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## Antimicrobial activity of honey against pathogenic bacteria (*Escherichia coli*)

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**Abstract**

Honey is the very old product that we get from bees. The medicinal property of honey was known to man kind in the ancient period itself, but the reason behind its amazing property was unaware. The present study reveals that zone of inhibition by different concentrations at 100 that is raw honey and 50% of honey samples of Bangalore were recorded against *E. coli* are presented. The zone of inhibition by various honeys collected from Bangalore region against *Escherichia coli* were recorded by measuring the diameter of zones of inhibition. 36 mm diameter zone of inhibition was achieved maximum in 100 % concentration against to 22.83mm in 50 % concentration. Unifloral honey was found to have higher zone of inhibition when compared to multifloral. Though, there were variations among the treatments statistically there was no significant variation between concentration and within the concentration with respect to the zone of inhibition. Also there was no correlation with the pH.

**Keywords:** Honey, *Escherichia coli*, zone of inhibition, flora

**1. Introduction**

Honey is one of the nature's best gift to mankind. Honey is defined as the natural sweet substance produced by honey bees from nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living part of plants or from extra floral nectaries of the plant, transform by combining with specific substance, ripen and store in the cells of the comb by them for their food (Mendes *et al.*, 1998) [7].

*A. cerana* is the domesticated species commonly known as Thudujejenu, Sakujenu, Potarejenu. This is the base for Indian beekeeping. It is found in India, Pakistan, Sri Lanka, Malaysia, Indo-China region, Philippines, China, Russia, Japan and Indonesia. This species is widely distributed upto 2500 mtr above sea level and accepted by the people for commercial beekeeping in the country. The natural colonies of *A. cerana* construct many parallel combs in semi circular shape and are found in hollow cavities of trees, cracks in rocks and old walls and internal galleries of termite mounds. The honey yield from single colony varies from 8-10 kg per year in plains and upto 20-25 kg per year in hilly areas.

Honey is used as a medicine since from pre historic period, but the factor influencing the antibacterial property is still in search of the result. But, it was just known to be a best remedy. But, not all honey have the same therapeutic effect due to large variation in its antibacterial activity (Molan, 2001) [8] and the variation in the antibacterial activity of honey is due to difference in its floral source (Allen *et al.*, 1991). The quality and medicinal value of honey is attributed to the floral source, honey bee species, region and season. The quality parameter of honey such as physico-chemical, antimicrobial and antioxidant properties with respect to different floral sources and seasons in Bangalore region needs indepth studies and therefore, the present study was undertaken with the following objectives.

**2. Material and Methods**

The honey samples from *A. cerana* were collected from different Bangalore region viz., Bagalur cross, Reva College, M. S. Palya and GKVK campus. A piece of the *A. cerana* comb completely sealed with honey collected from different places and brought to laboratory for extraction. The cut comb was unsealed using uncapping knife, filtered into a beaker using muslin cloth. The honey thus extracted was transferred to a air tight bottle which was labeled with respect to the place, date and time of collection. The bottles were stored in refrigerator at 4 °C for further studies The *A. cerana* honey was analysed for the presence of pollen following Acetolysis method suggested by Erdtman (1952, 1966) [2, 3]. One gram of honey was diluted in 9ml of water and centrifuged at 4000rpm for 15 min. The pollen so obtained at the bottom was collected, identified and counted in all the 25 cells of hemocytometer under microscope (Motic) and classified into unifloral and multifloral based on the number of pollen per 10g

of honey samples. Honey sample weighing 10 gram was diluted in 50 ml of distilled water and the solution was fed to the pH meter and the readings were recorded at room temperature (Cyber pH-14 pH meter, cyber lab).

