Antibacterial Activity of Natural and Commercial Honey- A Comparative Study

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Abstract: Honey forms part of the traditional medicine in many cultures. Honey has been extensively used as a healing agent throughout the human history in addition to its widespread usage as popular food. Honey is a sweet substance produced as a food source mainly from the nectar and secretions of plants by honey bees. In the present research, the antimicrobial activity of three different Honey samples viz., Kombu Honey, Malan Honey and Commercial Honey were evaluated against pathogenic bacteria (Staphylococcus aureus, Micrococcus luteus, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella flexneri, Proteus mirabilis, Pseudomonas aeruginosa and Acetobacter baumanii). The Natural and Commercial honey samples selected for the present study was collected from Chetheri Malai, Harur, Tamil Nadu, India. The Kombu honey, Malan honey and Commercial honey showed maximum zone of inhibition against Staphylococcus aureus. In conclusion, Kombu honey exhibited more antimicrobial activity against bacterial pathogens when compared to the Malan honey and Commercial honey.

Key words: Kombu Honey • Malan Honey • Commercial Honey • Bacterial Diseases and Antibacterial Activity

INTRODUCTION

Honey has been highly appreciated as an alimentary product and has been largely used, since ancient times, as well as in cosmetic manufacturing. Honey is a sugary substance obtained from the nectar of the flowers or from the secretions which come from or lie on the living parts of the plant and which honey bees crop, transform and combine with their own specific substances and store in the honeycomb of the beehive. Honey is a product extremely rich in sugars of which glucose and fructose are outstanding. It also possesses vitamins, mineral salts and microorganisms in honey have long been used to control the spoilage of honey. Microorganisms in honey may influence quality and safety. Due to the natural properties of honey and control measures in the honey industry, honey is a product with minimal types and levels of microbes. The microbes of concern in honey are primarily yeasts and spore-forming bacteria [1-3].

Honey has been extensively used as a healing agent throughout the human history in addition to its widespread usage as popular food. Honey is a sweet substance produced as a food source mainly from the nectar and secretions of plants by honey bees. Honey is used to feed bees during the winter [4]. For centuries, honey has been used as food and as natural medicine, being prescribed by physicians of many ancient cultures for the treatment of a wide variety of ailments. The art of apiculture and the benefits of honey have been known. Since, the Egyptian first dynasty [5] and ancient Greeks used honey as a sweetener [6-8]. In classical Greece, laws concerning apiculture were suggested and Plato included honey in their concept of a healthy diet [9]. The use of honey in folk medicine is thought to be as old as civilization, but in recent times there has been a renaissance in interest in its use as a medicinal product. The colour of honey can vary from clear to dark amber according to its floral source and mineral content and it
Inhibition of antibacterial activity of natural and commercial honey has a close relationship with its flavour and quality [10, 11]. Honeys may be viscous liquids or even solid with differing honeys identifiable by their colour, flavour, crystallization and the presence of pollen grains in honey sediment [12, 13].

Several authors reported that different honeys vary substantially in the potency of their antibacterial activity, which varies with the plant source [14, 15]. Thus, it has been shown that the antimicrobial activity of honey may range from concentrations < 3 % to 50 % and higher [16-18]. The bactericidal effect of honey was reported to be dependent on concentration of honey used and the nature of the bacteria [19, 20]. The concentration of honey has an impact on antibacterial activity; the higher the concentration of honey the greater its usefulness as an antibacterial agent [21, 22]. Taormina et al. [23] reported that the concentration of honey needed for complete inhibition of Salmonella typhimurium growth as <25 %.

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of the antibiotics is diminished. This type of bacterial resistance to the antimicrobial agents poses a very serious threat to public health and for all kinds of antibiotics including the major last-resort drugs, the frequencies of resistance are increasing worldwide [24, 25]. Therefore, alternative antimicrobial strategies are urgently needed and this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products, including honey [26-28]. The use of traditional medicine to treat infection has been practiced since the origin of mankind and honey produced by Apis mellifera is one of the oldest traditional medicines considered to be important in the treatment of several human ailments. Currently, many researchers have reported the antibacterial activity of honey and found that natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria [29, 30]. The present study was aimed to compare the antibacterial activity of natural and commercial honey against human pathogenic bacteria.

