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A PROSPECTIVE STUDY OF HONEY WITH SPECIAL REFERENCE TO ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

Background: Honey is a sweet food made by bees foraging nectar from flowers. The variety produced by honey bees by the genus Apis cerana indica is the one most commonly referred by most of the beekeepers and that honey is consumed by people in India. Many natural products like medicinal plants producing non-antibiotic drugs having antibacterial potentiality. Beside these products of some medicinal plants, the antibacterial activity of honey against many different life threatening bacteria has been reported.

Materials & Methods: The antibacterial activity of Bharat multi floral pasteurised honey obtained from Bharat Unani Pharmacy (Bharat honey co), Hyderabad, Andhra Pradesh, India was tested and evaluated against the bacterial strains of *Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus* by using agar-well diffusion method.

Results: It was observed that zone of inhibition was indirectly proportional with the dilution of honey as the dilution was less, the zone of inhibition was more in all 3 organisms tested. *P. aeruginosa, Esch.coli and S. aureus* were the most sensitive to undiluted honey samples tested with an average zone of inhibition of 39.96, 30.1 and 28.2 mm respectively.

Conclusions: The exact explanation for the antibacterial activity of honey is not known, but it is clear that the higher the concentration of honey the greater its usefulness as an antibacterial agent. Well documented clinical trials and researches are going on honey and nanotechnology which may provide promising results on therapeutic use of honey in the future.

KEYWORDS: Honey, Antibacterial Activity, Well Diffusion, *Pseudomonas aeruginosa*, *Escherichia Coli*, *Staphylococcus aureus*

INTRODUCTION

Honey is a sweet food made by bees foraging nectar from flowers. The variety produced by honey bees by

the genus Apis cerana indica is the one most commonly referred to, as it is the type of honey collected by most beekeepers and consumed by people in India. Honey bees convert nectar into honey by a process of regurgitation and evaporation: they store it as a primary food source in wax honeycombs inside the beehive. The common varieties of honey available in india are Rapeseed / Mustard Honey, Eucalyptus Honey, Lychee Honey, Sunflower Honey, Karanj / Pongamea Honey, Multi-flora Himalayan Honey, Acacia Honey, Wild Flora Honey, Multi and Mono floral Honey. Honey gets its sweetness from the monosaccharides fructose and glucose, and has about the same relative sweetness as granulated sugar[1,2]. Most microorganisms do not grow in honey so sealed honey does not spoil, even after thousands of years[3,4]. However, honey sometimes contains dormant endospores of the bacterium *Clostridium botulinum*, which can be dangerous to babies, as it may result in infantile botulism.[5] Historical documents indicates that ancient people used honey for medicinal purposes from locally available honeys, for example Ambroise Par (1510-1590) specifically advocated the use of rose honey for the production of a debriding agent for wounds[6]. Dioscorides advised the use of pale yellow honey from Attica for the treatment for rotten and hollow ulcers and Aristoles refers to pale honey as particularly useful for the preparation of salves for sore eyes and wounds. Even today in folk medicine some honeys are of more value than other, like strawberry honey in Sardinia, lotus honey in India (for the treatment of eye problems) and honey from the Jirdin valley in Yemen for their high therapeutic usefulness[7]. Multidrug resistant bacteria causing human infection was common in clinical practice due to continuous and indiscriminate use of antimicrobials[8]. To fight against such multi drug bacterial resistance to those antimicrobials, scientists discovered many natural products like medicinal plants producing non-antibiotic drugs having antibacterial potentiality[9,10]. Beside the products of some medicinal plants, the antibacterial activity of honey against many different life threatening bacteria has been reported by many scientists[11,12,13]. It has been reported that honey showed both bacteriostatic and bactericidal effect against many gram positive as well as gram-negative bacteria[14,15,16,17,18.] Full formed honey consists of 80% sugars, mainly glucose and fructose and some sucrose and maltose, and contains 18% moisture content causes osmotic stress, which prevents spoilage of honey by microorganisms and that sugar content of honey is sufficient to retain antibacterial activity of honey when diluted to approximately 30–40%. At higher dilutions, the antibacterial activity is due to other compounds than sugar. Later it was identified that H_2O_2 was identified as a major antibacterial compound in honey that was responsible for its antibacterial activity[7,19,20]. The enzyme glucose oxidase added by honey bees to the collected nectar during production of honey is activated on moderate dilution of honey and converts glucose into H_2O_2 and gluconic acid. However, some honeys have substantial antibacterial activity due to nonperoxide components. Recently, methylglyoxal and bee defensin-1 have been identified in manuka honey as antibacterial compound in honey[21,22.] Recently, exceptionally high levels of the antimicrobial compound methylglyoxal (MGO) have been found in manuka honey[21,22,23,24]. In addition, there are clear indications for the presence of additional honey antibacterial compounds of which the identity remains to be elucidated. An attempt was made to evaluvate the antibacterial activity of Bharat honey obtained from Bharat Unani Pharmacy (Bharat Honey Co), Hyderabad, India against the bacterial strains of *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923 by using agar-well diffusion method.

