e- ISSN: 2394-5532
p- ISSN: 2394-823X
General Impact Factor (GIF): 0.875
International Journal of Applied And Pure Science and Agriculture
www.ijapsa.com

Antibacterial activity of honeys produced by five major bee species in Kerala

Krishnasree V[1] and Mary Ukkuru P[2]

[1] *PhD Scholar, Department of Home Science, Kerala Agricultural University, College of Agriculture, Trivandrum.*
[2]*Professor & Head, Department of Home Science, Kerala Agricultural University, College of Agriculture, Trivandrum, Kerala, India.*

Abstract

The present study aimed to explore the antibacterial efficacy of the honey produced by the five bee species that prevail in Kerala namely, Apis cerana indica F. (Ac), Apis mellifera L. (Am), Apis dorsata F. (Ad), Apis florea F. (Af) and Trigona irridipennis S. (Ti). Antibacterial study was carried out against seven bacterial strains of which three were gram positive and four were gram negative. The results obtained were compared with standard antibiotic ampicilline. It was found that the antibacterial efficacy was more in the honeys when they were taken in the pure form. The antimicrobial activity was analyzed by measuring the zone inhibition diameter (ZID) exhibited by the honey against the pathogen.

Key words: antibacterial efficacy, zone inhibition diameter, Trigona irridipennis S. (Ti), Apis mellifera L. (Am), gram positive and gram negative bacterial strains.

I. INTRODUCTION

Honey has a long and varied relationship with humans, which goes beyond its nutritional attributes. Honey has been collected by humans for at least 8000 years and it also formed an important dietary and medicinal component of civilizations across the globe. This role of honey has continued throughout the ages into modern times (Allsop and Miller, 1996).

Honey extracted from combs and apiaries contain several undesirable materials like pollen, bee wax besides yerk, makes it susceptible for the presence of microorganisms as they are associated with specific foods or components of the ecosystem (Jay, 1992).

Organisms found in the environment around honey i.e. bees, hives, pollen, flowers, soil etc. are so likely to occur in honey. The intestine of the bees found to contain 1% yeast shaped microbes, 29% gram positive bacteria including *Bacillus, Streptococcus* and *clostridium* species; and 70% gram-negative bacteria including *Achromobacter, Citrobacter, Enterobacter, Erwina, Escherichia coli, Pseudomonas* etc (Tysset et al., 1970).

In spite of this scope for microbial proliferation in honey it has been identified as a natural antimicrobial agent being utilized for wound healing, preventing dental caries and improving the gastroenterological issues showing its efficacy in preventing the growth of bacteria that provoke such conditions.

English and co workers (2004) shown that honey prevents dental plaque, gingivitis, periodontitis. Sela *et al*., (1998) have been that honey is non-carcinogenic than sucrose, due to its anti bacterial property, which prevents the growth of bacteria that can cause dental caries.

Antimicrobial agents are essentially important as the evolution and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, is of great concern to the global health community. Long before mankind discovered the existence of microbes, the idea that certain plants and plant based foods had healing potential because of the presence of, what we would currently characterize as antimicrobial principles, was well accepted (Frey and Ryan, 2010).

Recently, the potent activity of honey against antibiotic- resistant bacteria has further increased the interest for its clinical applicability but incomplete knowledge about its antimicrobial activity pose a serious threat for its wide application (Al-Mamary *et al*., 2002; Levy and Marshall, 2004).

Hence the antimicrobial activity of honey is a subject for extensive analysis where the interest is based mainly on the activity against pathogens and its use as a natural medicine. The present study investigates the antibacterial activity of various honey types from different bee species that prevail in Kerala.

II. MATERIALS AND METHODS

a. Collection of honey samples

A total of five honey samples (in triplicates each) of , the Indian hive bee, *Apis cerana indica* F. (Apidae) (Ac), the European or Italian bee, *Apis mellifera* L. (Apidae) (Am), the rock bee, *Apis dorsata* F. (Apidae) (Ad), the little bee, *Apis florea* F. (Apidae) (Af) and *Trigona irridipennis* S. (Stingless bee) (Ti) were collected from the local beekeepers of different areas of southern zone of Kerala. The samples were stored in half liter pet containers duly labeled with name codes and date of collection. All chemicals used in this study were of analytical grade.

a.Animicrobial assay

2.2.1. Preparation of honey samples:

Honey solutions were prepared immediately before testing by diluting honey to the required concentrations (100, 75, 50 and 25%, v/v). All samples were then incubated for 30 minutes at 37°C in a shaking water bath that allowed aeration of the solutions. Incubation was carried out in the dark because both hydrogen peroxide and glucose oxidase are light sensitive (White and Subers, 1964).

