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Antimicrobial activity of some honey samples against pathogenic bacteria

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ABSTRACT

The aim of the present research work to investigate antimicrobial activity of some honey samples six winter honeys six summer honeys collected from different regions of Western Ghats. The microbes used in this study are *Staphylococcus aureus, Streptococcus pyogenes*, *Escherichia coli, Pseudomonas aeruginosa, and Proteus mirabilis*. Antibacterial activity of the honeys was assayed using the Disc diffusion method. Noticeable variations in the antibacterial activity of the different honey samples were observed. Among the microbes Staphylococcus aureus is the most sensitive against all honey samples shows the maximum inhibitor zone compare to summer honeys.

Keywords: Antibacterial activity; Disc diffusion; Honey

1. INTRODUCTION

Honey is one of the oldest traditional medicines considered as traditional remedy for microbial infections. It is also recognized as an efficacious topical antimicrobial agent in the treatment of burns and wounds (Brudzynski, 2006). This leads to the search for different types of honey with antibacterial activity (Mullai and Menon, 2007). The healing effect of honey could be due to various physical and chemical properties (Snow and Manley-Harris, 2004). The floral source of honey plays an important role on its biological properties (Molan, 2002).

Honey is being used in a few hospitals, especially in the clinical treatment of ulcers, bedsores, burns, injuries and surgical wounds. The antibacterial properties of honey may be particularly useful against bacteria which have developed resistance to many antibiotics, e.g. *Staphylococcus aureus*, which is a major cause of wound sepsis in hospitals (Armstrong and Otis, 1995). Honey is thus an ideal topical wound dressing agent in surgical infections, burns and wound infections (Betts and Molan, 2002).The use of honey as a medicine has continued into the present-day medicine. It has been shown that natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria (Bassom *et al*., 1994, Mundo *et al.,* 2004 and Lusby and Coombes 2005).

The antibacterial potency of honey has been attributed to its strong osmotic effect, naturally low P^H (Kwakman and Zaat, 2012), the ability to produced hydrogen peroxide which plays a key role in the antimicrobial activity of honey (Kacaniova *et al*., 2011 and Wahdam,1998)and phytochemical factors. Numerous reports and clinical studies have demonstrated the antimicrobial activity of honey against a broad range of microorganisms,

including multi-antibiotic resistant strains. Others studies demonstrated the antibacterial activity of honey against*: Escherichia coli, Campylobacter jejuni, Salmonella entercolitis, Shigella dysenteriae* (Adebolu, 2005 and Voidaou *et al.,* 2011), *Mycobacterium* (Asadi-Pooya *et al.*,2003), Methicillin-resistant *Staphylococcus aureus* and Vancomymin-resistant *Enterococci* (Cooper *et al.*,1999 & 2002 and Al-waili *et al.*,2005),Common gastrointestinal pathogenic bacteria(Lin *et al*.,2011),and the development of *streptococcus pyogenes* biofilms (Maddocks *et al*., 2012). The antifungal activity of the honey, especially anti-*Candida* activity (Irish et al., 2006, Koc *et al*., 2008 and Ahmad *et al*., 2012) has also been reported.

The present study aimed to evaluate the antibacterial activity of some honey samples collected from Theni District. The honey samples are classified into Summer honey (collected in the month of April) and Winter honey (collected in the month of October) Also antibacterial activities of Antibiotics like Commonly used in the treatment of infections cared by here resistant pathogenic bacteria were evaluated.

2. MATERIAL AND METHODS

2.1. Honey samples

Twelve honey samples (Six honey samples S1, S2, S3, S4, S5 & S6 were collected in the month of April and six honey samples-W1, W2, W3, W4, W5 & W6 collected in the month of October). All samples were collected from Thani District Western Ghats, India. Samples were stored in dark place at a room temperature (25-35c)

2.2. Bacterial Strains

Strains of *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus* and *Streptococcus pyogenes,* were obtained from Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai naga-608002, India.

2.3. Disc diffusion method

Bauer et al (1966) Filter paper discs of 6 mm diameter were prepared. The discs were impregnated with the different concentrations of each honey 0.5 McFarland standard was prepared by wing due method (Koneman *et al.,* 1992) and 5ml was into a sterile test tube. An inoculums of each isolate was prepared from subculture of bacterial suspension 4-5 colonies of each isolates were emulsified in sterile normal saline and the turbidity adjusted to 1.5×10^8 (Fu/ml(corresponding to 0.5 McFarland standers). A sterile cotton swab was dipped into the standardized bacterial suspension and used to evenly inoculate the Mueller Hinton agar plates. They were allowed to dry for 3 to 5 minutes. Thereafter, all discs were placed on the plates and µpressed gently to ensure complete contact with agar. A distance of at least15mm was maintained from the edges of the plates to present overlapping of inhibition zones. Amphicillin disc (5µg) was used as positive control. Fifteen minutes after the placement of discs, the plates were incubated for 24 h at 37 °C. After incubation the plates were examined and three diameter of the inhibition zone was measured in triplicates for each isolate.