MATERIALS AND METHODS

The antibacterial activity of Bharat multi floral pasteurised honey obtained from Bharat Unani Pharmacy (Bharat honey co), Hyderabad, Andhra Pradesh, India was tested and evaluated against the bacterial strains of *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923 by using

agar-well diffusion method[17,25,26,] A 4-6 hour incubated bacterial culture suspension matching with 0.5 Mc-Farland scale standard was prepared in 5ml peptone water and spread onto the sterile Mueller-Hinton agar (Himedia, Mumbai) plates to prepare a lawn culture. Dried in the incubator for half an hour and 5 wells were prepared in each plate using sterile 6 mm cork borer. Two such plates were prepared for each bacterial strain. To investigate the antibacterial activity, 50 µl of honey samples of serial dilutions (undiluted, 1:10, 1:20 upto 1:70) were added into each well. Sterile Normal saline as negative control and Dettol (Reckitt Benckiser, India) as positive control were also included. Plates were left for 1 h at 25 °C to allow a period of preincubation diffusion in order to minimize the effect of variation in time between the applications of different solutions. The plates were re-incubated aerobically at 37°C overnight to allow bacterial growth. After incubation, plates were observed and the zones of inhibition were measured to evaluate the antimicrobial activity for each of the tested honey dilution samples using a special scale obtained from Himedia, Mumbai, India. The experiment was carried out in triplicates for statistical relevance and the Mean± SD of results was calculated.

RESULTS

Tables 1 shows the Results of antibacterial activity of honey towards the 3 organisms tested. *P. aeruginosa, Esch.coli and S. aureus* were the most sensitive to undiluted honey samples tested with an average zone of inhibition of 39.96, 30.1 and 28.2 11.6 mm respectively. It was observed that zone of inhibition was indirectly proportional with the dilution of honey as the dilutin was less, the zone of inhibition was more in all 3 organisms tested. In P.aeruginosa, no zone of inhibition was observed with 1in 50, 60, 70 dilutions and dettol showing the resistant pattern. *Esch. coli* also showed resistance in I in 60 and 70 dilutions where as *Staphylococcus aureus* showed sensitive zones in all dilutions. No zone of inhibition was seen in all dilutions of normal saline (negative control) in all 3 organisms where as dettol (positive control) showed sensitive zones only for *Staph. aureus* and *Esch.coli* (Figure 1,2,3).

DISCUSSIONS

The antibacterial effect of honey samples on microorganisms increased as honey concentration was increased. The average inhibition zone of the undiluted honey samples (100%, w/w) on selected pathogenic microorganisms were 39.96, 30.1 and 28.2 11.6 mm for *P. aeruginosa, Esch.coli and S. aureus* respectively. Honey samples used in this study showed higher antibacterial activity for Gram negative than Gram positive microorganisms in accordance with the findings of Cooper et al[27,28]., in their two studies and also by Wilkinson and Cavanagh[29] and Vishnu Prasad et al[30]. where as the studies of Ogbaje et al [16] Agbagwa et al[17,] Rahmanian et al[31]., and Jeddar et al[32]., and Cooper et al[33]., found that Honey samples used in their study showed higher antibacterial activity for Gram positive than Gram negative microorganisms. The reason may be of using different types of honey in their study having different antibacterial activity. Any zone diameter havening less than 7mm shows that the organism is resistant to the honey sample but if the zone diameter is greater than 11 mm it suggests that the microorganism is sensitive to the honey Sample to the microorganisms tested[17]. At 1in 60 and 1in 70 dilutions none of the gram negative microorganisms ere inhibited. Differences in the level of sensitivity may be due to variation in the antibacterial potential of honey used in the present study and the source of honey samples. The source of the nectar used in the production of the honey may have caused the differences in the antimicrobial activities of honeys from different sources[34].

CONCLUSIONS

The exact explanation for the antibacterial activity of honey is not known, but it is clear that the higher the

concentration of honey the greater its usefulness as an antibacterial agent. However, it is excepted that the clinical significance of the antibacterial activity in honey will be unequivocally proven only if a clinical trial is conducted to compare dressings of sugar and selected honeys [35] Although more research is needed, as with many of the therapeutic interventions used in modern wound care, in the absence of data from well controlled clinical trials. Recent reviews on the successful usage of honey as a dressing on infected wounds shows that many authors support the use of honey in infected wounds and some suggest the prophylactic use of honey on the wounds of patients susceptible to MRSA and other antibiotic-resistant bacteria.[36,37,38,39] Well documented clinical trials and researches are going on honey and nanotechnology which may provide promising results on therapeutic use of honey in the future.

