells and were properly sealed when filled. Brood cells were compactly arranged in clusters. Larval cells were brown whereas pupal cells were creamy in colour [9]. The entrance of the colony was made up of resin and the newly built entrance was soft, later turned darker and became rigid due to maturation. The food storage zone was classified into honey zone and pollen zone. The pollen and honey were stored in separate pots, however, these pots were usually intermixed.

As mentioned above, antimicrobial activity of honey produced by Apis mellifera has widely been investigated [1, 10-12]. However, still there is no information available in the scientific literature on physico-chemical, microbiological and antibacterial characteristics on the honey produced by Trigona sp. Of course, there was a report on antimicrobial activity of propolis produced by Trigona sp. but not the honey [13]. Therefore, there is a need to study the physic-chemical and microbiological characteristics of Trigona sp. honey in comparison with honey produced by honeybee. On the other hand, the physic-chemical characteristics of honey from different sources have reported by many scientists [14-16].

In Ethiopia, honey produced by Trigona sp. is widely used for traditional treatment, such as respiratory ailments, surface infections and other diseases in line with treatments conducted using honeybee honey. Trigona sp. honey might be effective to treat different infectious disease with low concentrations. The main objective of this study was to investigate the antibacterial activity, microbiological and physico-chemical properties of Trigona sp. honey in comparison with honey produced by honeybee. The data generated in this study may help as baseline for empirical treatment for the various types of infectious diseases caused by pathogenic microorganisms.

**MATERIALS AND METHODS**

**Collection and Preparation of Honey Samples:** Honeybee honey and Trigona sp. were obtained from North Gondar, particularly Laye Armhcheho. Each honey sample was first purified and filtered with a sterile cloth mesh to remove fragment of propolis. Hundred gram of artificial honey was prepared from 1.5 g sucrose, 7.5 g maltose, 40.5 g fructose and 33.5 g glucose dissolved in 17 ml of sterilized and deionized water. This solution represents the proportions of the four well known predominant sugars in honey samples [17]. All samples were kept at 8°C in the dark. Solutions of honeys were prepared by 50% (v/v). Each sample of honey was diluted by sterilized water to give final concentrations of 50%, 25%, 12.5% and 6.25% and then kept at 0°C for further investigation.

**Bacterial Cultures:** All standard bacterial cultures, such as *L. monocyctogenes* (ATCC 19116), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Shigella flexneri* (ATCC 12022), *Proteus vulgaris* (ATCC 881), *Salmonella NCTC 8385* and *Streptococcus pneumonia* (ATCC 63) were obtained from Ethiopia Health and Nutrition Research Institute (EHNRI), while *Shigella dysenteriae* (clinical isolate) and *Salmonella typhi* (clinical isolate) were obtained from University of Gondar, teaching hospital. Most of them are commonly involved in causing pneumonia, gastroenteritis, urinary tract and wound infections.

**Antimicrobial Activity:** The antimicrobial activity of samples was studied by using agar well diffusion technique [18]. Standard and clinical strains of pathogenic bacteria were inoculated into 5 ml of sterile tryptocase soya broth and incubated at 37 °C for 24 hours. The cultures were subcultured on the surface of sterile Muller Hinton agar plates using sterile wire loop. From 2-3 colonies were peaked up by wire loop aseptically into sterile saline solution and the turbidity was adjusted to be equal with 0.5 Mcfarland standard solution (a concentration of 10^5 to 10^6 bacteria/ml) [19]. From the inoculated saline solution, test bacteria were swabbed on the surface of sterile Muller Hinton agar plates using a sterile cotton swab. Agar wells on the medium were prepared using sterilized cork borer with 6 mm diameter (17 shenkurt). By the help of micropipette,
100µl of different honey samples with the concentration of 50% were separately added to the wells in the plate. The plates were incubated at 37 °C for 24 h. Zones of the inhibition were measured by clipper in mm and the results were recorded. The inhibitions zones of samples with less than 12 mm (in diameter) were considered as having weak or no antibacterial activity.

**Determination of Minimum Inhibitory Concentration (MIC):** The determination of the minimum inhibitory concentration by the dilution solutions at the dose levels of 6.25, 12.5 and 25% was carried out by the method as reported by Malika et al. [20]. To each dilution of honey in nutrient broth tubes were seeded with 100 µl of the standard and clinical bacterial inoculum. Negative control tubes with no bacterial inoculation were simultaneously maintained. Tubes were incubated aerobically at 37°C for 24 h. The MIC was defined and considered as the lowest amount of concentration of honey which completely inhibits the growth of test organisms.