Statistical Analysis followed was completely randomized design (CRD)

### 3. Result and Discussion

Out of 21 samples analysed, 16 were unifloral, two were bifloral, and three were multi floral. The unifloral honeys were *Callistemon viminalis* Byrnes, *Areca catechu* L., *Citrus limson* L., *Mallotus philippensis* Lour., *Cocos nucifera* L., *Eucalyptus* sp. Labill., *Ocimum* sp. L., *Moringa olrifera* L. and *Pongamia pinnata* L. and the bifloral honeys were *Pongamia pinnata* L. and *Gliricidia* sp. L (sample-20), sample-13 with *Simarouba glauca* DC. *Eucalyptus* sp. and were unidentified pollens (Sekhar, P., 2000 and Marc, N., 2012) [10, 6]. The pH of honey is always acidic in nature. In the sample lot significantly it was estimated to be highest in the honey of *Eucalyptus* (5.06) and lowest in *Moringa oleifera* L. honey (3.10). Eight samples had pH between 3 to 4; twelve samples fell between 4 to 5; only one sample had more than pH of 5 (Fig. 1) (Azeredo *et al.*, 2003; Kayacier and Karman, 2008; Ouchemoukh *et al.*, 2007) [1, 4, 9].

The antimicrobial property of honey was estimated by measuring the diameter of zones of inhibition exhibited by honey on pathogen inoculated media. The results on inhibition by different concentrations (100 & 50%) of honey samples of Bangalore were recorded against *E. coli* are presented in Table 1. The zones of inhibition by various honeys collected from Bangalore region against *Escherichia coli* were recorded by measuring the diameter of zones of inhibition. Between 100 & 50% concentrations of honey, 100 % gave better effect on suppression of pathogen. 36 mm diameter zone of inhibition was achieved maximum in 100 % concentration against to 22.83mm in 50 % concentration. This are in agreement with Vijayakumar K. T., 2015 [11]. The maximum inhibition zone was observed in the multifloral honeys and bifloral honeys than unifloral honeys. The multifloral honey comprised with the pollens of forest species which were rich in antioxidants. The sample-1 which exhibited maximum inhibition zone of 36mm attributed to higher moisture in the sample which helps in release of hydrogen per oxide. The antimicrobial property of honey attribute to factors like low pH, high sugars, H<sub>2</sub>O<sub>2</sub> and other antioxidants. The honeys in our study which showed highest pathogen suppression ability had moderate level of antimicrobial factors.

### Standard antibiotics

The comparative analysis of antimicrobial property of various honey samples collected from our study area were compared with that of standard antibiotics *viz.*, Kenamycin (5mcg/disc), Tetracycline (30mcg/disc) and Streptomycin (10mcg/disc) (Table 2).

The zone of inhibition exhibited by standard antibiotics *viz.*, Kenamycin, Tetracycline and Streptomycin were shown 17,

23 and 28mm diameter, respectively, and control (distilled water) 0 mm which is compared with that of honey samples of Bangalore region.

