



## Antioxidant and Antibacterial Activity of Different Floral Honeys from Western Ghats of Karnataka

Shubharani R<sup>\*1</sup>, Anita M<sup>1</sup>, Mahesh M<sup>2</sup>, Sivaram V<sup>1</sup>

<sup>1</sup>Laboratory of Biodiversity and Apiculture, Department of Botany, Bangalore University, Bangalore-560056, India

<sup>2</sup>Azyme Biosciences Private Limited Jayanagar, Bangalore-560069, India

<sup>\*</sup>Corresponding author's E-mail: [shubharani.bolar19@gmail.com](mailto:shubharani.bolar19@gmail.com)

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### ABSTRACT

Honey has a wide range of therapeutic properties which depend largely on floral sources. In this study six honey samples collected from different locations of Western Ghats of Karnataka were screened for total phenolic and flavonoid content, potential antioxidant activity and antibacterial effects. The total phenol and flavonoids content was determined by Folin-Ciocalteu and Aluminum chloride method respectively and also honeys were analysed by HPLC. The antioxidant properties of these samples were assessed by DPPH\* (2, 2-diphenyl -1-picrylhydrazyl) and ABTS\* radical scavenging activity. The antibacterial tests were done by using gram-positive (*Staphylococcus aureus*) and gram-negative (*Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Escherichia coli*) bacteria. The result of the study suggests that, these honeys are not only interesting source for antibacterial activity but also potential source of antioxidant. All the 6 honey samples did not show the bacterial growth inhibition against *Staphylococcus aureus*. The difference between honey samples in terms of antioxidant and antibacterial activity could be attributed to the natural variation in composition and also to different floral sources of nectar.

**Keywords:** ABTS\*, Antibacterial, Antioxidant, DPPH, Honey, Therapeutic property.

### INTRODUCTION

Antioxidants being positive element protect key cell components from damage by neutralizing the free radicals. Free radicals are highly reactive compounds created in the body during normal metabolism or introduced from the environment. These are responsible for degenerative diseases, including carcinogenesis, aging, mutagenesis, etc<sup>1</sup>. Antioxidants that occur naturally in the body or consumed through diet prevent damage to the cells. Natural antioxidant has gained interest among consumers and scientific community because the studies have indicated that the frequent consumption of natural antioxidant is associated with lower risk of cardiovascular diseases and cancer<sup>2</sup>. The defense effect of natural antioxidant is related to vitamins, phenols and flavonoids<sup>3</sup>. Therapeutic qualities of honey have drawn the attention of researchers and extensive studies have been done in India and abroad<sup>4-11</sup> and have projected honey's unique antioxidant properties which contribute to better health.

Honey is a natural sweet viscid fluid produced by honeybees from nectar of blossoms and extra floral nectaries of plants. Since ancient time honey is highly valued throughout the world as nutritious food and medicine and reported to contain high level of natural nutrients<sup>12-13</sup>. It plays an important role in providing antioxidant and antibacterial effects. The amount and type of antioxidants in honey depends largely on the type floral sources<sup>14-15</sup>.

Honey also has a broad spectrum of antimicrobial activity<sup>16</sup>. Traditionally honey has been used in the topical treatment of infected wounds, burns, and also in

some common ailments<sup>17</sup>. Many researchers have suggested that this is due to acidity and hyperosmotic effects of its high sugar content<sup>18-20</sup>. Number of key properties of honey and other hive products have been described and supported in the scientific literature including the antimicrobial activities of honey. More recently honey has been reported to have inhibitory effects on the growth of more than 60 species of bacteria including aerobes, anaerobes, gram-positive, and gram-negative organisms<sup>21</sup>. Variation in the intensity of inhibitory activity has been observed between different honey types depending on the microbial species tested and honey source.

The aim of the present study is to determine and evaluate the total phenolic and flavonoid content, antioxidant assay and antibacterial effects of multifloral honey samples collected from Western Ghats of Karnataka which is one of the major honey producing centers in India.

### MATERIALS AND METHODS

Honey samples were collected at different locations from Western Ghats of Karnataka directly from domesticated bee hives and also from the local traditional honey hunters of Agumbe (Shimoga), Siddapura (Uttara Kannada), Puttur (Dakshina Kannada), Koppa (Chikkamagaluru), Virajpet (Coorg) and Jamboti (Belgaum) (Table-1). These honey samples were collected in the period from May to August 2012 using extractive method. Six honey samples were tested in order to assess total phenolic and flavonoid content, antioxidant, and antibacterial effects.