2.4. Statistical analysis

Data analysis results were expressed as means ± standard deviation and differences between means were analyzed statistically using an analysis of variance (ANOVA) according to Fisher's PLSD test. Differences were considered significant when$P \leq 0.05$.

Table 1. Antibacterial activity concentration [a] (200µl/Disc).

Bacterial strains	S1	S2	S3	S4	S5	S6	Am-B (5µg/disc)
Staphylococcus aureus	12.5 ±0.80	9.9±0.15	9.8±0.50	8.5±0.50	8.1±0.15	7.4±0.35	7.1±0.28
Streptococcus pyogenes	10.1±0.32	10.1±0.17	9.3±0.43	9.3±0.43	8.3±0.35	7.2±0.25	8.0±0.50
E. coli	9.4±0.45	9.2±0.34	8.3±0.20	8.2±0.25	7.3±0.40	7.1±0.28	7.3±0.57
Pseudomonas aeruginosa	9.1±0.15	9.1±0.17	8.2±0.11	9.0±0.80	8.1±0.28	7.1±0.28	9.3±0.57
Proteus mirabilis	9.3±0.30	9.1±0.15	9.2±0.25	8.2±0.25	8.1±0.15	8.0±0.51	9.0±0.50

Bacterial strains	W1	W2	W3	W4	W5	W6	Am-B (5µg/disc)
Staphylococcus aureus	13.3 ±0.40	11.5±0.25	10.5±0.49	9.4±0.40	8.2±0.25	8.2±0.25	7.3±0.57
Streptococcus pyogenes	12.1±0.15	11.4±0.40	11.3±0.30	10.0±0.57	9.2±0.46	9.2±0.52	8.1±0.28
E. coli	11.0±0.25	10.5±0.45	10.2±0.52	9.2±0.26	9.3±0.43	8.3±0.30	7.5±0.50
Pseudomonas aeruginosa	10.9±0.11	11.1±0.32	9.9±0.10	9.5±0.55	8.3±0.43	8.2±0.58	9.1±0.28
Proteus mirabilis	10.6±0.20	10.2±0.26	10.1±0.41	9.1±0.15	9.3±0.43	8.3±0.30	9.0±0.50

± = Standard deviation
[a] = including disc diameter of 6mm

3. RESULTS AND DISCUSSION

A total of twelve honey samples from different origins were evaluated for their antibacterial activity against the Gram positive species such as *Staphylococcus aureus, Streptococcus pyogenes*, and the Gram negative species such as *Escherichia coli, Pseudomonas aeruginosa, and Proteus mirabilis*. Among the twelve honey samples studied S1 and W1 honey samples shows maximum antibacterial activity especially against *Staphylococcus aureus*. The average diameter of the inhibition zones produced by these samples was 12.9mm. The growth of bacteria was also inhibited by these honey samples; although to a lesser extent. Among the honey samples normally the winter honey shows the maximum inhibition zone (average 11.55mm).

Among the summer honey samples S1 honey samples shows maximum inhibition zone (average 10.0mm),whereas the honey sample S6 shows minimum inhibition zone (average 7.42mm) Among the winter honey samples the honey sample W1 shows the Maximum inhibition zone (average 11.58mm),Whereas the honey sample S6 shows the Minimum inhibition zone (average 8.2mm).

Among the bacterial strains tested Staphylococcus aureus the most sensitive against all honey samples (average inhibition zone is 10.42mm), whereas the Pseudomonas aeruginosa shows the less sensitive against all honey samples.

Antibiotic resistance of bacteria is on increase the discovery of alternative therapeutic agents is urgently needed. Honey possesses therapeutic potential, including would healing properties and antimicrobial activity.

The antimicrobial properties of honey can be attributed to several factors like high osmotic pressure, low pH (Molan,1992a and 1992b).Among the twelve honey samples the honey samples collected in winter (W1, W2, W3, W4, W5 & W6) Shows the maximum inhibition zone.

4. CONCLUSION

The present study reveals that among the twelve honey samples tested against the pathogenic bacteria the six winter honey samples were more effective in inhibiting the pathogenic bacteria than the summer honey samples. All the honey samples were more effective against staphylococcus aureus than the other bacteria.

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