**Minimum Bactericidal Concentration (MBC):** The minimum bactericidal concentration of the honey on the standard and clinical bacterial isolates was done according to the method described in National Committee for Clinical Laboratory Standard [21]. Briefly, 1 ml was pipetted from the test sample obtained during the determination of MIC was streaked and incubated on the nutrient broth for 24 h. The least concentration of the honey with no growth was taken as the minimum bactericidal concentration [21].

**Determination of Microbiological Profiles in Honey Samples:** From each sample, 10 g was mixed with 90 ml of saline water (0.85 %) to prepare the initial dilution. This was used as stock solution for further serial dilutions. From the serial dilutions of 10−1 in saline water, 100 µl was spread on each standard plate count agar. The plates were incubated at 30°C for 48 h. For aerobic endospore bacteria counts, the initial dilution was activated by heat at 80°C for 10 minutes and cooled immediately using ice. Aerobic spore forming bacteria were cultured using plate count agar. The plates were incubated at 30°C for 48 h. Total coliforms were cultured on deoxycholate citrate lactose agar (APHA) and incubated at 37°C for 24 h. Media used for the culture of yeasts and moulds were supplemented with chloramphenicol (100 mg/l). Count of yeasts was carried out using surface plating dilutions on potato dextrose agar and incubating at 25°C for 72 h. Moulds were enumerated on Sabouraud agar (Pynamicro). The plates were incubated at 25°C for 7 days. Microbial counts were stated as colony forming units per gram of honey (cfu/g).

**Determination of Physico-chemical Characteristics of Honey:** The pH of honey samples were measured by pH-meter (JENWAY 4330) in a solution containing 10 g of honey in 75 ml of distilled water [22]. The lactonic, free and total acidity of honey samples were calculated using the titrimetric method. Ten grams of honey were weighed in a glass beaker and then 75 ml of deionized water were added. This solution was titrated with 0.05M NaOH until reaching pH 8.5 (free acidity) and measured with a pH-meter. Then 10 ml of 0.05M NaOH was added and titrated again with 0.05 M HCl until reaching pH 8.30 (lactonic acidity). Total acidity was obtained by adding free plus lactone acidities. Results were expressed as meq/kg [22]. The determination of moisture [22] was carried out by refractometry, using an Abbe refractometer (ABBE 60/DR). All measurements were performed at 22 °C, after waiting for 6 min for equilibrium and obtaining the corresponding percentage moisture (g/100 g sample) from the value of the refractive index of the honey sample using standard table designed for this purpose. Ash content was determined by ignition at 550°C in a furnace (Stuart, Bibby UK) to constant mass [22]. Five g of each honey sample was taken in a platinum dish and kept at 80°C for 4 h, after which the samples underwent calculations at 550°C in an electric laboratory furnace to the constant mass and lastly % of mass was calculated.

**Data Analysis:** The data were analyzed using SPSS version 16.0. Means and standard deviations of the triplicate analysis were calculated using two way analysis of variance (ANOVA) to determine the significance differences among variables (p ≤ 0.05) when the F-test demonstrated significance. The statistically significant difference was defined as p ≤ 0.05.

**RESULT AND DISCUSSION**

**Antimicrobial Activity of Different Honeys:** Antimicrobial activity of honey produced by tenegn (Trigona sp.) and honeybees (A. mellifera) was shown on Fig. 1. Antimicrobial activity of tenegn honey (16±2.12 mm) against pathogenic bacteria was significantly (P ≤ 0.05) greater than artificial honey (11.1±2.12 mm) and honeybee honey (11.1±2.31). The mean inhibition zone
Fig. 1: Antibacterial activity of different concentrations of Tenegn honey, honeybee honey and artificial honey (v/v) by agar well diffusion method. Values are means of triplicate determinations; Values within different colour of bars followed by different letters are significantly different at (p ≤ 0.05).

Fig. 2: Minimum inhibitory concentrations (MIC) of honey produced by honeybees (Apis mellifera), Tenegn honey and artificial honey using dilution solutions at the dose levels of 6.25, 12.5 and 25%. Values are means of triplicate determinations; Values within different colour of bars followed by different letters are significantly different at (p ≤ 0.05).