**MATERIALS AND METHODS**

**Collection of Honey Samples:** Three different types of honey samples were selected for the present study. They are: (a) Kombu Honey, (b) Malan Honey and (c) Commercial Honey. The Natural and Commercial honey samples selected for the present study was collected from Chetheri Malai, Harur, Tamil Nadu, India.

**Collection of Test Bacterial Cultures:** Ten different bacterial cultures of were procured from the Department of Biochemistry and Microbiology, Sacred Heart College (Autonomous), Tirupattur, Vellore district, Tamil Nadu, India. They are Staphylococcus aureus, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Shigella flexneri, Salmonella typhi, Acetobacter baumani, Proteus mirabilis, Micrococcus luteus and Pseudomonas aeruginosa.

**Maintenance of Bacterial Cultures:** The bacterial cultures were sub-cultured and maintained on Nutrient agar slants and stored in refrigerator at 4 °C.

**Bacterial Inoculum Preparation:** Bacterial inoculum was prepared by inoculating a loopful of bacteria in 5 ml of Nutrient broth and incubated at 37 °C for 12 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standard and then used for the determination of antibacterial activity.

**Determination of Antibacterial Activity of Honey Samples:** Mueller Hinton Agar plates were prepared and inoculated with test bacterial isolates by spreading the bacterial inoculum on the surface of the media. Wells (6 mm in diameter) were punched in the Mueller Hinton Agar. Honey samples with 75 µl/ml concentrations was mixed with 1 ml of Dimethyl sulfoxide (DMSO), mixed well and added into the well. Well containing DMSO alone act as a Negative control. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm).

**RESULTS AND DISCUSSION**

The antimicrobial activity of three different Honey samples viz., Kombu Honey, Malan Honey and Commercial Honey were evaluated against pathogenic bacteria (Staphylococcus aureus, Micrococcus luteus, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella flexneri, Proteus mirabilis, Pseudomonas aeruginosa and Acetobacter baumani). There are several studies investigating the antimicrobial activity of honey. The antibacterial activity of honey is usually associated with the release of hydrogen peroxide, from the oxidation of glucose to gluconolactone and then to gluconic acid in presence of the enzyme glucose oxidase. This activity was called peroxide-activity and constitutes, at variable extent, the mode of action of some honeys. The use of honey as a traditional remedy for microbial infections dates back to ancient times [31, 32].
The antibacterial activity of Kombu honey was investigated against bacterial pathogens and the results were given in the Table - 1. Antibacterial activity was observed for all the ten bacterial isolates at 75 µl concentration. The Kombu honey showed maximum zone of inhibition against *Staphylococcus aureus* (34 mm) followed by *Micrococcus luteus* (30 mm), *Bacillus cereus* (32 mm), *Escherichia coli* (28 mm), *Salmonella typhi* (24 mm), *Proteus mirabilis* (24 mm) and *Pseudomonas aeruginosa* (23 mm). Minimum zone of inhibition was noticed in the pathogen *Acetobacter baumanii* (22 mm). No zone of inhibition was seen in DMSO blind control.

Research has been conducted on manuka honey which has been demonstrated to be effective against several human pathogens, including *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhimurium*, *Staphylococcus aureus* [33-35]. Many laboratory studies have revealed that the honey is effective against Methicillin-resistant *Staphylococcus aureus* (MRSA), β- haemolytic *Streptococcus* and vancomycin resistant *Enterococcus* (VRE) [36-38]. However, the newly identified honeys may have advantages over or similarities with manuka honey due to enhanced antimicrobial activity, local production (Thus availability) and greater selectivity against medically important organisms [39-41]. The coagulase negative *Staphylococci* are very similar to *Staphylococcus aureus* in their susceptibility to honey of similar antibacterial potency and more susceptible than *Pseudomonas aeruginosa* and *Enterococcus* species [42-44].