### Quantitative phytochemical screening

**Total phenolic determination:** The total phenolic compounds were determined by using Folin-Ciocalteu method. One gm of honey sample was mixed with 10ml of distilled water, filtered. Different concentration of honey solution was mixed with 0.2N Folin- Ciocalteu reagent. After 5 min, 1.5ml of Na<sub>2</sub>CO<sub>3</sub> was added. The mixture was incubated at room temperature for 30 min. absorbance of the reaction mixture was measured at 670nm. Gallic acid was used as standard for the calibration curve<sup>22</sup>.

**Determination of Flavonoids:** The determination of total flavonoids was done by Aluminum chloride method<sup>23</sup>. Honey samples of different concentration were mixed with 0.5ml of 1.2% Aluminum chloride and 0.5ml of 120mM Potassium acetate. The mixture was allowed to stand for 30min. at room temperature. The absorbance was measured at 415nm. Quercetin was used as standard for calibration curve.

**High performance liquid chromatography (HPLC):** HPLC analysis was conducted with High performance liquid chromatography with Waters equipped of model 510, column C18, length 250 cm X 4.6 cm diameter and 5 micron variea. The phenols and flavonoids were detected at 254nm and 215nm using mobile phase 7:3 acetonitrile and water eluted isometrically. Phenols were compared with Gallic acid as standard and flavonoids with Quercetin as standard. Quantification was made according to the linear calibration curve of standard compound.

### Determination of free radical scavenging activity

**DPPH\* method:** The free radical scavenging activity in honey was determined by DPPH\* (2, 2-diphenyl -1-picrylhydrazyl)<sup>24</sup>. Honey was diluted with distilled water and different concentrations of honey solutions were mixed with 2ml of 0.1mM DPPH\* in methanol. The mixture was incubated in dark for 30 min. and absorbency

was determined at 517 nm using spectrophotometer. The analysis was performed in triplicate and the percentage of free radical scavenging activity of each sample was calculated as:

$$\% \text{ inhibition} = [\text{Abs (blank)} - \text{Abs (sample)} / \text{Abs (blank)}] \times 100$$

**ABTS<sup>+</sup> method:** The total antioxidant activity of the honey samples were also measured by ABTS<sup>+</sup> radical cation discoloration assay<sup>25</sup>. ABTS<sup>+</sup> was produced by reacting 7mM of ABTS<sup>+</sup> in distilled water with 2.4mM potassium per sulfate and kept in dark for 12-16 hr at room temperature. Prior to assay, the solution was diluted with ethanol and equilibrated to give absorbance at 734nm of 0.700 +/- 0.002. One ml of diluted ABTS<sup>+</sup> solution was added to honey sample extract of different dilutions. Absorbance was measured after 30 min. of incubation at room temperature. The capacity of radical scavenging was determined in triplicates and values are expressed as percentage of scavenging activity.

### Determination of antibacterial activity

The antibacterial activity of different concentrations of honey samples were studied against four microorganisms from gram-positive (*Staphylococcus aureus*) and gram-negative (*Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Eschiershea coli*) bacteria. Petri plates were prepared with Muller Hinton agar media. One ml of diluted bacterial culture was spread on each plate. Five wells of approximate 0.6 cm diameter were cut with sterile cork borer in the inoculated agar. The wells were filled with test samples with the help of sterile droppers. The plates were incubated for 24 hours at 37°C. At the end of incubation period the inhibition zones were measured.

### Statistical Analysis

The result were analysed statistically, all the standards were calculated the regressive r value and Pearson's correlation is less than 0.05.

