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Antibiotic Activity of Various Types of Honey of *Apis* Species

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Abstract: Honey is a natural and sweet substance produced by honeybees from the nectar of flowers. It is composed of sugars, acids, proteins, vitamins, enzymes and minor ingredients. It is a readily available source of glucose and fructose and thus has an important role in food and medicine. Honey also functions as an antibiotic agent on few strains of bacteria. To analyze this function, different strains of bacteria were collected and cultured under laboratory conditions. The effect of different concentrations of honey on these bacterial strains was tested. It is found that the honey samples showed antimicrobial activity on different bacterial strains. The minimum inhibitory zones of different strains of bacterial plates were recorded. The honey samples collected from Coorg district showed higher antimicrobial activity than the other regions of Karnataka.

Key words: Antibiotic activity · Bacterial strains · Concentration of honey · Minimum inhibitory zone

INTRODUCTION

Honey is one of the oldest and best-loved sweetening agents for foods and it has still retained a "natural image" over the centuries [1]. Honey, as defined by the Codex Alimentarius [2], is the natural sweet substance produced by honeybees from blossoms or from the secretions of living parts of the plants or excretions of plant-sucking insects living on parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature. Honey is a complex mixture and presents very great variations in composition and characteristics due to its geographical and botanical origin [3, 4]. The main components of honey include various saccharides, water, amino acids, mineral matter, proteins, vitamins and unstable compounds such as enzymes [5-8]. Honey has been broadly used in alimentations, medicine and pharmacology, as well as in various social, religious and other aspects [9, 10].

Honey has other functional applications in veterinary medicine [11]. The medicinal use of honey in wound treatment is derived from diverse ancient civilizations [12]. The antibacterial properties of honey were recognized more than a century ago and have subsequently been extensively studied [13]. A wide range of microbial species has shown to be inhibited by honey [11]. It is remarkable that ancient physicians were selective in the honey that they used in their remedies [12]. In the first laboratory demonstration of antimicrobial activity [14], it is seen that most of the bacteria cause wound infections, burn infections and sore throat. All these infections are cured naturally by the use of honey.

Thus, this study was carried out to investigate the effect of natural honey of different regions of Karnataka on bacterial species such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis* at different concentrations.

MATERIALS AND METHODS

Collection of Honey Samples: For the present study the honey samples of *Apis florea, Apis cerana, Apis dorsata* and *Apis mellifera* were collected from three different regions of Karnataka: Gandhi Krishi Vignan Kendra (GKVK), Bangalore; Pernur, Kushal Nagar of Coorg; and Champion reefs, K.G.F., Kolar. The collected samples were filtered through triple-fold musclene cloth and stored at 35°C.

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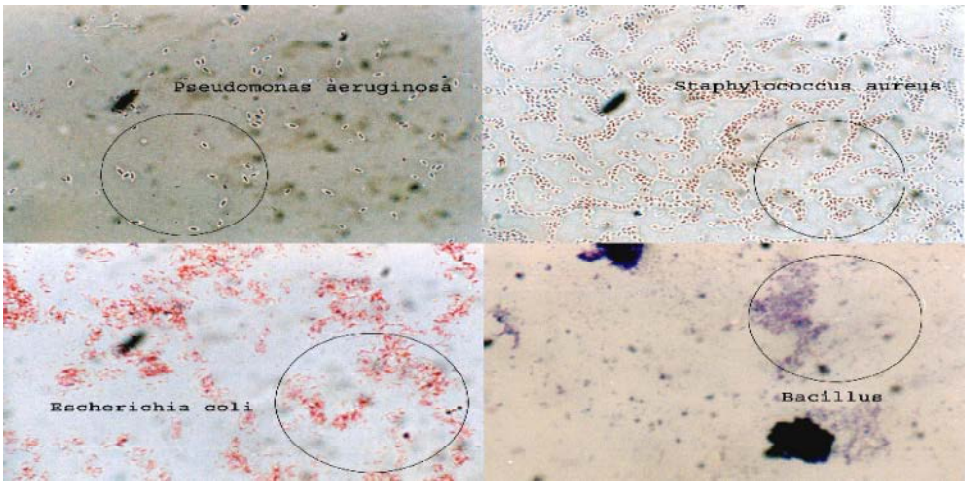

Fig. 1: Bacterial strains subjected for antimicrobial assay test.

Physicochemical Properties of *Apis* Honey Samples: The collected honey samples of *Apis* species were characterized for the following Physicochemical properties.

pH: Each stored honey sample was diluted for 10% (v/v) and pH was tested using digital pH meter [9].