Gram positive bacteria such as *Staphylococcus aureus*, the causal agent of a range of illnesses from skin infections to life-threatening diseases such as pneumonia and meningitis, did not grow in the presence of honeys produced by *A. mellifera* and *Tetragonisca angustula* bees in the Brazilian states of Paraná and Minas Gerais. In order to study this effect, honeys were analyzed by High performance liquid chromatography (HPLC) and the antimicrobial activity was connected to phenolic compounds, such as 4- hydroxybenzoic acid (HBEN). These compounds occur in higher concentrations in propolis than in honey and propolis was more effective against *Staphylococcus aureus* than honey [45]. This bacterium has proved to be variable in susceptibility to different honeys. Turkish honeys from Anatolia showed a moderate inhibition towards some strains of *Staphylococcus aureus* while Turkish rhododendron honeys partially inhibited the growth of this bacterium [46, 47].

Honey from stingless bees had powerful activity against *Staphylococcus aureus* when compared to that exhibited by manuka honey produced by honeybees belonging to Apidae family [48, 49]. Argentinean honeys from the province of Cordoba evidenced high activity against *Staphylococcus aureus*, which was considered to be of remarkable clinical importance, since an increase in difficult-to-treat skin infections had been reported in the last decade and resistance against several antibiotics had developed [50]. A study of the properties of several Cuban honeys demonstrated that Gram positive bacteria are more sensitive than Gram negative bacteria with *Staphylococcus aureus* the most sensitive bacterium [51, 52].

The antibacterial activity of Malan honey was studied against bacterial pathogens and the results were showed in the Table - 2. Antibacterial activity was noticed for all the ten bacterial isolates at 75 µl concentration. The Malan honey showed maximum zone of inhibition against *Staphylococcus aureus* (26 mm) followed by *Micrococcus luteus* (24 mm), *Bacillus cereus* (23 mm), *Shigella flexneri* (22 mm), *Pseudomonas aeruginosa* (21 mm), *Salmonella typhi* (21 mm), *Proteus mirabilis* (20 mm) and *Escherichia coli* (19 mm). Minimum

### Table 1: Antibacterial activity of Kombu honey against bacterial pathogens

<table>
<thead>
<tr>
<th>S.No</th>
<th>Bacterial pathogens</th>
<th>Kombu Honey at 75 µl/ml Zone of inhibition (mm in dm)</th>
<th>DMSO Zone of inhibition (mm in dm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>34</td>
<td>NZ</td>
</tr>
<tr>
<td>2</td>
<td><em>Micrococcus luteus</em></td>
<td>30</td>
<td>NZ</td>
</tr>
<tr>
<td>3</td>
<td><em>Bacillus cereus</em></td>
<td>32</td>
<td>NZ</td>
</tr>
<tr>
<td>4</td>
<td><em>Escherichia coli</em></td>
<td>28</td>
<td>NZ</td>
</tr>
<tr>
<td>5</td>
<td><em>Salmonella typhi</em></td>
<td>24</td>
<td>NZ</td>
</tr>
<tr>
<td>6</td>
<td><em>Shigella flexneri</em></td>
<td>25</td>
<td>NZ</td>
</tr>
<tr>
<td>7</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>26</td>
<td>NZ</td>
</tr>
<tr>
<td>8</td>
<td><em>Proteus mirabilis</em></td>
<td>24</td>
<td>NZ</td>
</tr>
<tr>
<td>9</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>23</td>
<td>NZ</td>
</tr>
<tr>
<td>10</td>
<td><em>Acetobacter baumanii</em></td>
<td>22</td>
<td>NZ</td>
</tr>
</tbody>
</table>

NZ – No zone of inhibition
Table 2: Antibacterial activity of Malan Honey against bacterial pathogens

