Eco. Env. & Cons. 30 (February Suppl. Issue) : 2024; pp. (S150-S153)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2024.v30i02s.029

Antimicrobial activity of honey

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(Received 2 June, 2023; Accepted 12 August, 2023)

ABSTRACT

The present study is aimed at determination of antimicrobial activity of honey. Honey is an effective remedy for healing of wounds as it shows antibacterial and antifungal activity. To study the antimicrobial activity of honey, four types of samples were collected from different sources and labelled as S1,S2,S3,S4. These samples were used to study the antimicrobial activity against laboratory cultures of known pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris, Candida albicans, Escherichia coli, Shigella spp., Bacillus subtilis, Micrococcus luteus, Micrococcus roseus*and *Bacillus megaterium.* Antimicrobial activity of honey samples was studied by using ditch test method and MIC was determined by agar cup diffusion method. All results were recorded and it was found that S1 and S3 honey samples showed maximum antibacterial activity even at 6 times dilutions.

Key words: *Honey, Proteus vulgaris, Antagonistic activity, Wound pathogens*

Introduction

Honey has a valued place in the human diet due to its unique taste, nutritional value and health promoting properties. Sugars, mainly fructose and glucose and minor amounts of oligosaccharides account for about 80% of its weight. As a consequence, it is an easily digestible and high energetic food product (Szweda, 2017). Honey can be used for the antibacterial activity as it contains high concentration of sugars and low pH value combined with the enzymatic production of hydrogen peroxide (Szweda 2017; Molan, 1997, Irish *et al*., 2011). Honey consists of iron and valuable antioxidants, also it do not show any side effect and cost effective which give advantage to use in medical field (Guruvu *et al*, 2021). Apart from antibacterial effect honey also exhibited antifungal effect also (Szweda, 2017; Guruvu *et al*., 2021). There are many reports of honey as an agent very effective as dressing of wounds, burns, skin ulcers and inflammations; the antibacterial properties of honey speed up the growth of new tissue to heal the wound (Mandal and Mandal, 2011)

Materials and Methods

Collection of honey samples

Following samples are collected and brought to the laboratory
S1 - Raw honey (Tutu plant, collected from Rethare, Karad)
S2 – Branded honey (Feaster brand, collected from Pharmacy, karad)
S3 – Raw honey (Jambule honey, collected from Khodashi, Karad) S4 – Mixed honey (collected from local market of Goa)

Test Cultures used for the antibacterial activity of honey

The laboratory cultures of known pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris, Candida albicans,*

Escherichia coli, Shigella spp., Bacillus subtilis, Micrococcus luteus, Micrococcus roseous, Bacillus megaterium were used to study antimicrobial activity of honey.

Detection of antibacterial activity by ditch test method

A rectangular ditch was cut in the agar along the diameter of the each plate separately. The each ditch was filled with samples S1, S2, S3 and S4 and kept for diffusion at 4 °C for 2-h and 4 old test cultures grown in nutrient broth were streaked across the agar surface at right angle to the ditch and incubated at 37 °C for 24- h and observed for zones of inhibition. After observation of zones of inhibition further proceed for Minimum inhibitory concentration (MIC) was done.

Determination of MIC by agar well diffusion method

The thick suspension of 4-h old culture of each test organisms were prepared in a separate nutrient broth test tubes. Then 0.1 ml of each suspension was spread inoculated on a separate sterile nutrient agar plate. Four wells were prepared in each plate. Each undiluted sample of honey was used and also as aqueous dilutions of 1:2, 1:4, 1:6 against the respective microorganisms available in our laboratory.

Sensitivity test

The agar cup diffusion method was employed to obtain the antibiotic susceptibility pattern of the 4-h old nutrient broth grown microbial test isolates against antibiotic discs. Comparative study was carried out against several antibiotics such as gentamycine, cloxacillin, chloramphenicol, tetracycline. Considerations for the sensitivity and resistance of bacteria and yeast were based on the extent of the presence or absence of zones of growth inhibition.

Results and Discussion

It could be seen from Table 1 that out of 11 isolates, 8 isolates were inhibited by raw honey sample (S1). Branded honey sample shows antibacterial activity against 6 isolates and honey sample (S3) was able to inhibit 10 isolates out of 11. Out of 4 samples S3 shows maximum antibacterial activity against most of the pathogens.

Sample S1 (Raw honey) showed significant inhibitory effects against all test organisms at 1:2 and 1:4 dilutions. While sample S1 exhibited moderate to minimal antimicrobial activity at 1:6 dilution. When it was compared with standard antibiotics it is found that it shows equivalent activity against test organisms. Sample S2 (Branded honey) showed antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* even at 1:6dilution while *Klebsiella pneumoniae, Proteus vulgaris, Escherichia coli, Shigella spp, Bacillus subtilis, Bacillus megaterium* were found to be resistant to sample S2 even at 1:2 dilution. All results were compared with standard antibiotics it is found that S2 shows maximum antimicrobial activity against *Pseudomonas aeruginosa.*

Sample S3 (Jambule honey) showed significant antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris, Candida albicans, Escherichia coli* while 3 organisms such as *Shigella spp, Bacillus subtilis, Bacillus*

Table 1. Antimicrobial activity of Honey against various laboratory culture by Ditch Test Method (Rice, W.G *et al.* 1950)

Sr. No.	Name of organisms	Raw honey	Branded honey	Jambule honey	Mixed honey
1	*Pseudomonas aeruginosa*	+	+	+	+
2	*Staphylococcus aureus*	+	+	+	+
3	*Klebsiella pneumoniae*	+	+	+	+
4	*Proteus vulgaris*	+	+	+	+
5	*Candida albicans*	+	+	+	-
6	*Escherichia coli*	-	-	+	+
7	*Shigella spp*	+	-	-	-
8	*Bacillus subtilis*	+	-	+	-
9	*Micrococcus luteus*	+	+	+	-
10	*Micrococcus roseus*	-	-	+	-
11	*Bacillus megaterium*	-	-	+	-

(+): indicates growth inhibition
(-): indicates no inhibition

Table 2. Determination of minimum inhibitory concentration of honey samples against laboratory pathogens.