2.2.3. Test organisms utilized:

The cultures *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa*, *Salmonella enterica* (MTCC 9844), *Listeria monocytogenes* (MTCC 839), *Escherichia coli* (MTCC 1698) and *Shigella flexneri* (MTCC 1457) were obtained from the MTCC (microbial type culture collection) and gene bank, institute of microbial technology.

Antibacterial activity was determined by agar-well diffusion method as reported by Booth (1971).Hundred µl of suspension containing 10^8 colony forming units ml^{-1} of bacteria was spread on nutrient agar medium. Wells (8 mm) were punched out of the solid of respective medium using sterile cork borer. 100 µl of different concentrations of the extracts were filled into each well. Petri dishes were incubated at 37°C for 24 hrs. At the end of the incubation period, inhibition zone formed on the medium were measured in mm. The assays were performed in triplicates and inhibition zone was also compared with ampicilline as a reference standard.

2.3. Statistical analysis:

The statistical processing of the data obtained from all studies is expressed as Mean ± standard deviation (SD) of three separate experiments using the computer programme Excel.

III. RESULTS & DISCUSSION

Since microorganisms became resistant over the period of time against antibodies, there is a need to explore the natural antibiotic sources for the prevention and treatment of several diseases. This was a

ystematic study carried out in this regard on analyzing the antibacterial properties of different bee honeys in Kerala.

According to Table 1, of the five bee honeys tested Ti honey yielded larger zones of inhibition growth for gram +ive bacteria namely *B. cereus*, *S. aureus* and *L. monocytogenes*. The ZID for these bacteria were 17mm, 18mm and 19mm respectively.

In all concentration (100-25%) Ti had the maximum inhibition against *B. cereus* and *L. monocytogenes* where as in case of *S. aureus* Am honey had the highest inhibition at a concentration of 75% and 50% which amounts to be 12mm and 10mm respectively. At 25% of concentration both the honeys have equivalent level (4mm) of inhibition towards *S. aureus*.

At their lowest concentration (25%) Ac and Ad honeys showed inhibition capacity only towards *L. monocytogenes* and *B. cereus* respectively with 6mm and 7mm of ZID. The overall antibacterial effect of Ac and Ad honeys were preceded by Ti and Am honeys.

Even though, the antibacterial activity of Af was relatively low when compared to the other honeys in the study, it showed growth inhibition for gram positive bacteria *L. monocytogenes* and *B. cereus* with an exemption to *S. aureus*.

Table 1: Antibacterial activity of five bee honeys against gram positive bacteria

Honey	**Gram positive bacteria**		
Ti	***B. cereus***	***S. aureus.***	***L. monocytogenes***
100%	17 ± 0.92	18 ± 0.23	19 ± 0.09
75%	16 ± 0.6	11 ± 0.25	17 ± 0.08
50%	15 ± 1.2	9 ± 0.04	16 ± 0.57
25%	11 ± 0.11	4 ± 0.6	14 ± 0.57
Am			
100%	16 ± 0.5	15 ± 0.43	14 ± 0.01
75%	14 ± 0.91	12 ± 0.67	12 ± 0.03
50%	12 ± 0.9	10 ± 0.12	10 ± 0.0
25%	7 ± 0.7	4 ± 0.33	8 ± 0.0
Ac			
100%	16 ± 1.0	15 ± 0.32	15 ± 0.12
75%	13 ± 1.0	11 ± 0.54	12 ± 0.4
50%	10 ± 0.9	7 ± 0.95	10 ± 0.11
25%	0 ± 0	0 ± 0	6 ± 0.8
Ad			
100%	15 ± 0.6	12 ± 0.0	15 ± 0.12
75%	13 ± 0.24	9.3 ±0.0	9 ± 0.0
50%	11 ± 0.5	0 ± 0.0	0 ± 0.0
25%	7 ± 0.4	0 ± 0.0	0 ± 0.0
Af			
100%	15 ± 0.9	8 ± 0.13	10 ± 0.0
75%	13 ± 0.9	5 ± 0.3	8 ± 0.02
50%	10 ± 0.8	0 ± 0.0	5 ± 0.0
25%	9 ± 0.9	0 ± 0.0	1 ± 0.0

Ti: *Trigona irridipennis* S. **Am:** *Apis mellifera* L. **Ac:** *Apis cerana indica* F. **Ad:** *Apis dorsata* F. **Af:** *Apis florea* F.

