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EVALUATION OF ANTIBACTERIAL ACTIVITY OF *APIS* HONEYS FROM PADERU FOREST DIVISION IN ANDHRA PRADESH INDIA

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ABSTRACT: Antibacterial activity of the two honey samples produced by the honey bee (*Apis florea*), was standardized by Disc diffusion method. The two honey samples were tested at three concentrations (5, 10 and 15µl v/v) and zone of inhibition were measured against *Staphylococcus aureus, Escherichia coli, Basillus subitis, Pseudomonas putida* and *Klebsiela pneumonia*; unifloral and multifloral honey samples were effected in inhibiting the growth of the gram positive and Gram negative organisms at (15 µl v/v). The inhibitory effect of unifloral honey on (15µl) *Staphylococcus aureus* and *Escherichia coli*; and multi floral honey on *E.coli* were comparable to that of Amphicillin (10µg/10µl). In conclusion, natural honey can be employed in treating, some common diseases especially those caused by the tested microorganisms and carry the potential of introducing new template into modern medicinal biology.

INTRODUCTION: The medicinal properties of honey have been reported and documented by the bee-keepers and medicinal practitioners [1, 2]. As a result of over-use and abuse of antibiotics, there has been an increase in the number of diseases, which seem to evolve to become more virulent with each generation. Investigations into natural and potent antibacterial seemed to be the right step to overcome the problem. Honey has been used as a medicine since ancient times by different people of various cultures and is still is used in folk medicine.

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Use of honey as a therapeutic substance has been rediscovered by the medical practitioners in more recent times and it is gaining importance as an antibacterial agent for the treatment of various diseases caused by different microorganisms, recent study examining the antibacterial properties of honey *in vitro* undertaken by[3, 4, 5].

The antibacterial activity of honey was recognized [6]. The antibacterial potency of honey has been attributed to its strong osmotic effect, naturally low pH [3]. Honey is produced from many sources and its antimicrobial activity varies greatly with origin and processing. Honey has been used as a medicine in many cultures for long time [7]. Honey is sweet syrup and collected by Bees from various flowers. It is considered to be natural syrup. The nectar is gathered by the bees and is slowly transformed into honey, through a long process involving the

addition of enzymes and the gradual reduction of moisture.

Honey is a rich source of carbohydrates mainly fructose and glucose. The chemical composition of honey varies depending on source plants, seasons and production methods. Therefore the colour, concentration and compounds vary depending on the floral sources. Other compounds which can be found in honey include proteins and acids such as Gluconic acid, minerals and Anti-oxidants such as Hydrogen peroxide (H_2O_2) and vitamins B6 &B12[8].

The present study aimed to evaluate the antibacterial activity of different origin of honey samples against different pathogenic microorganisms and whose activity is compared with selected broad spectrum antibiotic.

MATERIALS AND METHODS:

Collection of honey samples:

Two types of honey samples produced by *Apis florea* were evaluated. These honeys were collected from Paderu forest division, Visakhapatnam in Andhra Pradesh, India, which are aseptically collected in sterile container plastic bottles and stored in a cool and dry place at 35°C.

Acetolysis technique:

For pollen analysis of the honey samples, 5gm of honey was dissolved in 10 ml of distilled water and centrifuged for 10 min at 2500 rpm. The supernatant solution was decanted and sediment was treated with acetolysis mixture [9]. Three pollen slides were prepared from each sample, and pollen types were identified with the help of relevant literature. These pollen types were classified based on the recommendations of the International Commission for Bee-Botany [10] i.e. predominant pollen type having more than 45% of pollen count; secondary pollen type(16-45%); important minor pollen type(3-15%) and minor pollen type (< 3%).