<table>
<thead>
<tr>
<th>S. No</th>
<th>Bacterial pathogens</th>
<th>Malan Honey at 75 µl/ml Zone of inhibition (mm in dm)</th>
<th>DMSO Zone of inhibition (mm in dm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>26</td>
<td>NZ</td>
</tr>
<tr>
<td>2</td>
<td><em>Micrococcus luteus</em></td>
<td>23</td>
<td>NZ</td>
</tr>
<tr>
<td>3</td>
<td><em>Bacillus cereus</em></td>
<td>24</td>
<td>NZ</td>
</tr>
<tr>
<td>4</td>
<td><em>Escherichia coli</em></td>
<td>19</td>
<td>NZ</td>
</tr>
<tr>
<td>5</td>
<td><em>Salmonella typhi</em></td>
<td>21</td>
<td>NZ</td>
</tr>
<tr>
<td>6</td>
<td><em>Shigella flexneri</em></td>
<td>22</td>
<td>NZ</td>
</tr>
<tr>
<td>7</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>18</td>
<td>NZ</td>
</tr>
<tr>
<td>8</td>
<td><em>Proteus mirabilis</em></td>
<td>20</td>
<td>NZ</td>
</tr>
<tr>
<td>9</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>21</td>
<td>NZ</td>
</tr>
<tr>
<td>10</td>
<td><em>Acetobacter baumanii</em></td>
<td>17</td>
<td>NZ</td>
</tr>
</tbody>
</table>

NZ – No zone of inhibition

Table 3: Antibacterial activity of Commercial honey against bacterial pathogens

<table>
<thead>
<tr>
<th>S. No</th>
<th>Bacterial pathogens</th>
<th>Commercial Honey at 75 µl/ml Zone of inhibition (mm in dm)</th>
<th>DMSO Zone of inhibition (mm in dm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>22</td>
<td>NZ</td>
</tr>
<tr>
<td>2</td>
<td><em>Micrococcus luteus</em></td>
<td>20</td>
<td>NZ</td>
</tr>
<tr>
<td>3</td>
<td><em>Bacillus cereus</em></td>
<td>21</td>
<td>NZ</td>
</tr>
<tr>
<td>4</td>
<td><em>Escherichia coli</em></td>
<td>17</td>
<td>NZ</td>
</tr>
<tr>
<td>5</td>
<td><em>Salmonella typhi</em></td>
<td>19</td>
<td>NZ</td>
</tr>
<tr>
<td>6</td>
<td><em>Shigella flexneri</em></td>
<td>19</td>
<td>NZ</td>
</tr>
<tr>
<td>7</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>16</td>
<td>NZ</td>
</tr>
<tr>
<td>8</td>
<td><em>Proteus mirabilis</em></td>
<td>15</td>
<td>NZ</td>
</tr>
<tr>
<td>9</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>18</td>
<td>NZ</td>
</tr>
<tr>
<td>10</td>
<td><em>Acetobacter baumanii</em></td>
<td>17</td>
<td>NZ</td>
</tr>
</tbody>
</table>

NZ – No zone of inhibition

zone of inhibition was noticed in *Acetobacter baumanii* (17 mm). No zone of inhibition was seen in DMSO blind control.

Baltrusaityte et al. [53] observed that Lithuanian honeys exhibited antibacterial effects on *Staphylococcus aureus* and concluded that this was mainly due to the presence of hydrogen peroxide. Honeys produced in the Czech Republic are also antimicrobial effective with some honeydew honeys possessing the greatest effects. In a study with unifloral and multifloral Portuguese honeys, Henriques et al. [54] analyzed their antibacterial activity against a strain of *Staphylococcus aureus* and observed that all of the samples tested possessed peroxide activity except some honeys derived from *Lavandula stoechas* which revealed non-peroxide antibacterial activity.