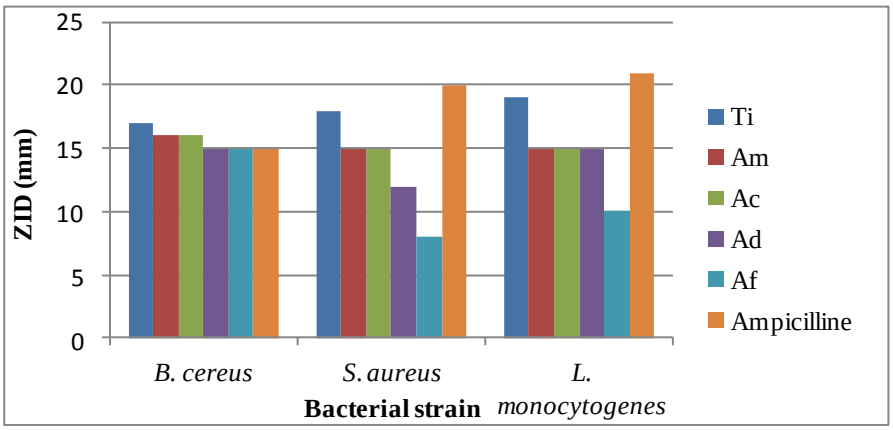

Figure 1: Antimicrobial activity of five pure bee honeys and standard ampicilline based on the ZID produced for gram positive bacterial strains.

The above figure indicates that the honey Ti had a better antibacterial activity when compared to the other honeys and also the standard on inhibiting the growth of bacterial strains of *B. cereus*. The ampicilline standard had highest growth inhibition against *S. aureus* and *L. monocytogenes* of 20mm and 21mm.

Table 2: Antibacterial activity of five bee honeys against gram negative bacteria

Honey	**Gram negative bacteria**		
Ti	***S. enterica***	***E. coli***	***S. flexneri***
100%	15 ± 0.01	20 ± 0.0	18 ± 0.3
75%	13 ± 0.04	15 ± 0.0	14 ± 0.21
50%	10 ± 0.12	11 ± 0.2	12 ± 0.4
25%	6 ± 0.9	8 ± 0.1	10 ± 0.22
Am			
100%	18 ± 0.11	16 ± 0.9	17 ± 0.4
75%	14 ± 0.0	12 ± 0.3	13 ± 0.4
50%	10 ± 0.0	10 ± 0.2	12 ± 0.5
25%	5 ± 0.2	4 ± 0.4	8 ± 0.4
Ac			
100%	10 ± 0.2	14 ± 0.4	11 ± 0.32
75%	8 ± 0.3	10 ± 0.3	9 ± 0.2
50%	0 ± 0.5	6 ± 0.21	0 ± 0.0
25%	0 ± 0.0	5 ± 0.15	0 ± 0.7
Ad			
100%	14 ± 0.0	11 ± 0.1	10 ± 0.45
75%	11 ± 0.1	10 ± 0.0	7 ± 0.22
50%	9 ± 0.1	8 ± 0.21	4 ± 0.3
25%	8 ± 0.51	5 ± 0.0	0 ± 0.0
Af			
100%	13 ± 0.6	12 ± 0.2	10 ± 0.2
75%	12 ± 0.23	10 ± 0.4	7 ± 0.11
50%	6 ± 0.21	5 ± 0.2	5 ± 0.1
25%	5 ± 0.0	5 ± 0.33	1 ± 0.0

The Am honey had highest antibacterial activity against the gram negative bacteria *S. enterica* followed by Ti, Ad, Af and the least activity was showed by the Ac honey when taken in the pure form. On dilution all the honey samples expressed a reduction in their zone inhibition against bacterial strains and in the Ac honey it had narrowed down to zero at their lowest concentration (25%).

The bactericidal effect of honey was reported to be dependent on its concentration; the higher the concentration of the honey the greater its usefulness as an antimicrobial agent (Badawy *et al*., 2004).

According to table 2, Ti had the highest zone inhibition diameter against *E.coli* and *S. flexineri* of 20mm and 18mm followed by Am (16mm, 17mm), Ac (14mm, 11mm). Af and Ad had almost similar inhibitory action against these two bacterial strains where as the zone inhibition diameter of Ad was zero at a concentration of 25%.