The antibacterial activity of Tenegn honey was ranged from 12 to 20 mm in diameter, while that of honeybee honey and artificial honey were both ranged from 7 to 14 mm. Antimicrobial activity of Tenegn honey was statistically (P ≤ 0.05) higher against S. flexneri (ATCC 12022) than other pathogens. Generally, antimicrobial activity of Tenegn honey against tested pathogens was greater than other honeys. This might be the reason that local society preferably uses Tenegn honey to treat patients infected with pathogenic bacteria. Its antimicrobial activity may not only depend on the osmotic effect of its high sugar content but other compounds originated from plants or production process of Trigona sp.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Honeys: Minimum inhibitory concentrations (MIC) of honey produced by Tenegn (Trigona sp.) honey, honeybees (A. mellifera) and artificial honey was shown on Fig. 2. MIC of Tenegn honey was 6.25% against all test organisms,
Table 1: Minimum bactericidal concentrations (MBC) of honey produced by honeybees (*Apis mellifera*) and tenegn (*Trigona sp.*) using dilution solutions at the dose levels of 6.25, 12.5 and 25%

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Tenegn honey</th>
<th>Honeybee honey</th>
<th>Artificial honey</th>
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<tbody>
<tr>
<td><em>E. coli</em> (ATCC 25922)</td>
<td>12.5</td>
<td>12.5</td>
<td>25.0</td>
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<tr>
<td><em>S. typhi</em> (clinical isolate)</td>
<td>6.25</td>
<td>25.0</td>
<td>25.0</td>
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<tr>
<td><em>L. monocytogenes</em> (ATCC 19116)</td>
<td>12.5</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td><em>S. aureus</em> (ATCC 25923)</td>
<td>6.25</td>
<td>6.25</td>
<td>25.0</td>
</tr>
<tr>
<td><em>S. flexneri</em> (ATCC 12022)</td>
<td>12.5</td>
<td>12.5</td>
<td>25.0</td>
</tr>
<tr>
<td><em>P. vulgaris</em> (ATCC 881)</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td><em>S. dysenteriae</em> (clinical isolate)</td>
<td>12.5</td>
<td>12.5</td>
<td>25.0</td>
</tr>
<tr>
<td><em>Salmonella</em> NCTC 8385</td>
<td>12.5</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td><em>S. pneumonia</em> (ATCC 63)</td>
<td>6.25</td>
<td>25.0</td>
<td>25.0</td>
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</tbody>
</table>

Table 2: Psycho-chemical characteristics of honey

<table>
<thead>
<tr>
<th></th>
<th>Honey</th>
<th>Tenegn Honey</th>
<th>Codex draft, 2001</th>
<th>European Commission, 2002</th>
<th>ANONN, 2001-2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.8±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.87±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>3.4-6.1</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>18.5±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;21</td>
<td>&lt;21</td>
<td>13.4-26.6</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.2±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>0.020-0.028</td>
</tr>
<tr>
<td>Electrical conductivity (mS/cm)</td>
<td>0.488±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.27±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Free acidity (meq Kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>6.25±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>-</td>
</tr>
<tr>
<td>Lactone (meq Kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.23±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.75±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total acidity (meq Kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>6.48±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.75±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are means of triplicate determinations; Values within the same raw followed by different letters are significantly different at (p < 0.05).

while honeybee honey and artificial honey were ranged from 6.25-12.5% and 12.5-25%, respectively to all tested bacteria. Tenegn honey and honeybee honey were collected from the same area. However, MIC of tenegn honey (6.25%) against tested organisms was significantly (P ≤ 0.05) lower than honeybee honey (6.25-12.5%). The reason for this was may be due to species variation of producing insect, chemical composition and production process.

Minimum bactericidal concentrations (MBC) of honey produced by honeybees (*Apis mellifera*) and tenegn (*Trigona sp.*) was shown on Table 1. A minimum bactericidal concentration (MBC) of tenegn against all tested pathogenic bacteria was 33.33% at 6.25% concentration, while that of honeybee honey was 11.11% at the same concentration. Tenegn MBC against all pathogenic bacteria was 66.67% at 12.5% concentration of solution, while honeybee honey was 44.44% at 12.5% concentration of solution. With regard to artificial honey, the MBC activity against tested pathogenic bacteria was 11.11% at 12.5% concentration. In contrast to honey produced by honeybee, tenegn honey could inhibit and kill all test organisms at very low concentrations (6.25-12.5%). In brief, a minimum bactericidal concentration of tenegn against all (100%) tested pathogenic bacteria was ≤ 12.5% concentration, while that of honeybee honey was killed only 55.55% at the same concentration. A minimum bactericidal concentration to *S. pneumonia* (ATCC 63) was 6.25% of tenegn solution; however, a MBC of honeybee honey solution to the same pathogenic bacterium was 25%. This was may be one of the reasons that the society frequently uses tenegn honey in traditional treatment of respiratory ailments than honey produced by honeybee. Surprisingly, the MBC of tenegn and honeybee honey against *S. aureus* (ATCC 25923) was 6.25%. This might be one of the basic reason that the society widely use honey frequently for treatment of wound. The antibacterial activity of honey to strains of *S. aureus* taken from infected wounds was reported by Cooper *et al.* [23].