**Table 1:** Correlation between total phenolic content and antioxidant capacity of honey samples

| Location of honey samples collected | Total phenolic (mgGAE/Kg) | Total flavonoids (mg QE/Kg) | DPPH*IC <sub>50</sub> (μl/ml) | ABTS <sup>+</sup> IC <sub>50</sub> (μl/ml) |
|-------------------------------------|---------------------------|-----------------------------|-------------------------------|--|
| Agumbe                              | 10.80 ± 0.18              | 0.94 ± 0.045                | 59.50 ± 0.050                 | 13.58 ± 0.145                              |
| Siddapura                           | 10.30 ± 0.20              | 0.63 ± 0.028                | 78.48 ± 0.084                 | 09.64 ± 0.682                              |
| Puttur                              | 10.36 ± 0.12              | 0.98 ± 0.067                | 75.75 ± 0.090                 | 09.15 ± 1.012                              |
| Koppa                               | 10.83 ± 0.46              | 0.98 ± 0.070                | 63.54 ± 0.125                 | 09.76 ± 0.982                              |
| Virajpet                            | 70.72 ± 0.21              | 1.63 ± 0.160                | 42.62 ± 1.542                 | 24.95 ± 1.984                              |
| Jamboti                             | 40.98 ± 0.36              | 2.27 ± 0.292                | 48.46 ± 0.982                 | 16.31 ± 1.086                              |

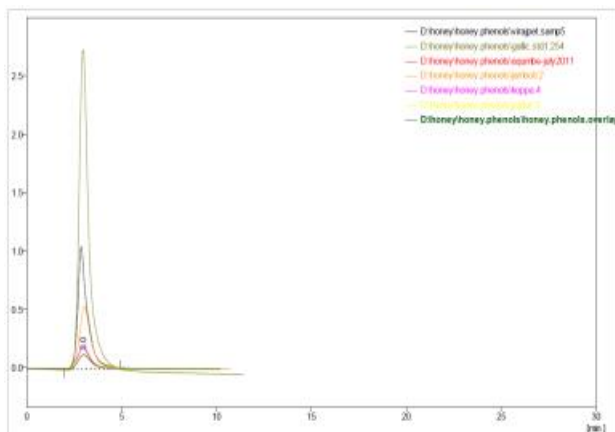
**Table 2:** Inhibition zones produced by different honey samples

| Location of honey samples collected | <i>Staphylococcus aureus</i> | <i>Escherichia coli</i> | <i>Pseudomonas aeruginosa</i> | <i>Klebsiella pneumonia</i> |
|-------------------------------------|------------------------------|-------------------------|-------------------------------|-----------------------------|
| Agumbe                              | --                           | ++                      | +                             | --                          |
| Siddapura                           | --                           | +++                     | +++                           | ++                          |
| Puttur                              | --                           | ++                      | +++                           | ++                          |
| Koppa                               | --                           | +                       | ++                            | --                          |
| Virajpet                            | --                           | +++                     | ++                            | ++                          |
| Jamboti                             | --                           | ++                      | +++                           | --                          |

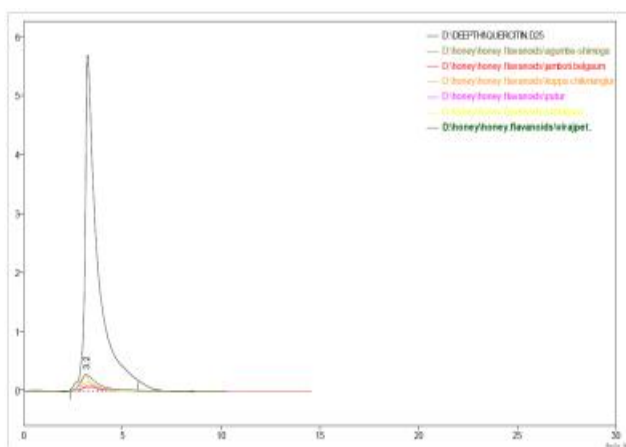


## RESULTS AND DISCUSSION

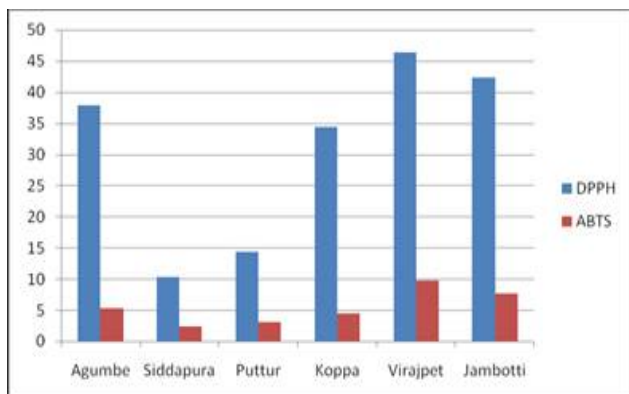
The result of total phenolic and total flavonoid content obtained by HPLC of these honey samples are represented in table 1. It was calculated high phenolic content in honey sample 5, moderate in sample 6 and less in sample 1, 2, 3 and 4. According to the result obtained by HPLC, the total flavonoids content ranged from 0.63 to 2.27mg of Quercetin/Kg. The total flavonoids content was high in sample 6. The value obtained is in accordance with previously reported result<sup>26-27</sup>. HPLC chromatogram of total phenol and flavonoid of all the 6 honey samples and standards are shown in Figure 1 and 2.



