



## Studies On Antimicrobial Properties Of Wildbee Honeys Of Different Seasons

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### ABSTRACT

The studies on antibacterial activity of honey was conducted during the season 2012-13 due to its low pH, osmolarity and hydrogen peroxide accumulation. The aim of this work was investigate the antimicrobial activity of honeys from different seasons, specifically to evaluate their non-hydrogen peroxide derived activity, against *Staphylococcus aureus*(Rosenbach), *Escherichi coli*(Migula ) and *P. aeruginosa*(Schroter.) . Antibacterial activity of honeys was assayed using disc diffusion succptibility methods. Noticeable variations in the zone of inhibitions of the different honey samples were observed. Studies shown that honeys had broad spectrum of antibacterial activity, particularly Greek Pine, Scottish Heather, Chilean Ulmo, New Zealand Beech and Jarrah Honey. and none of the tested honey had a detectable non-peroxide activity.

**Keywords:** Antibacterial activity, Non-peroxide activity, Honey, *Staphylococcus aureus*(Rosenbach. ), *E. Coli*(Migula. ), *P. aeruginosa*(Schroter. ).

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### INTRODUCTION

Honey is a sweet substance prepared by the bee by foraging on variety of flowers. It is regarded as the ambrosia in the Indian mythological books. (Abrol, 1997). It is known to man since the early times as the natural medicines. It is the naturally complex substances containing varied composition of fructose, glucose, sucrose, proteins, fats, vitamins, minerals, enzymes, amino acids, volatile aromatic substances, ashes, water etc (Al-Waili, 2004). However, these constituents differed as per the variety of flowering vegetations in different geographical region. There are different varieties of bees and accordingly, they have different types of nests, nesting patterns and composition of honey. Honey gets its sweetness from the monosaccharide fructose and glucose. Most micro-organisms do not grow in honey because of its low water activity. Infestation by bacteria, fungi and viruses are responsible for various ailments in the human beings. The disease like cough and cold, fever, blood pressure, asthma, anemia fattiness etc. These diseases can be cured by herbal medicines that is with the help of honey. Which have many curative properties(Dustman, 1979., (Molan, 1992; Molan, 1998; Chinakwe, 2006; Al-Waili, 2004; Abd-Elaal *et al.*, 2007; Al-Somail *et al.*, 1994;Molan *et al.*, 2000; Ndaisaba *et al.*, 1993;Subrahmanyam *et al.*, 2001; Taormina *et al.*, 2001).Therefore, the present work has been under taken to investigate the comparative antimicrobial activity of honeys of *A. dorsata* (Fabricius.) bee- species that gathered during

different seasons against three different micro-organisms namely *Staphylococcus aureus*(Rosenbach. ), *Escherichia coli* (Migula. ) and *P. aeruginosa*(Schroter) (Molan, 1992).

### **Honey: A versatile natural bee-product –**

Honey is the most precious edible commodity obtained that of honey. Wax and pollen. Honey, the most precious edible commodity obtained by man from the bees. Besides honey, the bee-products like wax, propolis, royal jelly, and pollen have been obtained by a man since from very old time.

Bees forage on variety of flowers to gather nectar & pollen, and transform it in to honey. It means complex sucrose solution (nectar) is converted by bees into simple glucose and fructose by its own digestive enzymes while pollen grains are added from outside to enrich the stuff. It is composed of water (20%), glucose and fructose (65-78%), sucrose (1-5%), proteins (1%), fats (1%) and growth hormones, enzymes, minerals, vitamins etc. in traces(Bogdnov, 1989).

### **Antimicrobial activity in honey:**

Use of honey as a therapeutic substance has been rediscovered by the medicine profession in more recent times, it is gaining acceptance as an antibacterial agent for the treatment of various ailments like urinary treat infection, diarrhea, Necrotic Breast Ulcers, Gastrointestinal problems and other skin infections resulting from burns and wound. The use of honey as a wound dressing material, an ancient remedy that has been rediscovered (Molan, P.C.,1998).

It is reported that honey inhibits growth of bacteria due to a high sugar concentration i.e. osmosis, hydrogen peroxide and proteinaceous compounds present in it. It is thought that light, temperature, oxygen, processing and storage may also affect the antibacterial activity of honey. In addition to that, the other factors like high osmotic pressure, low water activity, low pH, acidic environment, low protein content, high carbon to nitrogen ratio, low redox-potential due to the high content of reducing sugars, viscosity, anaerobic environment and other phyto-chemicals are also likely to play some role in determining antibacterial activity of honey.

### **Objectives of Study:**

To evaluate antimicrobial activity of experimental materials (sugar syrup and wild bee honey of different seasons) against the infectious microbes like *E. Coli*(Migula), *Pseudomonas aeruginosa*(Schroter. ) and *S. aureus*(Rosenbach. ).To explore the comparative bactericidal potentialities of experimental materials to understand their therapeutic applications.