**Physicochemical Analyses:** Table 2 shows the physicochemical characteristics of honeybees (*A. mellifera*) and tenegn (*Trigona sp.*) honey samples. The pH of tenegn honey (2.87±0.01) was statistically (P ≤ 0.05) greater (3.8±0.02) than honeybee honey. The pH of tenegn honey (2.87±0.01) was not found to be within the range (3.15-4.66) of different stingless bee honeys [24]. According to standard of ANONN, pH of honey should be between 3.4 and 6.1 and this finding was not within this range. The low pH of honey inhibits the presence and growth of microorganisms and mostly increases shelf life of different food products. This characteristic of honey influences the texture, stability and shelf life of food [25].
The acidic pH of honey is significant since acidification has been shown to encourage curing by causing oxygen release from hemoglobin [26]. In this study, the pH (2.87±0.01) was highly reduced in comparison with other types of honeys. It can play a great role as an antibiotic substance against different pathogenic bacteria. The values of pH in honey help to determine its origin such as honey originated from flowers with lower pH, while from forest show higher pH values [27].

The moisture content of tenegn honey was significantly (P < 0.05) greater (25 ± 0.4%) than honeybee honey (18.5± 0.3%). The moisture content of tenegn honey (25 ± 0.4%) was within the range (19.0-41.9%) of different stingless bee honeys reported by Bruno et al. [24] and within the standard of ANONN (13.4-26.6%). The moisture content of any type of honey can be influenced by various factors; harvesting season, the degree of maturity reached in the hive and environmental factors [28]. In this study, moisture content of tenegn honey (25 ± 0.4%) was significantly (P < 0.05) higher than the 20% maximum established for A. mellifera honey [29, 30]. Moisture content of honey is the most important factor for the determination of quality of the product, since it affects storage life and processing characteristics of the product. The strong association of sugar in honey with molecules of water may reduce free water available for microorganisms and thereby protects honey from attack by microorganisms.

The ash content and EC of the honey samples were shown on Table 2. Ash and EC values depend on the mineral content of the honey sample. Ash gives a direct measure of inorganic residue after carbonization, while electric conductivity measures all ionizable organic and inorganic substances present in the given sample. In this study, the ash content of tenegn (1.8±0.02) was significantly (P < 0.05) greater than honeybee honey (0.2±0.01%). Tenegn honey ash content was also greater than honeys reported by different authors [31, 32, 33, 34, 35]. The ash content of honey was entirely depended on floral origin of honey [36]. In this study, as value of ash was increased, the value of electrical conductivity was also increased in comparison with honeybee honey.

The electrical conductivity values of the tenegn honey (3.27±0.01 mS/cm) was statistically (P < 0.05) greater than honeybee honey (0.488±0.01 mS/cm). At the same time, tenegn honey electrical conductivity (3.27±0.01 mS/cm) was greater than the value of 0.80 mS/cm and 0.49-8.77 mS/cm reported by Bruno et al. [24], respectively. It is well known that the amount of ash is positively correlated with electrical conductivity values bound to honey minerals content [37, 38, 39]. As mentioned above, electrical conductivity measures all ionisable organic and inorganic substances present in honey, while, ash represents a direct measure of the inorganic residue after honey carbonization. The differences in the electrical conductivity of tenegn honey and honeybee honey may be attributable to the difference in insect species, botanical origins of honey and geographical region. All honey samples were found to be within the limit required by honeys quality of European Legislation [40] in all parameters [33].

The values for free, lactone and total acidities were shown on Table 3. Free acidity of honeybee honey and tenegn honey were 6.25±0.01 meq Kg⁻¹ and 36±0.02 meq Kg⁻¹, respectively. The values of free acidity of all samples were within the range or permissible limit that reported in the literature [41, 42]. Lactone and total acid value of honeybee honey were 0.23±0.02 meq Kg⁻¹ and 6.48±0.01 meq Kg⁻¹, respectively. Moreover, lactone and total acid value of tenegn honey were 0.75±0.01 meq Kg⁻¹ and 36.75±0.02 meq Kg⁻¹, respectively. The free acidity of honey may be described by considering or due to the presence of organic acids, specially the gluconic acid in equilibrium with their esters or lactones and inorganic ions such as chloride and phosphate [43, 44]. The total acidity values found in this study were below the maximum limits of 40 meq/kg set internationally for honey. Generally, all the values in this study for total acidity fall within the range reported for Moroccan honey [35]. The acidity of honey contributes a lot to impart flavor and help to increase its stability against microorganisms. All of the samples analyzed in this study were in agreement with the demands set out in the guideline [32, 33], which should not be more than 50 meq/kg.

