Research Article

Antibacterial Activity of Honey against Selected Pyogenic Bacteria

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ABSTRACT

Honey is an ancient remedy for wounds and skin infection. A present study was performed on antibacterial activity of different types of honey against the selected pyogens. Monofloral Honey, Polyfloral Honey, Forest Honey, Commercial Honey were collected and organoleptically evaluvated. Antibacterial activity of honey was determined by agar well diffusion method against *Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa* and showed higher antibacterial activity with zone of inhibition range about 16-30 mm diameter. When comparing four honey samples Polyfloral Honey was having higher antibacterial activity.

Keywords: Honey, Organoleptic evaluation, *Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa*, Antibacterial activity.

INTRODUCTION

Honey is an ancient remedy for the treatment of wound infection, which has recently been 'rediscovered' by the medicinal profession. Honey is gains acceptance as an agent for the treatment of ulcers, bed sores and other skin infections resulting from burns and wound. [1] Honey is a unique gift of nature. It is a natural product of super saturated sugars produced by honeybees from nectars of flowers. In ancient communities it was regarded as an important medical treatment for all kind of health problem. Honey is used to treat infected wounds as long as 2000 years before bacteria were discovered to be cause of infection. Honey has been used for wound treatment, healing and cleaning wounds, against different internal and external infection. It possesses high concentration of sugars such as fructose (38.19%), Glucose (31.28%), disaccharides like maltose (7.3%), Sucrose (1.31%) and Moisture (17.2%). [2] It is acidic in nature with a pH of 3.2- 4.5. The pure honey contains alkaloids, auter quinone, glycosides, flavonoids, and reducing compounds. [3] Honey possesses inherent antimicrobial properties some of which are due to high osmotic pressure/ low water activity. High sugar content (osmolarity), Hydrogen peroxide,[4] acidic nature,[5] was responsible for antibacterial activity.

A wound disrupts the barrier of the skin and provides a portal of entry through which microorganisms can enter the circulatory system and deep body tissue. Pyogenic infection may endogenous or exogenous. There are various ways by which pyogenic infection may become infected by different types of microorganisms. The commonest pyogenic bacteria often associated with infected wounds are *Staphylococcus aureus, Streptococcus pyogenes,* and coliform bacilli, such as *Escherichia coli, Proteus sp., Pseudomonas aeruginosa* and other enteric bacilli. [6] In chronic infections they are slow to heal and in pus show other microbes. Skin infections are often the result of a break in the integrity of the skin. Bacterial skin infections include erythema and related diseases, impetigo, folliculitis, erysipelas and cellulitis. While there are certainly other bacterial skin infections, they are either uncommon or result from systemic illness.

Staphylococcus aureus is considered to be one of the most important wound pathogen. It is a Gram positive cocci, occurs generally in bunches, non motile, non capsular, and non-sporulating. [7] *Staphylococcus aureus* causes supportive, pus forming infections and toxins in human. It can produce skin diseases like superficial skin lesions such as boils, carbuncles, and furnculosis, wound infection. *S. aureus* is a leading cause gastroenteritis resulting from consumption of food. It is one of the organisms causing chronic mastitis on bovine *Staphylococci* rapidly develop resistance to many antimicrobial agents and present therapeutic problems. Sir Alexander Ogston noticed that nonvirulent staphylococci were also often present on skin surface.

Streptococcus pyogenes is spherical Gram positive bacteria that cause pyogenic infections. It is non motile and nonsporinig microorganism. Cellular division occurs along a single axis in these bacteria, and thus they grow in chains or some strains of *Streptococcus pyogenes* and some group C stains are having capsules composed of hyaluronic acid. *S.pyogenes* is the cause of many important human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases. Infections typically begin in the throat or skin. [7]

Pseudomonas aeruginosa is a common bacterium which causes disease in animals and humans. It is a large group

of aerobic, nonsporing Gram negative bacilli. [8] It found in soil, water, skin flora most manmade environments throughout the world. It is implicated in hot tab rash. *P.aeruginosa* secretes a variety of pigments, including pyocyanin (blue green), fluorescein (yellow green) and fluorescent. Definitive clinical identification of *P. aeruginosa* often includes identifying the production of pyocyanin and fluorescein, as well as ability to grow at 42°C. This organism can be achieve anaerobic growth with nitrite as a terminal acceptor, and in its absence, it is also ferment arginine by the substrate level phosphorylation. Adaptation to micro aerobic or micro anaerobic environments is essential for certain life styles of *P.aeroginosa*. [9] The main aim of the present study is to determine the antibacterial activity of honey against selected pyogenic bacteria.