**Figure 1 :** The HPLC chromatogram of total phenol content



**Figure 2:** The HPLC chromatogram of total flavonoids content



**Figure 3:** Percentage of radical scavenging activity of DPPH and ABTS.

DPPH\* undergo reduction by an antioxidant is measured in terms of decrease in its absorbance at 517nm. As DPPH\* radicals react with a suitable reducing agent, the electron becomes paired and the solution changes from purple to yellow color. Table 1 shows the scavenging ability expressed as IC<sub>50</sub> on DPPH\* radical. Antioxidant activity of the present investigation showed a fall in the absorbance of DPPH\* indicating significant antioxidant activity in all the honey samples. The highest percentage of DPPH\* radical scavenging ability was 46.31% in sample 5 and 42.35% in sample 6 when compared to standard Gallic acid. The results clearly indicate that honey sample 2 and 3 has less free radical scavenging potential of 10.26% and 14.41% compare to sample 1 and 4 has moderate radical scavenging activity of 37.83% and 34.41% (Figure 3) that prevents or decrease the free radical damage occurring in human body.

ABTS<sup>+</sup> assay is an excellent tool to determine the antioxidant activity. In the present study, honey samples showed notable ABTS<sup>+</sup> radical scavenging activity. Among 6 samples, sample 5 and sample 6 exhibited the highest activity. The IC<sub>50</sub> scavenging activity of ABTS<sup>+</sup> was observed ranging from 9.15 to 24.95µl/ml. A good correlation is found between ABTS<sup>+</sup> assay and DPPH\* method. The percentage of inhibition of DPPH\* and ABTS<sup>+</sup> radical of each sample is indicated in Figure 3. The difference between the antioxidant activities of honey samples may be attributed to natural variation in composition and also to different floral sources of nectar and different location<sup>28-29</sup>. The result obtained support that honeys of Western Ghats serve as a good source of natural antioxidant that may have therapeutic potential.

### Antibacterial assay of honey samples

The present study reveals that the gram-negative bacteria *Escherichia coli* were inhibited by all the honey samples. Maximum inhibition was observed in sample 2 and 5. The samples 1, 3, and 6 showed moderate inhibition and samples 4 showed less inhibitions. Similar results were reported with *Pseudomonas aeruginosa*, sample 2, 3 and 6 very effectively inhibited the growth. Samples 4 and 5 showed moderate inhibition, and sample 1 showed less inhibition. Studies on *Klebsiella pneumonia* indicated that samples 2, 3, 5 and 6 were moderately inhibited and no activity was found from the samples 1 and 4 against the organism. All the 6 honey samples did not show any promising activity against gram-positive bacteria *Staphylococcus aureus* (Table2).

Among the different samples of crude honey used in the study, sample 2, 3, 5 and 6 collected from Siddapura, Puttur, Virajpet and Jamboti respectively showed maximum bacterial growth inhibition activity. The above mentioned regions have rich vegetation, agricultural farms, plantations and forest with beekeeping as a subsidiary source of income. As far as gram-negative organisms are concerned, the present report confirms the previous findings<sup>30</sup>.

**CONCLUSION**

The results obtained justify the correlation between the phenolic and flavonoid content, and antioxidant capacity. Phenolic compounds play a major role in antioxidant activity of natural products. Honey is a supersaturated solution of carbohydrates and it has a high degree of acidity, low pH and high osmotic pressure which prevents the growth of many bacterial forms<sup>31-32</sup>. Various honeys have been shown to inhibit the growth of microbes, including important clinical wound isolates like *Pseudomonas aeruginosa*<sup>33</sup>. It can be concluded that honey at various dilutions has different bacterial growth inhibition activities. From this study we found that honey samples from Virajpet and Jamboti had high antioxidant properties. Western Ghats honey samples studied proved to be a good source of antioxidant and antibacterial agent that might fight against diseases and safeguard health.

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