**Microbial Analysis of Different Honeys:** Microbial count and profiles (in cfu/g) on tenegn and honeybee honey samples was shown on Table 3. The intrinsic properties of honey determine the growth and survival of microorganisms due to the effect of bacteriostatic or bactericidal action caused by the low pH and high content of honey’s sugars [45]. The microbial profile in the honey
produced by tenegn and honeybee was presented on Table 3. The standard plate counts (SPC) were found in low numbers total aerobic mesophilic bacteria counts in tenegn honey (1.54 x 10^1 cfu/g) in comparison with honey produced by honeybee (excessive colony counts). The total aerobic mesophilic bacteria counts in tenegn honey were in agreement with the report of Omafuvbe and Akanbi [46]. However, the colony count of mesophilic bacteria in this study was higher than that reported by Tysset and Roussean [47], Iurlina and Fritz [45] and Malika et al. [35]. According to Snowdon and Cliver [48], total aerobic viable count values for honeys can range from zero several thousand per gram. This variation in bacterial counts may be due to the type of sample, the freshness of the honey, the time of harvest and the analytical techniques used [48].

In this study, Bacillus species were found in all samples. They were detected significantly (P < 0.05) less colony count in tenegn honey (1.30 x 10^3) than honeybee honey (5.6 x 10^3). Low colony count was may be due to low pH and chemical composition of tenegn honey than honeybee honey. The result obtained for bacteria endospore indicates that the total aerobic mesophilic bacterial count comprised mainly of spore formers than vegetative cells. This study outcome agrees with the reports of Malika et al. [35] and Omafuvbe and Akanbi [47]. Previously, different species of Bacillus was reported from honey of A. mellifera [49] which was in line with this study.

Total coliforms were not detected in any one of the honey samples. In a similar study, total coliform were not detected in honey reported by different authors [45, 50]. This may be explained by the evidence that honey is well preserved against bacteria so that theses microorganisms would not survive in unfavorable conditions. As the result, the shelf life of honey is relatively longer than perishable food substances. In this study, yeasts (2.0 x 10^3) were detected in any of the samples which were in line with Malika et al. [35] report but there were no any yeast colony on honey samples reported by Omafuvbe and Akanbi [4]. The colony count of moulds in honeybee honey was (1.0 x 10^4). In contrast, no mould colony was observed on tenegn honey. There were some reports that quantify the levels of moulds and yeasts in honey. According to Tysset et al. [51] report, among 14 French honey samples, the mean counts of moulds were 254 cfu/g with values varied from 0 to 2500 cfu/g. Colony number of moulds provides basic information on the quality and shelf life as well as spoilage potential of honey [48]. In this study, there was no found to be detected any mold on tenegn honey sample than honey produced by honeybee (1.0 x 10^3). The microorganisms found in tenegn honey and honeybee honey were spore forming bacteria but no coli forming species have been found in both honey samples. The high counts of microorganisms detected in honey are usually due to contamination from exogenous sources.

**CONCLUSION**

Generally, antimicrobial activity of tenegn honey against tested pathogens was greater than other honeys. This might be the reason that local society preferably uses tenegn honey than other honeys to treat patients infected with pathogenic bacteria. The reason for this might be due to species variation of producing insect and production process. In this study, the pH was highly reduced in comparison with other types of honeys. It can play great role as antibiotic substance against different pathogenic bacteria. Moisture content of honey is the most important factor for the determination of quality of the product, since it affects storage life and processing characteristics of the product. The strong association of sugar in honey with molecules of water may reduce free water available for microorganisms and thereby protects honey from attack by microorganisms. The moisture content of tenegn honey was much greater than moisture content of honeybee honey in this study. However, the antimicrobial activity was much higher in tenegn honey than honeybee honey. This might be due to chemical composition, production process and type of spices. As mentioned above, this is may be one of the main reasons that the society preferentially uses tenegn honey for treatment of infections than the honey produced by honeybee. The differences in the electrical conductivity of tenegn honey and honeybee honey may be attributable to the difference in insect spices, botanical origins of honey and geographical region. Low colony count was may be due to low pH and chemical composition of tenegn honey than honeybee honey. To come up with a comprehensive conclusion, in vivo study is significant for antimicrobial activity of the samples under investigation.

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REFERENCES