Preparation of test organisms:

Stocked culture of Gram positive and Gram negative bacteria's (*Staphylococcus aureus, Escherichia coli, Bacillus subitis, Pseudomonas putida* and *Klebsiela pneumonia)* used in this study were obtained from the Department of Biochemistry, Osmania University, Hyderabad. The isolates were identified based on standard Microbiological techniques, and sub-cultured in nutrient agar slopes at 37°C for 24 hrs. Colonies of fresh cultures of the different microorganisms from overnight growth were picked with sterile inoculating loop and suspended in 10 ml nutrient broth contained in sterile and incubated for 6 hrs at 37°C.

Antibacterial assay:

The agar diffusion technique (disc diffusion method) was employed. The honey samples were first inoculated separately on standard nutrient media with no test organisms so as to evaluate their possible contamination. Thereafter, solidified nutrient agar plates were separately flooded with the liquid inoculums of the different test organisms using the pour plate method. The plates were drained and allowed to dry at 37° C for 30 min after which four equidistant What-man paper of 6 mm in diameter were pleased at different sites on the plates. 50 µl volume of honey sample prepared as stock.

Different concentrations of (5µl, 10µl and 15µl v/v) of the honey samples were separately placed on the different What-man paper disc with micropipette. The plates were allowed to stay for 15 min for pre-diffusion to take place followed by an overnight incubation that lasted for 24 hrs at 37° C. The resulting zones of inhibition were measured with the use of a caliper and recorded. The Minimal inhibitory Concentrations (MIC) of honey samples were carried out using the modified method[11] and reported as the least concentration that inhibited the growth of the test organisms and also conventional antibiotic (Amphicillin 10µg/10µl) as a standard drug disc was placed on them. The diameters of the zones of inhibition were measured (mm).

RESULT AND DISCUSSION:

The unifloral honey was characterized by the presence of *Schleichera oleosa*, (85%) as predominant pollen type. *Mimosa pudica* (15%), *Eucalyptus globulus* (7.6%), *Gnaphalium polycaulon* (6.8%) *Clerodendrum inerme* (3%) and *Feronia elephantum* (3.6%) are recorded as important minor pollen types.The minor pollen types are *Psidium guajava* (2.6%), *Saccharum officinarum* (1.2%), *Syzygium heyneanum* (1.6%),

Leucas aspera (1.3%), *Dillenia pentagyna* (1.6%), and *Pavetta crassicaulis* (2.3%) (**Plate 1; Fig. 1**).

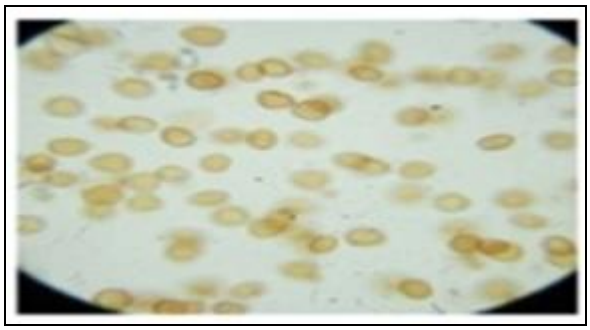


FIG 1 : UNIFLORAL HONEY

The multifloral honey was characterized by the presence of *Mimosa pudica* (37.3%), *Schleichera oleosa* (35.9%), *Tridax procumbens* (18%), *Psidium guajava* (18.3%), *Bauhinia purpurea* (21.6%), *Syzygium cumini* (19%), *Commilina suffruticosa* (32.3%), *Crotalaria juncea* (25%), *Eucalyptus globulus* (28%) and *Centipeda minima* (20%) are recorded as secondary pollen types. *Ageratum conyzoides* (15%), *Conyza strica* (15%), *Cassia auriculata* (11.6%), *Celasrtus paniculata* (15.3%), *Sapindus emarginatus* (15.7%), *Terminalia arjuna* (10%), *Lannea coramandalica* (15.3%), *Citrullus lanatus* (4%), *Erythrina indica* (5%) and *E. suberosa* (7.3%) are recorded as important minor pollen types. *Acacia chandra* (2.3%), *Albizia lebbeck* (1.3%) and *Lantana camara* (2%) are recorded as minor pollen types (**Plate1; Fig. 2**).