## **MATERIALS AND METHODS**

### **Materials :**

#### **Honey from different flowering seasons:**

The Vidarbha region of Maharashtra state in India is unique in hag total three major species of bees *Apis florea*(Fabricius. ), *Apis cerana*(Fabricius. ), *Apis dorsata*(Fabricius. ) known for the commercial production of honey and wax. Among them, the giant rock bee (*Apis dorsata*(Fabricius.)) thrives well in both the plains and hilly regions especially in the forest areas. The locals people harvest honey and sell it to the local market. The production of honey from this bee variety has been seen in all three seasons depending upon the flowers available in that geographical area. As per change in a seasons, flowering composition also changes, which has a direct impact on chemical composition of honey that varies with different seasons.

#### **Collection of honey samples from different honey flow seasons:**

In order to explore the antimicrobial sensitivity of honey of different seasons against some selected microorganism the respective samples of freshly processed honey and syrup of plain sugar with 20-21% moisture were collected from local agency namely, Centre for Bee Development, Nalwadi, Wardha (MS). All these three samples were preserved for experimentation in the airtight pet jars of 250ml and labelled as A, B and C respectively, viz. A – Winter honey, B. Summer honey, and C – Rainy season honey.

#### Collection of bacterial strains for experimentation:

Following test organisms were collected from the PG Department of Microbiology, Agnihotri College of Science, Wardha (MS) for investigation against different honey, *Escherichia coli*(*Migula.* ), *Pseudomonas aeruginosa*(*Schroter.* ), and *Staphylococcus aureus*(*Rosenbach.* ). These are the common microorganisms responsible for ill health of human beings.

#### Select media used for studying test microorganism :

- i) Eosin – Methylene Blue Agar (EMB) for *E.coli*(*Migula.* ).
- ii) Mannitol Salt Agar (MSA) for *S. aureus*(*Rosenbach.* ).
- iii) Pseudomonas Isolation Agar (PIA) for *P. aeruginosa*(*Schroter.* ).

#### Media Composition :

- i) Eosin-Methylene Blue Agar (EMB):

Ingredients	gms/lit
• Peptic digest of animal tissue	10.0gms
• Dipotassium phosphate	02.0gms
• Lactose	05.0gms
• Sucrose	05.0gms
• Eosine	0.40gms
• Methylene blue	0.065gms
• Agar	13.50gms
• pH	7.2+0.2

- ii) Mannitol Salt Agar (MSA)

Ingredients	gms/lit
Proteus peptone	10.0gms
Beef extract	01.0gms
Sodium chloride	7.5gms
D- mannitol	10.0gms
Phenol red	0.25gms
Agar	15.0gms
PH	7.4+0.2

- iii) Pseudomonas Isolation Agar :

Ingredients	gms/lit
• Peptic digest of animal tissue	20.0gms
• Magnesium chloride	1.40gms
• Potassium sulphate	10.0gms
• Triclosan (Irgasan)	0.025gms
• pH	7.2+0.2

#### Method:

The samples of honeys harvested from different bee-species were collected in winter seasons from Wardha area. The Whatman's filter paper no. 1 punch disc (5mm) soaked in pure natural honey of different seasons and sugar syrup were placed aseptically on colonies of selected strains of bacteria separately grown by disc A diffusion method. Immediately after incubation period. i.e. the optimum duration of incubation (1 or 2 days), the 'Zone of inhibition', was carefully measured around the disc. It was observed that quantification of microbial growth inhibition was determined by measuring the diameter of zones clear of microbial growth around the disc in the agar. The experiment was carried out for the test microorganisms namely *Escherichia coli*(Migula), *Pseudomonas aeruginosa* (Schroter) and *Staphylococcus aureus*(Rosenbach) and were organised in triplicates for the verification of the earlier observation reports.

#### Disc diffusion Susceptibility Methods :

##### Principles:

When a filter paper disc impregnated with a chemicals and is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will placed the chemicals in the agar only around the disc. The solubility of the chemicals and its molecular size will determine the size of the area of chemicals infiltration around the disc. If an organism is placed on the agar it will not grow in the area around disc, if it is susceptible to the chemicals, This area of no growth around the disc is known as a "Zone of inhibition."

##### Working with the disc diffusion method:

Selective agar plates seeds with 0.1ml of the pure culture using spread plate technique was used. Plates were allowed to stand for 20 minute before discs were applied sterile absorbent disc were placed into different honey samples for 10 minute before being applied directly onto inoculated agar plates. Control sterile discs were also applied. Plates were incubated at 37<sup>0</sup>C for 24-hours and zone of inhibition were measured.