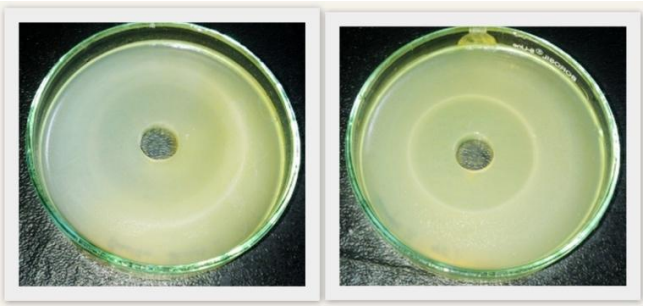

Figure 2: ZID of Ti honey against *E.coli* (left), ZID of Am honey against *S. enteric* (right)

From the below figure it is evident that the standard ampicilline had low antibacterial activity against the gram negative bacterial strains analyzed except for *P. aeruginosa*, where none of the honey samples tested exhibited an inhibitory zone.

Am honey was found to have larger zones of inhibition against *S. enterica* followed by Ti and Ad. Whereas, Ti showed greater inhibitory zone against *E.coli* followed by Am, Ac and Af. Maximum zone inhibitory diameter for *S. flexineri* were obtained by Ti and Am. All the other honeys Ac, Ad and Af had more or less similar antibacterial activity against *S. flexineri*.

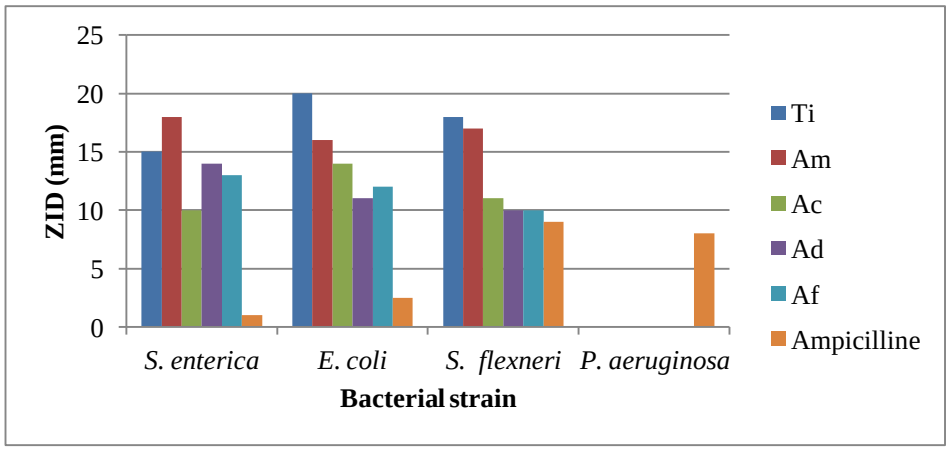

Figure 3: Antimicrobial activity of five pure bee honeys and standard ampicilline based on the ZID produced for gram negative bacterial strains.

The antibacterial activity of the honey is predominantly due to the hydrogen peroxide, which is formed by the glucose oxidase system. When the system breaks down it releases a small amount of glucose, producing gluconic acid and hydrogen peroxide (Weston, 2000).

The Ac, Ad and Af honey analyzed are having low pH which might have contributed to their antibacterial activity. Because the low pH of honey inhibits the presence and growth of microorganisms due to the presence of oraganic acids (Finola *et al*., 2007).

Among the honeys analyzed Ti and Am exhibited highest antimicrobial activity while having a moderate pH might be because of the higher amounts of phytochemicals present in them as these bees forage on a larger number of plants for nectar and most of them are having medicinal value.

Several antibacterial phenolic compounds have been identified in honeys which originate from the plant nectar where they have been foraging (Isla *et al*., 2011).

The low redox potential of honey due to its high content of reducing sugar was found to discourage the growth of moulds and aerobic bacteria that make them antibacterial. The viscosity of honey is another prominent factor as it opposes convection currents and limits the entry of dissolved oxygen it makes the survival of microbes at bay (Molan, 1992).

As the osmotic pressure is high, the microbes in honey shrivel as water flows out of their cells into the surrounding honey (Mundo *et al*., 2004).

IV. CONCLUSION

In general, all the five honey species selected for the study exhibited antibacterial activity against gram positive and gram negative bacterial strains even though a reduction in zone inhibition diameter was found on lower concentrations.

Among all the honey species analyzed *Trigona irridipennis* S. (Ti) honey had th maximum antibacterial activity and *Apis florea* F.(Af) showing the least. There was striking similarity observed between the *Apis mellifera* (Am) and *Trigona irridipennis* S. (Ti) honey in their sensitivity towards the gram negative bacterial strains namely; *S. enterica* and *S. flexineri*.

It was found that all honey was not equally effective against all microbes at all time. Hence their relative merits need to be tested in clinical trials for it to be an effective antibacterial agent with wide application. The whimsicality of antibacterial activity of non-standardized honey may hamper its introduction as an antimicrobial agent due to variation in the in vitro antibacterial activity of various honeys.

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