Sr. No.	Honey samples	Test organisms	Zone of inhibition in mm at various dilutions			Antibiotics (1µg/ml)			
			1:2	1:4	1:6	G	CX	CH	TE
1	S1	*Staphylococcus aureus*	22mm	18mm	16mm	16mm	11mm	15mm	12mm
		Pseudomonas aeruginosa	17mm	15mm	14mm	20mm	13mm	12mm	18mm
		Klebsiella pneumoniae	25mm	18mm	-	20mm	13mm	12mm	18mm
		Proteus vulgaris	27mm	18mm	-	12mm	14mm	-	18mm
		Candida albicans	22mm	20mm	19mm	-	-	-	-
		Escherichia coli	15mm	11mm	-	12mm	-	-	-
		Shigella spp	16mm	15mm	11mm	13mm	-	-	-
		Bacillus subtilis	16mm	15mm	14mm	-	17mm	16mm	16mm
		Bacillus megaterium	14mm	12mm	11mm	-	12mm	-	-
2	S2	*Staphylococcus aureus*	12mm	11mm	11mm	16mm	11mm	15mm	12mm
		Pseudomonas aeruginosa	25mm	23mm	17mm	20mm	13mm	12mm	18mm
		Klebsiella pneumoniae	-	-	-	20mm	13mm	12mm	18mm
		Proteus vulgaris	-	-	-	12mm	14mm	-	18mm
		Candida albicans	13mm	15mm	11mm	-	-	-	-
		Escherichia coli	-	-	-	12mm	-	-	-
		Shigella spp	-	-	-	13mm	-	-	-
		Bacillus subtilis	-	-	-	-	17mm	16mm	16mm
		Bacillus megaterium	-	-	-	-	12mm	-	-
3	S3	*Staphylococcus aureus*	24mm	24mm	23mm	16mm	11mm	15mm	12mm
		Pseudomonas aeruginosa	13mm	11mm	-	20mm	13mm	12mm	18mm
		Klebsiella pneumoniae	12mm	-	-	20mm	13mm	12mm	18mm
		Proteus vulgaris	26mm	20mm	-	12mm	14mm	-	18mm
		Candida albicans	26mm	20mm	18mm	-	-	-	-
		Escherichia coli	26mm	23mm	-	12mm	-	-	-
		Shigella spp	-	-	-	13mm	-	-	-
		Bacillus subtilis	-	-	-	-	17mm	16mm	16mm
		Bacillus megaterium	-	-	-	-	12mm	-	-
4	S4	*Staphylococcus aureus*	18mm	15mm	13mm	16mm	11mm	15mm	12mm
		Pseudomonas aeruginosa	18mm	15mm	13mm	20mm	13mm	12mm	18mm
		Klebsiella pneumoniae	-	-	-	20mm	13mm	12mm	18mm
		Proteus vulgaris	18	-	-	12mm	14mm	-	18mm
		Candida albicans	11	-	-	-	-	-	-
		Escherichia coli	11	-	-	-	12mm	-	-
		Shigella spp	-	-	-	13mm	-	-	-
		Bacillus subtilis	-	-	-	-	17mm	16mm	16mm
		Bacillus megaterium	-	-	-	-	12mm	-	-

(-): indicates no inhibition
S1: Raw honey, S2: Branded honey, S3: Jambule honey, S4: Mixed honey
G: Gentamycine, CX: cloxacillin, CH: Chloramphinicol, TE: Tetracycline

megaterium were found to be resistant to sample S3. When results were compared with standard antibiotics it was found that sample S3 was showing maximum activity than standard antibiotics.

Out of nine isolates sample S4 (Mixed honey) showed antimicrobial activity against 5 organisms such as *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Candida albicans, Escherichia coli,* remaining four organisms are found to be resistant to sample S4. Sample S4 also inhibits test organisms more efficiently than standard antibiotics.

Conclusion

All the undiluted honey samples S1, S2, S3, S4 showed antimicrobial activity against test organisms. S1 is a raw honey sample extracted from honey comb collected from Tutu plant Rethare, Karad, showed antimicrobial activity against 10 out of 11 test organisms. S2 is a Branded honey sample

collected from local market of Karad showed antimicrobial activity against 6 out of 11 test organisms, S3 is a raw honey extracted from honey comb collected from Jambule plant Khodashi, Karad. Showed antimicrobial activity against 10 out of 11 test organisms, S4 is a mixed honey sample collected from local market Goa, showed antimicrobial activity against 4 out of 11 test organisms. S1 and S3 honey sample showed maximum antimicrobial activity even at 6 times dilutions. This activity is found to be equivalent to the activity if standard test antibiotics such as Gentamycin, Cloxacillin, Chloramphenicol and Tetracycline.

These studies substantiate the earlier reports of others that honey has good antimicrobial power and can be used for the healing of wounds and burns.

Conflict of interest: There is no conflict of interest among authors.

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