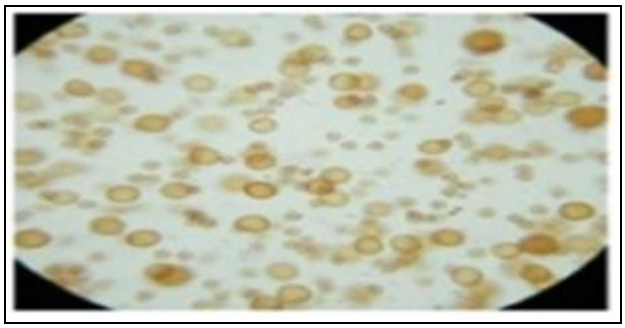
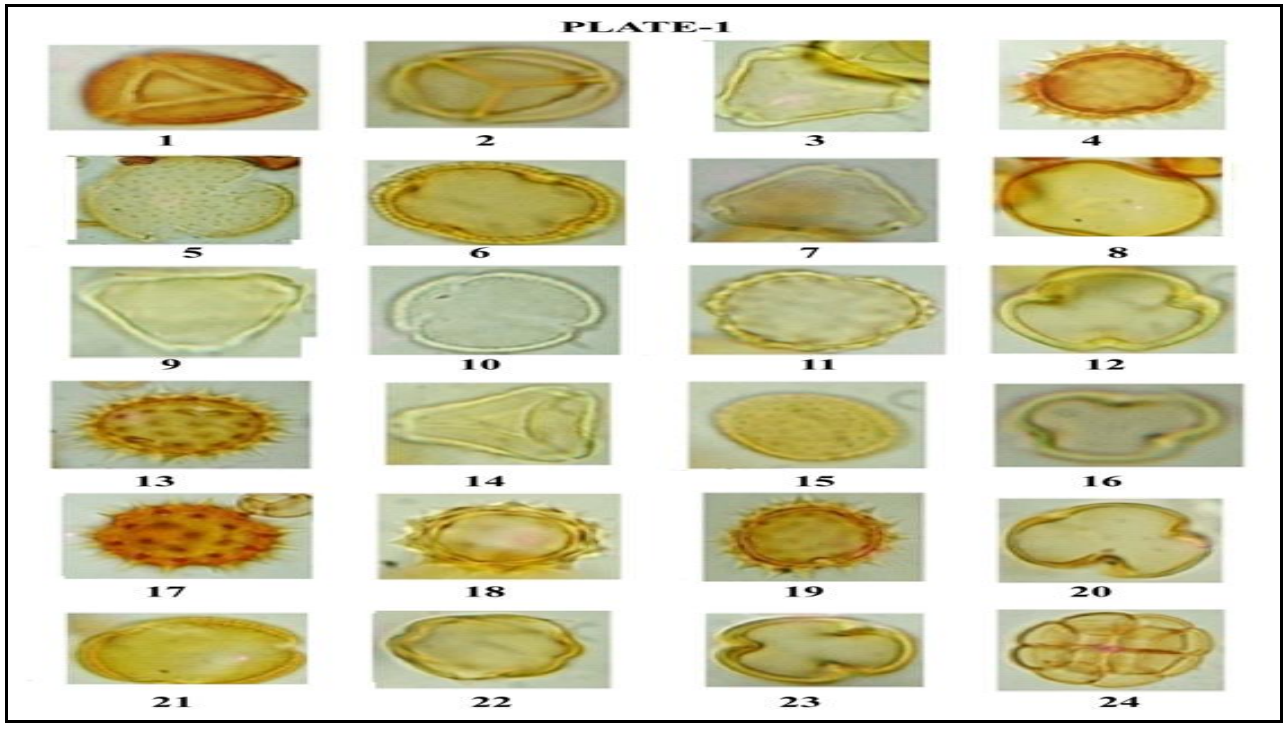


FIG 2: MULTIFLORAL HONEY



EXPLANATION OF PLATES -1

1. *Schleichera oleosa* **2.** *Mimosa pudica* **3.** *Eucalyptus globulus* **4.** *Gnaphalium polycaulon*