HMF: Hydroxy Methyl Furfural content of honey samples was determined according to the method of [15].

Moisture Content: The moisture content was measured according to the harmonized methods published by the European Honey Commission [10].

Reducing Sugars and Apparent Sucrose Content: The reducing sugars and apparent sucrose content were estimated according to the titration method of Codex Alimentarious Commission [2].

Ash Content: The ash content was determined according to the method [16].

Peroxide Content: The peroxide content was estimated according to the methods of [17] and [9].

Collection of Microbial Strains: The bacterial strains were procured from the Department of microbiology, St. John's Hospital, Bangalore and Don Bosco Institute of Bio-Sciences, Bangalore.

The following four different bacterial strains were included: *E. coli, P. aeruginosa, S. aureus* and *B. subtilis* (Fig. 1).

Preparation of Medium: The collected bacterial strains are initially subcultured on the slants by streak–slant technique. The slant consists of nutrient agar medium (NA).

Culture of pathogens was in nutrient broth medium (Pathogenic suspension).

Chemicals required	*Quantity*
(a) Peptone	5 g
(b) Beef extract	3 g
(c) NaCl	5 g
(d) Agar–Agar	15 g
(e) Distilled water	1000 ml

Weigh various quantities of different components as given in the above chart and dissolve in distilled water. Adjust pH to 7.2; plug the flask with cotton wool. Sterilize the medium at 15 lbs pressure for 15–20 min using an autoclave.

Culture of Pathogens in Nutrient Broth Medium (Pathogenic Suspension)

Composition of Nutrient Broth Medium:

Peptone, 5 g
Beef extract, 2 g
Sodium chloride, 5 g
Distilled Water, 1000 ml (pH 7.0±0.2)
Autoclave for sterilization. The slants were incubated at 37°C for 24 h.

Antimicrobial assay test by Well–Diffusion Technique

Culturing of Pathogens on NA Plates for Antibacterial Assay Test: The sterilized NA medium was poured into the sterile plates and cooled within the Laminar Air Flow.

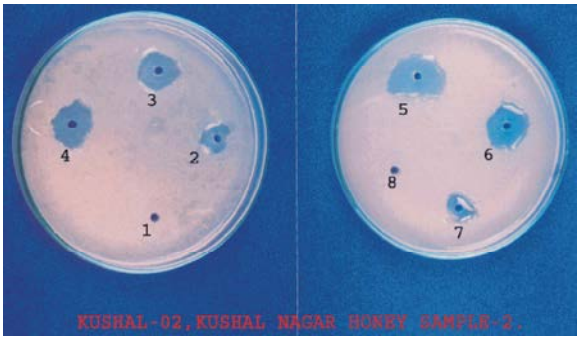

Fig. 2: Antibacterial assay by Agar Well-Diffusion technique.

The pathogenic suspension of 50 µl from nutrient broth culture was spread over the medium using a sterile glass spreader and incubated at 37°C for 24 h. Eight wells were made by using sterile gel puncture in the plates and filled with different concentrations of honey sample. The plates were later incubated at 37°C for 36 h. After the incubation, the clear zones formed were measured by using an 'mm' scale to mark antibacterial zone (Fig. 2).

RESULTS

The results of physicochemical characteristics of different honey samples are summarized in the Table 1 and are in the form of mean±SD with statistical significance at $P<0.01$.

The pH of *A. dorsata* honey samples from Bangalore was highest with 6.1±0.3 and of *A. florea* from Coorg was least with 3.8±0.2. The HMF content of *A. dorsata* honey samples from Bangalore was highest with 19.9±0.3% and of *A. florea* from Coorg was least with 7.6±0.5%. The moisture content of *A. mellifera* honey samples from Kolar was highest with 6.1±0.3% and of *A. mellifera* from Coorg was least with 14.8±2.0%.

The reducing sugar content of *A. florea* honey samples from Coorg was highest with 78.6±2.9% and of *A. dorsata* from Kolar was least with 61.3±0.7%. The sucrose content of *A. mellifera* honey samples from Kolar was highest with 6.1±1.1% and of *A. florea* from Coorg was least with 0.9±0.2%. The peroxide content of *A. dorsata* honey samples from Kolar was highest with 14.3±0.5 µg/g/h at 20°C and of *A. florea* from Coorg was least with 4.2±0.2 µg/g/h at 20°C. The ash content of *A. mellifera* honey samples from Coorg was highest with 0.43±0.3% and of *A. dorsata* from Kolar was least with 0.12±0.3%. The antibiotic sensitivity with inhibitory zone size of the bacteria used in this study is presented in Tables 2-5.

