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Research Article

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Antimicrobial Potential of Honey Samples of *Apis cerana indica*

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ABSTRACT

Honey is an ancient remedy for the treatment of infected wounds, which has recently been rediscovered by the medical profession, particularly where conventional modern therapeutic agents are failing . Honey samples were collected from two different geographical regions , i.e Hill (kodaikkanal) and plain (Mannargudi) . The antimicrobial activity of honey was performed by well diffusion assay against pathogens i.e, bacteria [Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa Enterobacter aerogenes, klebsiella], Fungi [Aspergillus niger, Aspergillus flavus, Trichoderma viride], yeast [Saccharomyces cerevisiae, candida], Actinomycetes [Streptomyces] and antibiotic sensitivity test was also performed for these pathogens using some antibiotics [Gentamycin, Auxamine, Tetracycline, Ampicillin].

Key words: Honey, Well diffusion assay, Pathogens, Antibiotic sensitivity.

INTRODUCTION

Honey produced by honeybees is one of the oldest traditional medicine Considered to be important in the treatment of respiratory ailment, gastrointestinal infection and various other diseases. It is been used effectively as a dressing for wound,(including surgical wounds), burns and skin ulcers to reduce pain and odor quickly[1].

Honey is the substance made from the gathering of nectar, sugary deposits from plants and animals by honeybees which in their natural scientific model, synthesized, purified and stored in comb in a vicious or gelly liquid. Most people think of honey as excellent food, but some others consider it an elizir and still others as medicine[2]. Honey has been discovered for the treatments of bacterial infections by medical profession, particularly, where conventional modern therapeutic agents are failing[3]. Honey like other saturated sugar syrups and sugar pastes, has an osmolarity sufficient to inhibit microbial growth[4].

The high antimicrobial activity of honey has been attributed to its properties such as osmotic effect, acidity, hydrogen peroxide and other phytochemical factors , further more, it appears that the honey from certain plants has better antibacterial activity than from others[5]. So honey samples from Hill and Plain regions with differently flowering plants should differ in then antibacterial potentials.

Honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes ,gram-negative and gram-positive[6-7]. This paper explains the efficacy of honey samples of *Apis cerena indica* collected from two different regions of Tamil nadu, South India viz., samples collected from hills and plains, against some pathogenic microbial strains. Study has revealed varying responses of tested strains susceptibility towards the hill and plain regions of honey as an antimicrobial agent.

EXPERIMENTAL SECTION

Honey samples were collected from two different geographical regions, i.e., Hill (kodaikanal) and plain (Mannargudi), Were collected in sterile containers and kept at room temperature (24 – 26^{o}C) for 30 days before experimentation Each sample was diluted to various concentrations (50,75, and 100% in v/v) in order to text their antimicrobial potential.

Samples were collected from soil,sewage,milk sample and then serially diluted. Nutrient agar medium and potato dextrose agar medium, glycerol yeast extract agar medium was poured onto the sterile petriplates. After solidification, the selected dilution factors from 10^{-2} to 10^{-4} and 10^{-3} to 10^{-5} were spreaded on the medium. Then the plates were incubated at 37^{o}C for 24hrs and 72hrs respectively. *Saccharomyces cerevisiae* were collected from MTCC (Microbial type culture collection) Chandighar, Stain No: 170. The isolated colonies were identified by cultural, morphological and biochemical characteristics.

Bacteria: *Bacillus subtilis, Bacillus cereus, Enterobacter aerogenes, Pseudomonas aeruginosa, Klebsiella* sp,
Fungi: *Aspergillus niger, Aspergillus flavus, Trichoderma viride* , **Yeast** : *Saccharomyces cerevisiae, Candida,*
Actinomycetes: *Streptomyces spp*

The bacterial, fungal, yeast and actinomycetes cultures were used for well diffusion method, suspension of microbial colonies were mixed thoroughly with respective agar medium and poured into petridishes, wells were made in petridishes with the help of sterile cork borer under aseptic condition in laminar air flow chamber and 0.2ml of honey sample of different concentrations were poured in each well [8-10].The bacterial plates were observed after 24hrs for clearing zone around the well, fungal and yeast plates were observed after 48hrs for clearing around the well. The *Streptomyces* plates were observed after 48 – 72hrs for clearing around the well. The zone of inhibition was calculated by measuring the diameter of inhibition zone around the well, the zone of diameter was measured.