5. *Clerodendrum inerme* **6.** *Feronia elephantum* **7.** *Psidium gaujava* **8.** *Saccharum Sp.*

9. *Syzygium heynearum* **10.** *Leucas aspera* **11.** *Dillenia pentagyna* **12.** *Pavetta crassicaulis*

13. *Tridax pocumbens* **14.** *Syzygium cumini* **15.** *Commilina suffruticosa* **16.** *Crotalaria juncea*

17. *Centipeda minima* **18.** *Ageratum conyzoides* **19.** *Conyza Strica* **20.** *Cassia auriculata*

21. *Cealastrus paniculata* **22.** *Terminalia sp.* **23.** *Lannea coramandalica* **24.** *Albizia lebbeck*

The result of the *in vitro* susceptibility of the test organisms on some unifloral and multifloral honey samples were differed in their activity. All the test organisms were sensitive to 15µl (v/v) concentration of both honey samples, but higher susceptible at 5µl (v/v) concentration (**Fig. 3, 4; Table. 1, 2 & Graph-1, 2**). In unifloral honey sample had higher antibacterial activity on Gram positive *Staphylococcus aureus* when lower activity on *Pseudomonas putida* at 15µl (v/v) (**Graph-1**). In this observation *Pseudomonas putida* was higher susceptible among the all test organisms in all concentrations (5, 10 and 15µl v/v) in this unifloral honey.

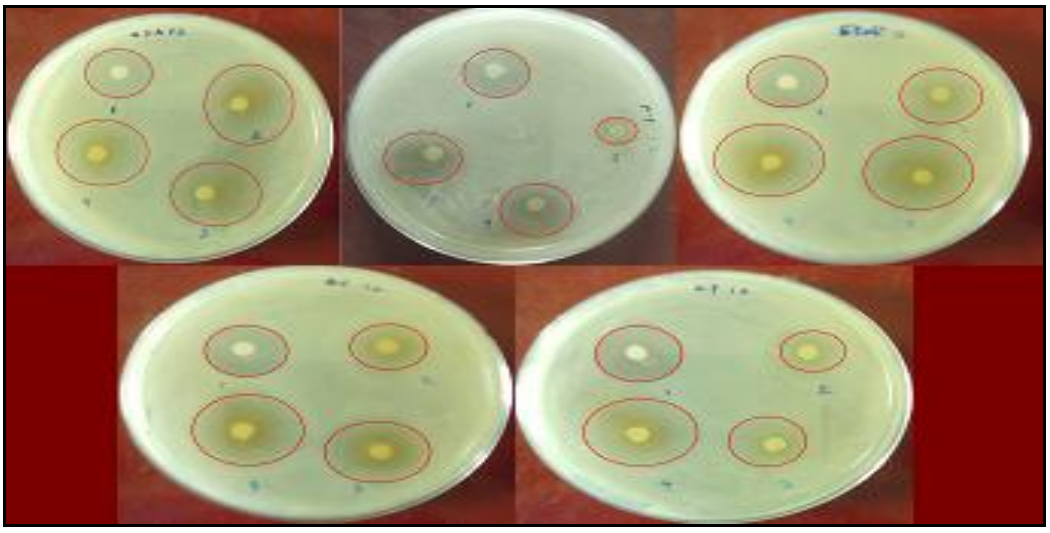


FIG. 3: BACTERIAL INHIBITION UNIFLORAL HONEY DIFFERENT CONCENTRATIONS

(1)Amphicillin (10µg/10µl), (2). Honey 5% µl v/v, (3). Honey 10% µl v/v, (4).Honey 15% µl v/v.