E. coli, P. aeruginosa, S. aureus and *B. subtilis* strains of bacteria did not show any inhibitory zones for honey samples of *A. florea, A. mellifera, A. cerana* and *A. dorsata* from Bangalore and Kolar regions. None of the bacterial strains showed inhibitory zones for all the types of honey samples of *Apis* species at 100% (v/v) concentration. The minimum inhibitory concentration values for *P. aeruginosa* was found to be remarkably consistent. The *P. aeruginosa* strain was inhibited at 100, 50 and 40% dilutions of *A. florea* honey samples; 100 and 40% by *A. mellifera*; 100, 95 and 40% by *A. cerana* and 100, 95, 90%; and 40% by *A. dorsata* honey samples. The maximum inhibitory zone with 17.8±0.19 mm was recorded for *P. aeruginosa* strain in *A. florea* honey samples from Coorg at 60% dilution and the minimum inhibitory zone with 6.1±0.04 mm at 80% dilution.

Table 1: Physicochemical properties of different *A.* honey samples

Regions	Honey types (n=18)	pH	HMF (mg/kg)	Moisture (%)	Reducing sugars (%)	Sucrose (%)	Ash (%)	Peroxide µg/g/h at 20°C
Coorg	*A. florea*	3.8±0.2	7.6±0.5	14.8±2.0	78.6±2.9	0.9±0.2	0.41±0.1	14.3±0.5
	A. mellifera	4.2±0.1	8.2±1.2	14.9±0.0	75.9±1.3	3.4±1.1	0.43±0.3	12.3±0.1
	A. cerana	4.6±0.2	9.1±0.3	15.3±3.5	70.5±0.8	5.1±0.6	0.39±0.1	11.8±0.3
	A. dorsata	4.9±0.2	9.8±1.8	15.6±0.0	61.8±0.6	5.9±0.8	0.40±0.1	10.9±0.1
Bangalore	*A. florea*	5.0±0.3	12.5±0.5	16.2±0.0	71.32±1.8	2.8±0.9	0.36±0.2	10.4±0.3
	A. mellifera	5.4±0.1	16.3±0.3	16.0±2.3	68.6±1.9	3.7±1.1	0.28±0.1	9.2±0.8
	A. cerana	5.4±0.1	18.2±0.4	15.9±0.0	63.5±0.9	3.9±1.4	0.38±0.1	10.6±0.5
	A. dorsata	6.1±0.3	19.9±0.3	16.3±5.1	63.2±2.8	3.8±1.8	0.32±0.2	9.3±0.3
Kolar	*A. florea*	5.2±0.3	18.2±1.3	20.0±4.4	70.6±1.0	4.2±0.3	0.34±0.2	6.1±0.1
	A. mellifera	5.6±0.2	16.4±0.3	20.1±0.0	69.3±1.6	6.1±1.1	0.22±0.1	5.3±0.3
	A. cerana	5.5±0.1	18.5±1.2	18.5±3.9	68.7±1.4	5.6±2.3	0.16±0.1	4.8±0.1
	A. dorsata	5.9±0.2	19.3±1.8	19.6±2.9	61.3±0.7	4.9±2.3	0.12±0.3	4.2±0.2

Mean±SD of five replicants. Significance at $P<0.05$.

Table 2: Minimum inhibitory concentrations of honey samples of *A. florea* showing inhibitory zones in 'mm' with control organisms

	Honey samples of A. florea:			
	Diameter of zones of inhibition (mm)			
	Bacterial strains			
Honey concentration,% (v/v)	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
100	0.0	0.0	0.0	0.0
95	8.2±0.35	9.4±0.33	0.0	8.5±0.51
90	9.8±0.16	12.7±0.22	7.8±0.21	11.6±0.18
85	11.4±0.21	14.9±0.12	10.5±0.16	7.5±0.34
80	9.4±0.11	17.8±0.19	13.8±0.11	6.7±0.16
75	6.7±0.17	11.4±0.06	15.7±0.19	0.0
70	6.1±0.19	8.7±0.17	11.7±0.28	0.0
60	0.0	6.1±0.04	9.5±0.54	0.0
50	0.0	0.0	6.7±0.24	0.0
40	0.0	0.0	0.0	0.0

Mean±SD of five replicants. *P*-value significant at 0.01%.