### 4. Conclusion

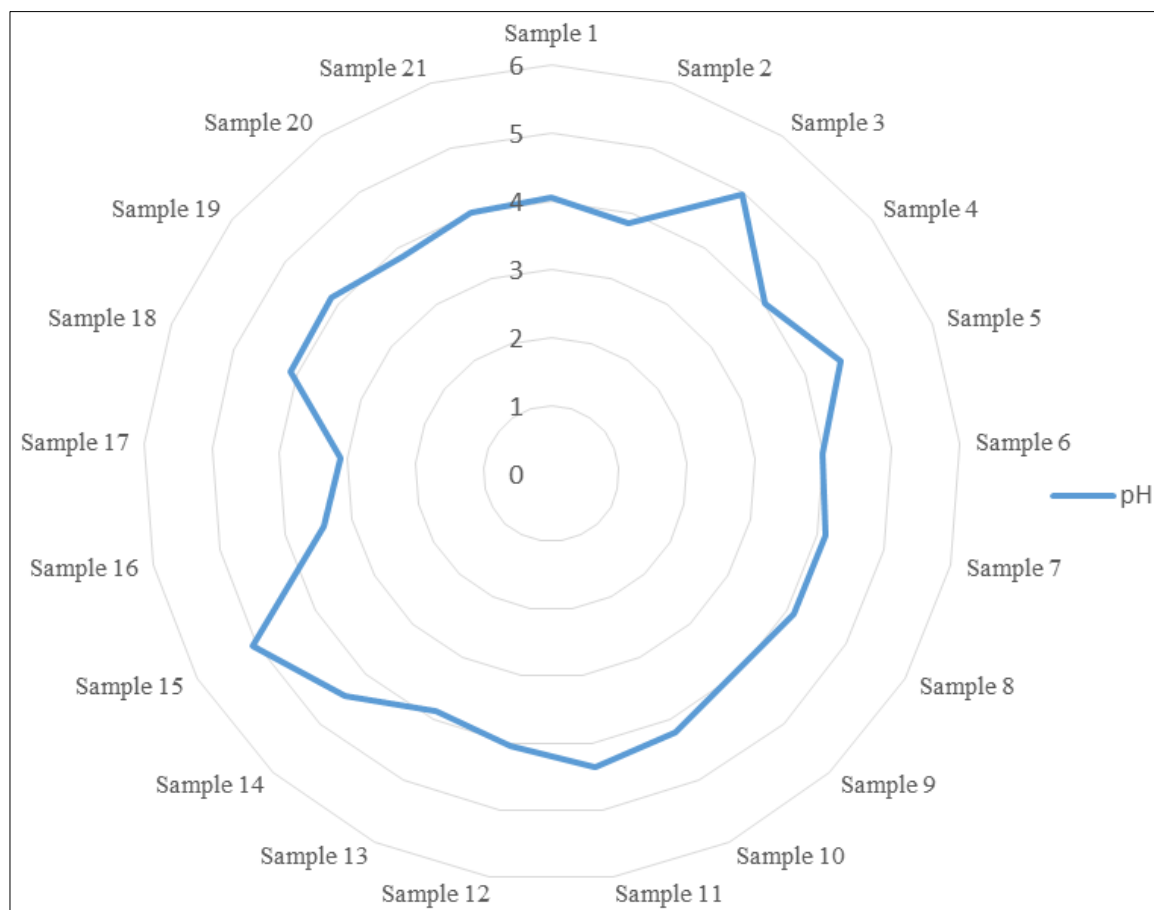
The present study reveal that there was no significant variation between concentration and within the concentration with respect to the zone of inhibition. Also there was no correlation with the pH. Many literature (Manisha Deb Mandal and Shyamapada Mandal, 2011) [5] show the correlation between pH and the zone of inhibition but the result obtained were very interesting and demands for the more and more study in India.

**Table 1:** Antimicrobial property of honey from *A. cerana* against *E. coli*

Sample collection	Type of Flora	pH	<i>E. coli</i>	
			100% dilution	50% dilution
Sample 1	Unifloral	4.06	36.00	20.33
Sample 2	Unifloral	3.85	24.00	13.00
Sample 3	Unifloral	4.96	29.67	14.50
Sample 4	Multifloral	4.00	24.33	13.17
Sample 5	Multifloral	4.55	31.17	22.83
Sample 6	Unifloral	3.99	21.17	11.83
Sample 7	Unifloral	4.12	24.33	16.67
Sample 8	Unifloral	4.11	13.17	19.83
Sample 9	Unifloral	3.97	29.17	16.67
Sample 10	Unifloral	4.22	26.50	20.50
Sample 11	Unifloral	4.36	22.00	13.00
Sample 12	Unifloral	4.04	23.33	16.92
Sample 13	Unifloral	3.88	32.00	20.83
Sample 14	Unifloral	4.45	27.67	11.48
Sample 15	Unifloral	5.06	21.33	8.00
Sample 16	Unifloral	3.43	25.17	9.17
Sample 17	Multifloral	3.10	19.67	12.83
Sample 18	Multifloral	4.11	31.33	13.83
Sample 19	Multifloral	4.14	23.67	10.33
Sample 20	Multifloral	3.86	26.67	19.08
Sample 21	Multifloral	4.01	33.50	8.50
Mean		4.11	25.99	14.92
SD		0.43	2.38	1.08
Max.		5.06	36.00	22.83
Min.		3.10	13.17	8.00
S.Em±		0.021	1.187	1.799
CD@1%		0.081	4.529	6.865

**Table 2:** Zone of inhibition of standard antibiotics against *E. coli*

Antibiotics	Diameter of inhibition zone (mm)
Kenamycin	17
Tetracycline	23
Streptomycin	28
Control	0
Baglur Sample	36
Gkvc Sample	33.5
Mean	22.92
S Em±	3.243
CD@1%	14.007



**Fig 1:** The variation of pH with different samples

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