Basson and Grobler [55] found poor antibiotic activity of South African honeys from indigenous *L. cordifolium* and Erica species. Brudzynski and Miotto [56] investigated twenty Canadian unheated honeys, quantified Maillard reaction - like products (MRLPs) and total phenol contents, apart from assessing the antioxidant activity using the ORAC method. One of their results indicated that both the recorded antioxidant activity and the content of MRLPs of these honeys mainly contributed to the antibacterial activity against *Escherichia coli*. This antibacterial activity confirms the work of Rufian Henares and De La Cueva [57] who suggested that the antibacterial activity of coffee melanoidins against *Escherichia coli* is due to their behavior as metal chelators.

The antibacterial activity of Commercial honey was tested against bacterial pathogens and the results were tabulated in the Table - 3. Antibacterial activity was recorded for all the ten bacterial isolates at 75 µl concentration. The Commercial honey showed maximum zone of inhibition against *Staphylococcus aureus* (22 mm) followed by *Micrococcus luteus* (21 mm), *Bacillus cereus* (20 mm), *Salmonella typhi* (19 mm), *Shigella flexneri* (19 mm), *Pseudomonas aeruginosa* (18 mm), *Escherichia coli* (17 mm), *Acetobacter baumanii* (17 mm) and *Klebsiella pneumoniae* (16 mm). Minimum zone of inhibition was noticed in *Proteus mirabilis* (15 mm). No zone of inhibition was seen in DMSO blind control.

Isla et al. [58] observed that algarroba honey (*Prosopis nigra*) and a multifloral honey from the northwestern provinces of Argentina had activity against *Staphylococcus aureus*. They identified at least five antibacterial compounds in the algarrobo honey and four...
compounds in the multifloral honey and found that most of them corresponded to flavonoids. One of these flavonoids was identified as pinocembrin. The authors also pointed out that the antibacterial activity of the analyzed honeys might be mainly due to their phenol content because of the significant correlation observed between the phenolic content and the antibacterial activity.

Some other honey sensitive - pathogens described in the literature are Bacillus anthrasis (Anthrax), Corynebacterium diptheriae (Diphtheria), Klebsiella pneumoniae (Pneumonia), Mycobacterium tuberculosis (Tuberculosis), Salmonella typhi (Typhoid fever), Vibrio cholerae (Cholera) and different Streptococci, among others. For example, Klebsiella pneumoniae was found to be sensitive to Argentinean [59] and Thai honeys [60]. The growth of Enterococcus faecalis was inhibited by stingless honeybee honey [61] and this bacterium also showed sensitivity to honey according to the research performed by Gallardo Chacon et al. [62].

The initial event in the development of bacterial infections of the gastrointestinal gut is the attachment of bacteria to the mucosal epithelial cells and the blocking of this event represents an interesting strategy for the prevention of diseases. Alnaqdy et al. [63] studied the antimicrobial activity of Omani honeys against Salmonella enteritidis and the ability of these honeys to prevent the bacterium from adhering to intestinal epithelial cells. Among the Gram negative bacteria, Escherichia coli are of great concern from a health point of view. It may cause life threatening gastric infections and diarrhea, following consumption of contaminated food, as well as other infections such as cystitis, meningitis, peritonitis and pneumonia. There are several studies on the antibiotic effect of honey towards Escherichia coli. The Escherichia coli exhibited sensitivity to Stingless honeybee honey, Spanish honeys, Cuban honeys and Thailand honeys.

CONCLUSION

The present research concluded that the Kombu honey exhibited more antimicrobial activity against bacterial pathogens when compared to the Malan honey and Commercial honey. Comparatively, the activity of Commercial honey was very less when compared to the Natural honey samples. Further, since honey is a cheap, easily available and also a non-toxic antimicrobial agent due to its properties, it can be very effectively used for medical purposes. On the basis of present study, it is suggested that further broad spectrum studies as well as clinical trials should be conducted before the use of these products as routine medicines. New studies based on a chemical, pharmacological and clinical approach must be conducted.

REFERENCES