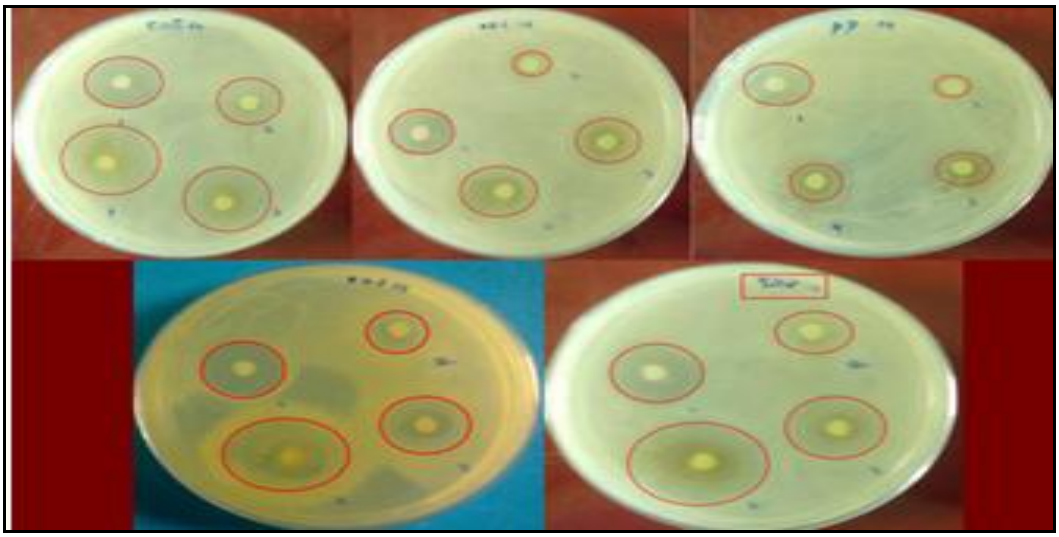
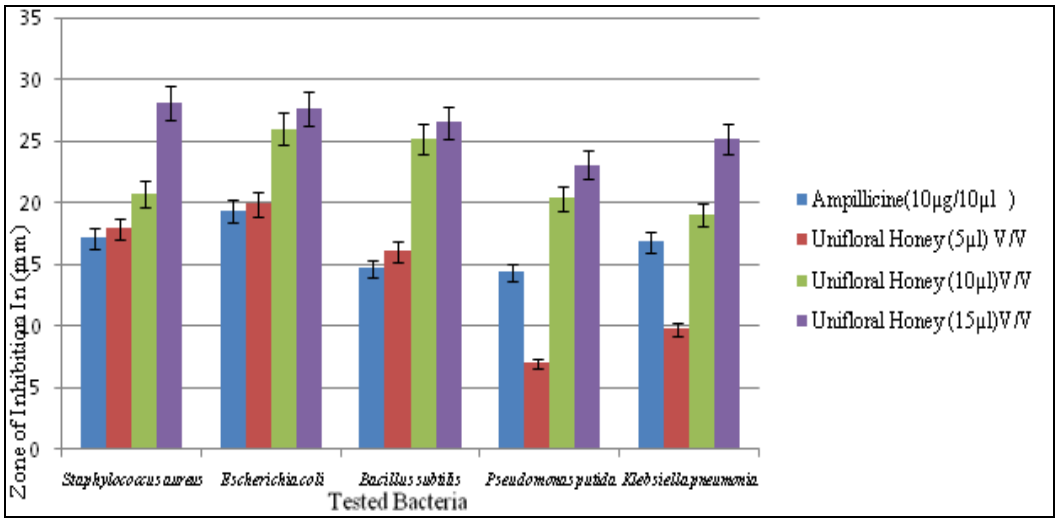


FIG. 4: BACTERIAL INHIBITION MULTIFLORAL HONEY DIFFERENT CONCENTRATIONS

(1) Amphicillin (10µg/10µl), (2).Honey 5%µl v/v, (3). Honey 10%µl v/v, (4). Honey 15%µl v/v.