Antibiotic disc were used to detect antibiotic sensitivity of bacterial, fungal, yeast and actinomycetes suspension from respective agar medium. The antibiotic disc [Gentamycin, Tetracycline, Ampicillin, Auxamine] were placed on the inoculated plates containing microbial cultures [Bacteria, Fungi, Yeast, Actinomycetes] and incubated for 24 – 72hrs. Antibiotic sensitivity was assayed from the diameter of zones.

RESULTS AND DISCUSSION

Honey samples were collected from two different geographical regions, i.e Hill (Kodaikkanal) and plain (Mannargudi). Each sample was diluted to various concentrations (25, 50,75 and 100% in v/v) in order to test their antimicrobial potential .Test organisms are isolated from soil, sewage, milk sample *saccharomyces cerevisiae* were collected from MTCC (Microbial type culture collection) Stain No: 170. The isolated colonies were identified by cultural, morphological and biochemical characteristics. The bacterial, fungal, yeast and Actinomycetes culture used for well diffusion method and observe the zone of inhibition.

Zone of inhibition was observed and measured in bacterial colonies, both diluted and undiluted honey showed zone of inbibition for *Bacillus cereus* 8.3±0.57, 12.8±0.23, 20.2±0.29, 25.3±0.57(Hill), 8.7±0.26, 16.4±0.64, 19.8±0.76, 25.7±0.57(Plain) at 25%, 50%, 75%, 100%, v/v respectively. *Bacillus subtilis* 8.0±0.50, 13.0±0.23, 20.2±0.23, 24.3±0.57(Hill Region), 8.5±0.26, 16.0±0.57, 19.5±0.76, 25±0.57 (Plain Region) at 25%, 50%, 75%, 100% (v/v) respectively.(Table-1)Zone of inhibition was noted in *Pseudomonas aeruginosa*, 8.8±0.76, 10.2±0.72, 14.3±0.57, 31.2±1.05 (Hill Region), 8.0±0.35, 11.7±0.57, 21.5±1.00, 29±1.0 (Plain Region) at 25%, 50%,75%, 100% (v/v) respectively.(Table-1)Zone of inhibition was noted in *Enterobacter aerogenes* 6.8±0.76, 14.5±0.50, 18.0±1.00, 25.0±1.00 (Hill Region), 8.0±0.10, 14.0±1.00, 20.0±1.00, 22.0±1.00(Plain Region) at 25%, 50%, 75%, 100% (v/v) respectively.(Table-1)Zone of inhibition was noted in *Klebsiella*, there is no zone of inhibition in 25% and 50% in both Hill and plain Region honey, 16±0.39, 19±0.56 (Hill Region), 16±1.00, 18±0.36(Plain Region) observed at 75% and 100% dilutions.(Table-1). There was no zone of inhibition in fungal *(Aspergillus niger, Aspergillus flavus, Trichoderma viride),* yeast *(Saccharomyces cerevisiae, Candida),* Actinomycetes *(Streptomyces)* inoculation.

Table-1 : Antibacterial activity of Honey

Name of organisms	Zone of Inhibition (in mm)							
	Hill Region				Plain Region			
	25%	50%	75%	100%	25%	50%	75%	100%
Bacillus cereus	8.3± 0.57	12.8 ± 0.23	20.2 ± 0.29	25.3 ±0.57	8.7 ± 0.26	16.4 ± 0.69	19.8 ± 0.76	25.7 ± 0.57
Bacillus subtilis	8.0 ± 0.50	13.0 ± 0.23	20.2 ± 0.23	24.3 ± 0.57	8.5 ± 0.26	16.0 ± 0.57	19.5 ± 0.76	25.0 ± 0.57
Pseudomonas aeruginosa	8.8 ± 0.76	10.2 ± 0.72	14.3 ± 0.57	31.2 ± 1.05	8.0 ± 0.35	11.7 ± 0.57	21.5 ± 1.00	29± 1.00
Enterobacter aerogenes	6.8 ± 0.76	14.5 ± 0.50	18.0 ± 1.00	25.0 ± 1.00	8.0 ± 0.10	14.0 ± 1.00	20.0 ± 1.00	22.0 ± 1.00
Klebsiella	NZ	NZ	16 ± 0.39	19 ± 0.56	NZ	NZ	16 ± 1.00	18 ± 0.36

Values are expressed as Mean ± standard deviation . *NZ = No zone*

Antibiotic disc [Gentamycin, Auxamine, Tetracycline, Ampicillin] Were used to detect the antibiotic sensitivity of bacterial fungal, yeast and actinomycetes suspension. Antibiotic sensitivity was assayed from the diameter of zones.