Table 3: Minimum inhibitory concentrations of honey samples of *A. mellifera* showing inhibitory zones in 'mm' with control organisms

	Honey samples of A. mellifera:			
	Diameter of zones of inhibition (mm)			
	Bacterial strains			
Honey concentration,% (v/v)	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
100	0.0	0.0	0.0	0.0
95	0.0	8.9±0.57	0.0	0.0
90	6.4±0.14	11.5±0.19	0.0	0.0
85	8 .7±0.11	15.8±0.47	7.6±0.31	7.4±0.11
80	10.3±0.42	16.3±0.11	11.7±0.58	10.4±0.22
75	7.8±0.19	13.8±0.04	14.8±0.67	9.3±0.81
70	6.3±0.84	10.8±0.13	10.1±0.22	6.2±0.96
60	0.0	9.8±0.56	7.4±0.95	0.0
50	0.0	6.7±0.24	0.0	0.0
40	0.0	0.0	0.0	0.0

Mean±SD of five replicants. *P*-value significant at 0.05%.

Table 4: Minimum inhibitory concentrations of honey samples of *A. cerana* showing inhibitory zones in 'mm' with control organisms

	Honey samples of A. cerana			
	Diameter of zones of inhibition (mm)			
	Bacterial strains			
Honey concentration,% (v/v)	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
100	0.0	0.0	0.0	0.0
95	0.0	0.0	0.0	6.9±0.34
90	7.3±0.11	6.7±0.35	7.1±0.94	9.5±0.67
85	8.2±0.33	7.9±0.55	8.9±0.61	12.3±0.17
80	9.4±0.97	11.4±0.81	11.7±0.17	8.6±0.51
75	7.2±0.12	14.9±0.64	9.7±0.66	6.4±0.53
70	6.8±0.64	11.7±0.11	7.4±0.67	0.0
60	0.0	8.5±0.31	6.9±0.53	0.0
50	0.0	6.6±0.81	0.0	0.0
40	0.0	0.0	0.0	0.0

Mean±SD of five replicants. *P*-value significant at 0.01%.

Table 5: Minimum inhibitory concentrations of honey samples of *A. dorsata* showing inhibitory zones in 'mm' with control organisms

	Honey samples of A. dorsata			
	Diameter of zones of inhibition (mm)			
	Bacterial strains			
Honey concentration,% (v/v)	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
100	0.0	0.0	0.0	0.0
95	0.0	0.0	0.0	0.0
90	6.8±0.54	0.0	0.0	6.8±0.54
85	9.7±0.65	6.7±0.94	6.9±0.37	8.4±0.68
80	8.9±0.14	7.8±0.38	8.4±0.44	9.7±0.83
75	6.4±0.92	10.5±0.86	7.1±0.92	7.5±0.81
70	0.0	13.7±0.84	0.0	6.1±0.57
60	0.0	11.7±0.11	0.0	0.0
50	0.0	7.2±0.28	0.0	0.0
40	0.0	0.0	0.0	0.0

Mean±SD of five replicants. *P*-value significant at 0.05%.

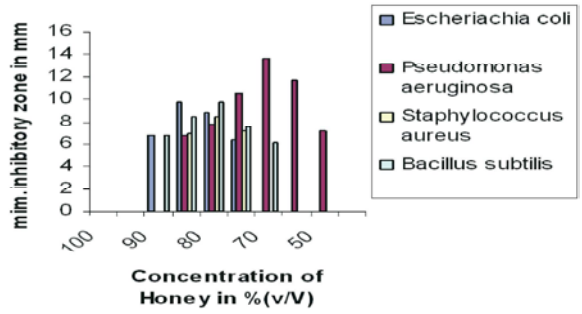

Fig. 3: Degree of inhibitory action of honey sample of *A. florea* against various bacterial strains at different concentrations.

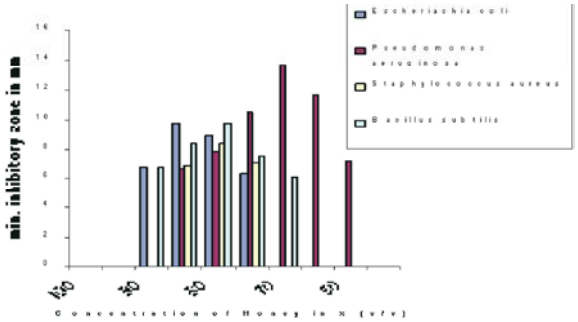

Fig. 5: Degree of inhibitory action of honey sample of *A. cerana* against various bacterial strains at different concentrations.