## RESULTS AND DISCUSSIONS

A growth inhibitory reactivity of honeys of different seasons and plain sugar against some gram-positive and gram negative organisms has been undertaken by employing the diffusion method. The identities of the isolates were cross-matched with those present in a standard manual(Buchanan and Gibbon, 1974; Carter and Chengappa,1991). It has been observed that the 'Zone of inhibition' has been recorded against all three types of microbes due to all the four experimental materials (honey and sugar). However, a scale of sensitivity against the each type of honey and sugar has been found varied and distinctive.

**Table 2. Measurement of Zone of inhibition by disc diffusion method :**

Microbes tested	Types of honey tested to explore the antimicrobial effect of honeys of different bee varieties			
	Winter honey	Summer honey	Rainy honey	Sugar
<i>E. coli</i> (Migula. )	27	36.66	27.66	11.33
<i>P.aeruginosa</i> (Schroter.)	32	32	17.5	10
<i>S.aureus</i> (Rosenbach.)	10.5	10.5	10	10

**Sensitivity against *E. Coli*(Migula. ) :**

A zone inhibition against *E. Coli* (*Migula.*) in summer honey was maximum while in winter and rainy seasons honey was much less than that occurred in case of summer honey. However, sugar syrup has been found inhibitory for the *E coli* but the zone of inhibition was recorded comparatively very less than any type of honey.

#### **Sensitivity against *Pseudomonas aeruginosa*(*Schroter.* ) :**

A zone of inhibition against *P. aeruginosa* (*Schroter.* ) due to summer and winter honeys was recorded maximum, while in rainy season honey it was much less. However, the sugar syrup has been found inhibitory for the *P. aeruginosa* ( ) but the zone of inhibition was recorded comparatively very less than any type of honey (Abd-Elaal, A.M., El-Hadidy, M.R., El-Mashad, N.B., El-Sabaie, A.H.2007).

i) A zone of inhibition against *S. aureus*(*Rosenbach.* ) in winter honeys was recorded maximum while in case of summer and rainy seasons honey, and sugar syrup it was much less as compared to other types of honey (Chinakwe, E.C., 2006).

ii) Comparative analysis of antimicrobial sensitivity of honey and sugar syrup :

All the three microorganisms tested against alternative antibiotics like honey of different seasons and sugar syrup showed the mixed sensitivity. Although, a rainy season honey has been better than the sugar syrup but poor antibiotic activity than the honeys of winter and summer seasons. Amongst the microorganisms, *E. Coli* has been found more sensitive followed by *Pseudomonas aeruginosa* (*Schroter.* ) while *S. aureus*(*Rosenbach.* ) responded very marginally against all the experimental material were employed to test antimicrobial property.

Under the most cases of infections *E. Coli*, (*Migula.* ) *P. aeruginosa* (*Schroter.* ) and *S. aureus* (*Rosenbach.* ) are the causative organisms responsible for further worsening the problems especially of the gastro intestine, diarrhoea and wounds. During the present study, we profoundly demonstrated the comparative antimicrobial activity of honeys collected during different seasons against these pathogens (Molan, P, C.,1992).

In the present study, an idea behind employing the honeys of different seasons was to establish the fact that antibacterial properties in honey are derived as per the floral base. During the winter-summer and rainy seasons, there are different flowering plants. Bees forage on them to collect nectar and pollen, and make the honey. Therefore, as the basic chemical composition of honeys was different for each season. Based on the observation in the present study, the honey from different phyto-geographic regions (varied seasons) differ in their ability to inhibit the growth of bacteria and yeasts suggesting that botanical origin play an important role in influencing a honey's antimicrobial activity (Abd-Elaal, A. M., El-Hadidy, M. R., El-Mashad, N. B., and El-Sabaie, A.H.,2007).

### **CONCLUSION**

- The study broadly indicated the antibiotic property of honeys of all seasons, however, honeys of winter and summer season were noticed more effective than the honey of rainy season and the pure sugar syrup.
- As compared to bacteria *P. aeruginosa* (*Schroter.* ) was showed to be less sensitivity against all the materials tested in the present study as the alternative antibiotic substitutes.
- The use of honey in food formulation or to treat the ulcers, burns and wounds can help to control some food pathogens like *E. Coli*(*Migula.* ), *P. aeruginosa*(*Schroter.* ).

- Since honey is non-toxic antimicrobial agent due to its properties, it can be very effectively used for medical purposes.

### ACKNOWLEDGEMENTS

Many thanks to Prof. Dr. Abd El-fatah Badawy for valuable guidance and for all the members of Egypt petroleum research institute (EPRI) and to Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt, for their valuable assistance and facilities they provided.

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