Bacillus subtilis and *Bacillus cereus*, was sensitive to gentamycin and showed the zone of inhibition, 14.3±0.6, 11.5±0.01 respectively. *Pseudomonos aeruginosa* was sensitive to gentamycin (12.6±0.1), Ampicillin (10.3±0.1), Tetracycline (13±1.15) and showed zone of inhibition.*Enterobacter aerogenes* was sensitive to gentamycin (9.7±0.5) and Tetracycline (10.8±0.05), showed zone of inhibition.*Klebsiella was* Sensitive to Gentamycin (12.6±0.1), Ampicillin (10.3±0.1) and Tetracycline 13±1.15) and showed zone of inhibition. *Aspergillus flavus* Sensitive to gentamycin (11.3±0.04) and showed zone of inhibition. Fungi such as *Aspergillus niger*, *Trichoderma viride*, is namely *Saccharomyces cerevisiae* does not sensitive to these antibiotics (Gentamycin, Auxamine, Ampilillin, Tetracycline)(Table-2). *Candida* was sensitive to Tetracycline (12.4 $\pm$ 0.05) and showed zone of inhibition. *Streptomyces* was sensitive to tetracycline (10.6 $\pm$ 0.40) and showed zone of inhibition. (Table-2)

Table-2: Sensitivity of microorganisms to standard antibiotics

Name of organism	Zone of inhibition (in mm)			
	Gentamycin	Auxamine	Ampicillin	Tetracycline
Bacillus cereus	11.5 ± 0.01	NZ	NZ	NZ
Bacillus subtilis	14.3 ± 0.6	NZ	NZ	NZ
Pseudomonas aeruginosa	12.6 ± 0.1	NZ	10.3 ± 0.1	13 ± 1.15
Enterobacter aerogenes	9.7 ± 0.5	NZ	NZ	10.8 ± 0.05
Klebsiella	19.3 ± 0.1	NZ	NZ	15.5± 0.01
Aspergillus niger	NZ	NZ	NZ	NZ
Aspergillus flavus	11.3 ± 0.04	NZ	NZ	NZ
Trichoderma viride	NZ	NZ	NZ	NZ
Saccharomyces cerevisiae	NZ	NZ	NZ	NZ
Candida	NZ	NZ	NZ	12.4 ± 0.05
Streptomyces	NZ	NZ	NZ	10.6 ± 0.40

Values are expressed as mean ± standard deviation *NZ= No zone*

Among the study all the isolated bacterial strains was sensitive to Gentamycin, the fungi namely *Aspergillus flavus* was sensitive to Gentamycin. Yeast strain *Candida* and Actinomycetes strain such as *Streptomyces* was sensitive to tetracycline. In this present study, various honey samples was used to detect the antimicrobial potential against some bacteria, Fungi, yeast, actinomycetes, and assess the antibiotic sensitivity of these microbial strains.

It is evident that Indian honey possess considerable antibacterial potency and honey offers advantages in controlling bacterial growth and in the treatment of certain health problems.

Our study reports to antibacterial potential of honey samples of hill and plain region against five bacterial species was recorded. All the bacterial species showed significant differences in their susceptibility with regard to different concentrations of honey samples whereas for *E.coli and P.aeruginosa*, the variation due to sample collection sites(region) was not significant [11]

Our study correlated with the findings of Honey is applied to a wound and it is kept in place if a good therapeutic effect is to be obtained. For the optimal MIC of the antibacterial components of honey to be reached at the deepest sites of infection there needs to be the highest concentration possible on the surface, and a reservoir of sufficient quantity that it is not substantially depleted by diffusion into the wound tissues [3].

CONCLUSION

The present study highlights the antimicrobial activity of honey sample from hill and plain region, high antimicrobial activity has been attributed to its properties such as asmatic effect acidity, hydrogen peroxide and other phytochemical factors. Thus we concluded that the honey could be used as a drug for respiratory ailment, gastrointestinal infection wound infection and various other diseases caused by bacteria.

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