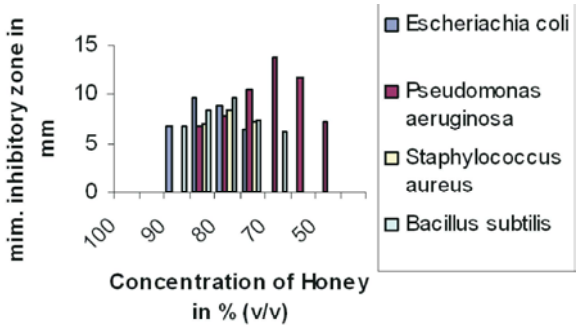

Fig. 4: Degree of inhibitory action of honey sample of *A. mellifera* against various bacterial strains at different concentrations.

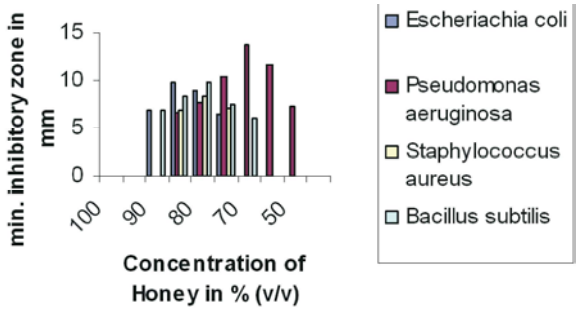

Fig. 6: Degree of Inhibitory action of Honey sample of *A. dorsata* against various Bacterial strains at different concentrations

The maximum inhibitory zone with 11.4±0.21 mm was recorded for *E. coli* strain in *A. florea* honey samples from Coorg at 85% dilution and the minimum inhibitory zone with 9.4±0.97 mm was recorded for *A. mellifera* honey at 80% dilution. The maximum inhibitory zone with 15.7±0.19 mm was recorded for *S. aureus* strain in *A. florea* honey samples from Coorg at 75% dilution and the minimum inhibitory zone with 8.4±0.44 mm was recorded for *A. dorsata* honey at 80% dilution. The maximum inhibitory zone with 12.3±0.17 mm was recorded for *B. subtilis* strain in *A. mellifera* honey samples from Coorg at 85% dilution and the minimum inhibitory

zone with 9.7±0.83 mm was recorded for *A. dorsata* honey at 80% dilution. The data were significant at $P<0.01$ for *A. florea* and *A. cerana* honey samples and $P<0.05$ for *A. mellifera* and *A. dorsata* honey samples.

DISCUSSION

This study reports on physicochemical properties that were in accordance with the published results of [19]. The pH values ranged from 3.8±0.2 to 6.1±0.3 were in accordance with the results of [20] and [9]. The moisture content varied from 14.8±2.0 to 20.3±4.4%. The minimum range of moisture content is due to the low rate of fermentation. The HMF content, sucrose levels and reducing sugars were within the range of Current Codex Standard and confirmed the freshness of samples [21]. The ash and peroxide contents were also within the range in accordance with [9, 20, 22]. In conclusion, physicochemical characteristics of all the collected honey samples of Coorg showed much closer values of Codex Standard, indicating good quality. However, low standards were noticed for the honey samples of Kolar and Bangalore.

The medicinal use of honey in wound treatment is derived from diverse ancient civilizations [12]. Several authors are of the opinion that the saccharide content of honey is exclusively responsible for its antibiotic activity [13, 23-28]. Whereas, the present study results depict the results controversial to these authors as the honey samples were quite fresh. However, according to [15], variation in antibiotic activity of these natural honeys are not attributable to sugars content alone. [13] reported that the acidity (low pH) and peroxide contents are important for antibacterial activity. Slow generation of peroxide content inhibits the bacterial growth [24]. According to [13], as the dilution of Manuka honey increases (up to 50%), the size of the inhibitory zones also increases. This was proved in the present paper report for the Coorg honey samples. In conclusion, low pH (acidity), high reducing sugars, moisture and peroxide contents upon slow dilution of honey samples assures that *A. florea* and *A. mellifera* honey samples from Coorg are of good quality as they show good range of physicochemical characteristics and highest antibacterial activity. On the basis of our studies, we suggest that the honey samples from Coorg (Karnataka) may serve as a potent antibacterial agent on the above-described bacterial strains.

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