TABLE 1: MINIMAL INHIBITORY CONCENTRATION OF UNIFLORAL HONEY

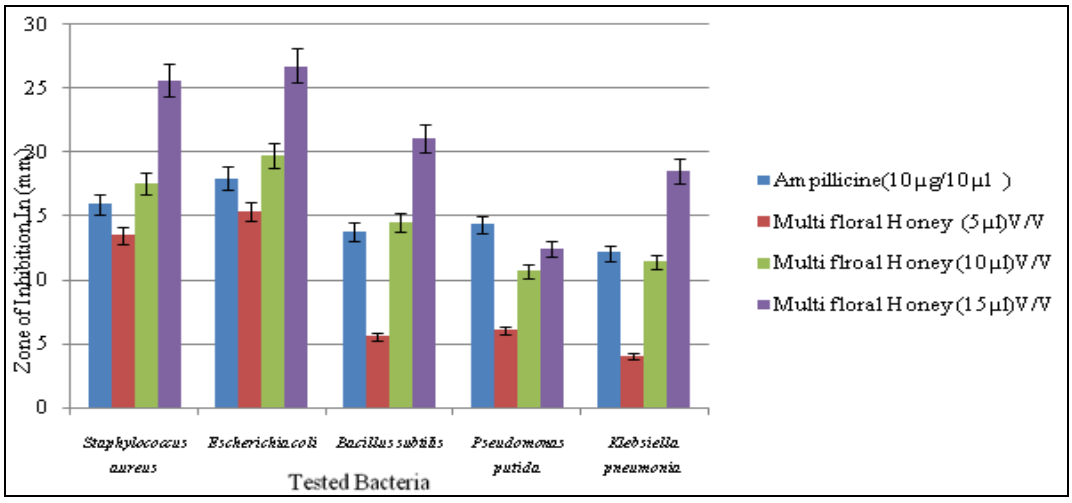
S.No	Name of Micro Organism	Amphicilli (10µg/10µl)	Unifloral Honey (V/V) (5%µl)	(10%µl)	(15%µl)
1	*Staphylococcus aureus*	17.05	17.62	20.73	28.09
2	*Escherichia coli*	19.32	19.84	25.97	27.58
3	*Bacillus subtilis*	14.63	16.00	25.17	26.50
4	*Pseudomonas putida*	14.27	6.95	20.32	23.02
5	*Klebsiella pneumonia*	16.74	9.71	18.96	25.19



GRAPH-1: ANTIMICROBIAL ACTIVITY OF UNIFLORAL HONEY

TABLE 2: MINIMAL INHIBITORY CONCENTRATION OF MULTIFLORAL HONEY

S.No	Name of Micro Organism	Amphicillin (10µg/10µl)	Multifloral Honey (V/V)		
			(5%µl)	(10%µl)	(15%µl)
1	*Staphylococcus aureus*	15.92	13.53	17.54	25.62
2	*Escherichia coli*	17.97	15.35	19.73	26.74
3	*Bacillus subtilis*	13.77	5.58	14.48	21.07
4	*Pseudomonas putida*	14.33	6.08	10.68	12.42
5	*Klebsiella pneumonia*	12.14	4.05	11.42	18.53



GRAPH 2: ANTIBACTERIAL ACTIVITY OF MULTIFLORAL HONEY

Klebsiella pneumonia was shown higher susceptible among the all test organisms in all concentrations (5, 10 and 15 µl v/v) in multifloral honey, which had shown higher antibacterial activity in *E.coli* in all concentrations compared with Amphicillin standard. Honey samples in the different concentrations were more effective against *E.coli* than other bacteria [12]. Collectively, present findings revealed that the honey had higher antibacterial activity against *E. coli* in all concentrations except 15 µl (v/v) in unifloral honey; *Klebsiella pneumonia* and *Pseudomonas putida* were more susceptible in among the all test organisms.

Hence the unifloral honey was more antibacterial activity than compared with the multifloral honey at 15µl % (v/v) in the 6 hrs incubation period. In which the both honey samples were showed more

zone of inhibition activity than Amphicillin (10µg/10µl) standard drug; the unifloral honey will be used in human pathogens and evolution in biomedical applications.

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