MATERIALS AND METHODS

Collection of Bacterial Samples

The Pyogenic microorganisms *Staphylococcus epidermidis* (MTCC 3068)*, Streptococcus pyognes* (MTCC 14289) and *Pseudomonas aeruginosa* (MTCC 741) were procured from Microbial type culture collection centre. These organisms were subcultured on to their selective media.

Collection of Honey Samples

Four types of natural honey were collected from Sencheri hills, Senthuruthi pirivu, Coimbatore and stored in sterile container.

- Monofloral Honey (MH)
- Polyfloral Honey (PH)
- Forest Honey (FH)
- Commercial Honey (CH)

Organoleptic Evaluation of Natual Honey

The natural honey was organoleptically evaluated for various properties like appearance (transparent, Semi-transparent, glossy, and opaque), colour (Amber, light amber, extra light amber, dark amber, yellow) and consistency (thick ,thin) of honey differs depending on the nectar source (the blossom) visited by the honey bees, smell (good, pleasant), Taste (Sweet, sour, bitter), spreadability,the spreading diameter of 0.1mL of honey placed between two glass plates (10x10cm) was measured after 1 min with a mass of plate 50gm.[10]

Agar well diffusion method

The agar well diffusion method is used to determine the growth inhibition. [11] The honey was prepared in the concentration of 0.9, 0.7, 0.5, 0.3mL dissolved in sterile distilled water. The sterile Muller Hinton Agar was prepared and poured in sterile petridishes and allowed to solidify. Using sterile well cutter puncture 6mm diameter wells with uniform spacing for various concentrations for various types of honey. The log phase culture broth was taken and swabbed over the agar using sterile cotton swab to obtain uniform lawn of culture. The wells were filled with 10µL of mentioned concentration of various honeys respectively. The plates were then incubated at 37°C for 24 hours.

Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration of the various honey samples were found using broth dilution assay method. [12] Seven test tubes containing 1mL of sterile nutrient broth were prepared. For assaying honey, the starting concentration is kept at 8mL in the first tubes containing 1mL of broth. This was serially diluted to other tubes and finally 1mL is discarded from the sixth test tube. So, the dilution will be 8, 4, 2, 1, 0.5, 0.25mL respectively. To all these test tubes, 0.1mL of the cultures of target microorganism were added separately and incubated at 37°C for 24 hours. After incubation the tubes were examine for visible turbidity after incubation and plated on to nutrient medium and result were tabulated.

RESULTAND DISCUSSION

Organoleptic Evaluation of Honey

The natural honey samples were organoleptically evaluated; the various properties like appearance, colour and consistency, smell (odour), taste, spreadability were evaluated and tabulated in Table 1.

Mono floral honey appeared as semi-transparent, golden amber with thick consistency. It has pleasant smell with sweet ends with bitter taste. Poly floral honey appeared as semi-transparent, light amber with hick consistency had sweet taste. Forest honey appeared as opaque, dark amber with thin consistency and sweet ends with sour taste. Commercial honey appeared as glossy, bright yellow with thin consistency. It has pleasant smell with sweet ends with sugary taste.

Table 1: Organoleptic Evaluation of Honey

Parameters	Monofloral Honey	Polyfloral Honey	*Forest Honey*	*Commercial Honey*
Colour & Consistency	Semi-transparent	Semi-transparent	Light amber/ thin	Bright yellow/ thin
Smell	Golden Amber / Thin	Light Amber / Thick	Pleasant	Pleasant
Appearance	Pleasant	Pleasant	Opaque	Glossy
Taste	Sweet Ends With Bitter	Sweet	Sweet ends with sour	Sweet ends with sugary
Spreadability	44 Mm	44mm	41mm	45mm

Agar Well Diffusion Method

In the present study antibacterial activity of honey were tested against pyogenic bacteria at different concentration using agar well diffusion method. The results were observed in the form of zone of inhibition and tabulated in Table 2-5.

Among all the honey the poly floral honey has higher antibacterial activity against *S. aureus.* Mono floral honey has moderate activity on *S.aureus.* Commercial honey has lowest activity *S.pyogenes*.

Among all the honey the poly floral honey and forest honey has higher activity against *S.pyogenes*. Mono floral honey has moderate activity on *S.pyogenes.* Commercial honey has lowest activity on *S.pyogenes.*

Among all the honey the poly floral honey has higher antibacterial activity against *P. aeruginosa.* Forest honey has moderate activity on *P. aeruginosa.* Commercial honey has lowest activity against *P. aeruginosa.* When comparing all the honey the poly floral honey has higher activity against all the pyogens.