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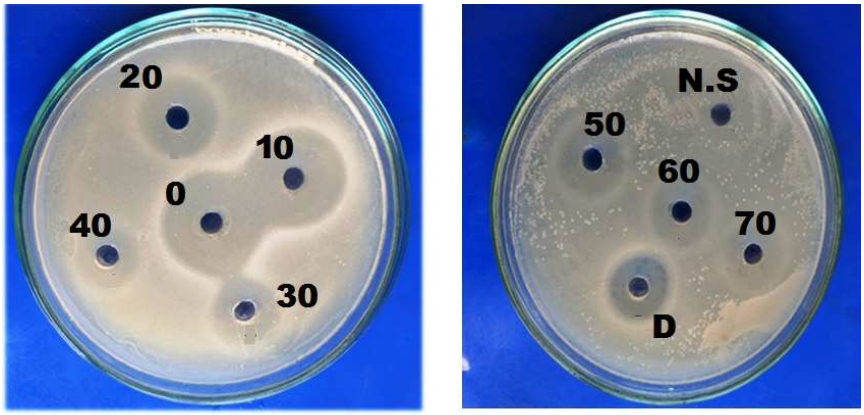
APPENDICIES

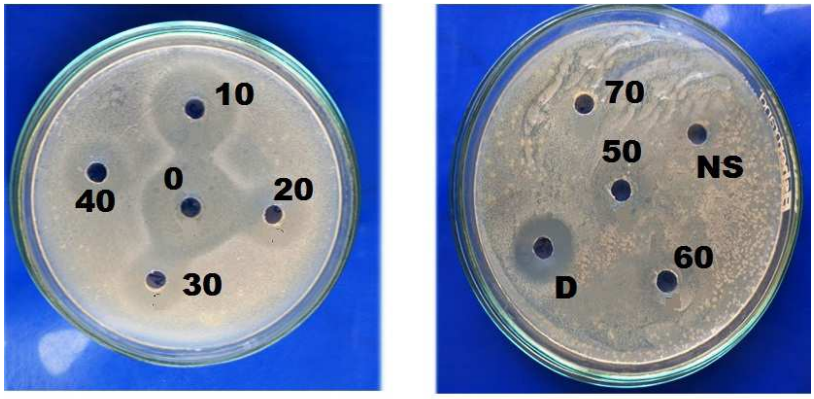
Table 1: Antibacterial Activity of Western Nigerian Honey against Selected Microorganisms

Name of the Organism	Undiluted Honey	Diluted Honey with Different Concentrations (v/v) with Diameter Zone of Inhibition (mm)							Positive Control	Negative Control
	0	10	20	30	40	50	60	70	Dettol (D)	Normal Saline (NS)
Staphylococcus aureus	28.2±0.20	25.03±0.15	22.2±0.20	18.13±0.15	16.2±0.20	15.06±0.20	13±0.20	10±0.20	22±0.20	0
Escherichia coli	30.1±0.10	28±0.20	20.1±0.10	18.1±0.10	16.16±0.15	11.23±0.20	0	0	20.13±0.15	0
Pseudomonas aeruginosa	39.96±0.15	25.2±0.20	18.25±0.20	15±0.20	10.16±0.15	0	0	0	0	0



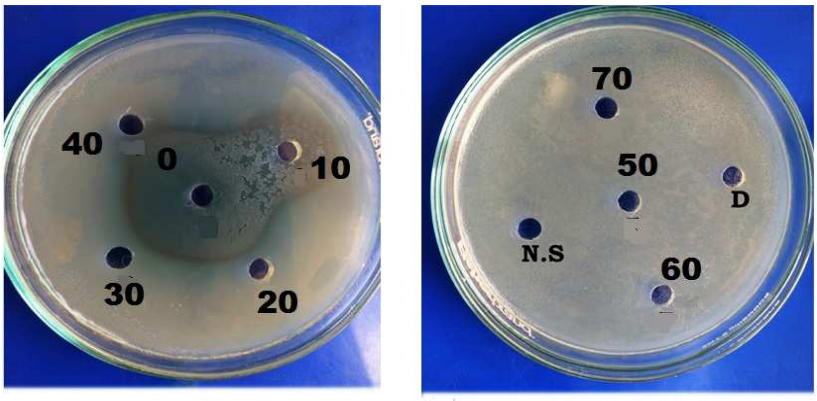
O = UnDilution , N.S= Normal Saline, D= Dettol

Figure 1: Inhibitory Zones of *Staphylococcus aureus*



O = UnDilution , N.S= Normal Saline, D= Dettol .

Figure 2: Inhibitory Zones of *Escherichia Coli*



O = UnDilution , N.S= Normal Saline, D= Dettol

Figure 3: Inhibitory Zones of *Pseudomonas aeruginosa*