Table 2: Antibacterial activity of mono floral honey against bacterial pathogens

Inhibition zone diameter in millimeter				
Organisms	**Concentration(mL)**			
	0.9mL	**0.7mL**	**0.5mL**	**0.3mL**
S.aureus	23mm	22mm	20mm	19mm
S.pyogenes	22mm	20mm	19mm	18mm
P.aeruginosa	21mm	20mm	17mm	16mm

Table 3: Antibacterial activity of poly floral honey against bacterial pathogens

Inhibition zone diameter in millimeter				
Organisms	**Concentration(mL)**			
	0.9mL	**0.7mL**	**0.5mL**	**0.3mL**
S.aureus	23mm	22mm	21mm	20mm
S.pyogenes	22mm	21mm	20mm	19mm
P.aeruginosa	30mm	22mm	21mm	20mm

Table 4: Antibacterial activity of forest honey against bacterial pathogens

Inhibition zone diameter in millimeter				
Organisms	**Concentration(mL)**			
	0.9mL	**0.7mL**	**0.5mL**	**0.3mL**
S.aureus	23mm	21mm	19mm	18mm
S.pyogenes	22mm	21mm	20mm	19mm
P.aeruginosa	30mm	23mm	21mm	21mm

Table 5: Antibacterial activity of commercial honey against bacterial pathogens

Inhibition zone diameter in millimeter				
Organisms	**Concentration(mL)**			
	0.9mL	**0.7mL**	**0.5mL**	**0.3mL**
S.aureus	21mm	20mm	18mm	17mm
S.pyogenes	22mm	20mm	19mm	16mm
P.aeruginosa	24mm	20mm	18mm	17mm

Table 8: Minimal Inhibitory Concentration

Sample	**Organism**	**Dilution (Plating method)**					
		T1 (8ml)	T2 (4ml)	T3 (2ml)	T4 (1ml)	T5 (0.5ml)	T6 (0.25ml)
MH	*S.aureus*	NG	NG	NG	NG	NG	G
	S.pyogenes	NG	NG	NG	NG	NG	G
	P.aeruginosa	NG	NG	NG	NG	NG	G
PH	*S.aureus*	NG	NG	NG	NG	NG	G
	S.pyogenes	NG	NG	NG	NG	NG	G
	P.aeruginosa	NG	NG	NG	NG	NG	G
FH	*S.aureus*	NG	NG	NG	NG	NG	G
	S.pyogenes	NG	NG	NG	NG	NG	G
	P.aeruginosa	NG	NG	NG	NG	NG	G
CH	*S.aureus*	NG	NG	NG	NG	NG	G
	S.pyogenes	NG	NG	NG	NG	NG	G
	P.aeruginosa	NG	NG	NG	NG	NG	G

MH - Monofloral Honey, PH - Polyfloral Honey,FH - Forest Honey, CH - Commercial Honey, NG - No Growth, G - Growth

Minimal Inhibitory Concentration

The minimum inhibitory concentration (MIC) of natural honey samples were done by broth dilution method. MIC values were taken as the lowest honey concentration that prevented bacterial growth after 24 hours of incubation. The MIC of four different natural honey samples were less than 2mL inhibitory concentration which indicates that the honey is effective and were highly active even in low concentration. This result showed that the honey has antibacterial activity against pyogenic microorganisms. The results were tabulated in table 6.

The present study was carried out to assess the antibacterial activity of honey against pyogenic microorganism and it was organoleptically evaluated to ensure the property of the honey. The organoleptic evaluations of honey provide information about the type of honey collected for this study. Honey possesses inherent antibacterial properties against pyogenic pathogens due to high osmotic pressure and low water activity. Osmotic effect, hydrogen peroxide, acidic nature as well as phelonic acids, flavonoids and lysozyme are responsible for antibacterial activity of honey. [13] Mono floral honey, poly floral honey, forest honey, commercial honeys were used for this study. Among the honey the poly floral honey with 0.9ml concentration showed highest antibacterial activity, when compared with other honey. The poly floral honey shows activity against all the selected pyogenic pathogens. Among the selected pyogenic pathogens it was highly active against *P.aeruginosa* and *S.aureus.* Commercial honey found to less active then all the other honey, even though the antibacterial properties rendered good. The enzyme glucose oxidase found in honey produced hydrogen peroxidises which is the inhibitory agent against the bacterial strains.[14] In 2006, it was reported that the antibacterial efficacy of the honey was increased due to Hydrogen peroxide; an inhibine component of honey was produced when honey is diluted with water. [15,16] The MIC value of honey with different concentration (8, 4, 2, 1, 0.5, and 0.25 ml) against selected pathogen, the minimal concentration about 0.25mL inhibits the growth of bacteria. From this information it is proven that these honeys have antibacterial activity.

CONCLUSION

From the present study, the antibacterial potential of different honey was determined against pyogenic pathogens. Most of the commercial antibiotics may have side effects and some allergic reactions to the individuals and the drug resistance nature of the pyogens increases day by day. Herbal remedies like honey will serve as an alternative medicine without any side effects and used for skin treatments and pyogenic infection.

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