Research Paper

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Antibacterial Activities of Different Brands of Unifloral Honey Available at the Northern Region of Bangladesh

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The antibacterial activities of five different brands of unifloral honey from the Northern region of Bangladesh were investigated. The honeys were from Litchi (*Litchi shinensis*), Kadom (*Anthocephalus cadmba*), Til (*Seasamum indicum*), Plum (*Zizyphus mauritiana*) and Mustard (*Brassica campestris*) flowers. It was found that the tested unifloral honeys showed a significant antibacterial activity against the wound infecting and enteric pathogens. Among them honey from Mustard flower was more active.

Key words: Honey, antibacterial activity, unifloral honey

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Introduction

Knowledge on the basic mechanism of healing has grown rapidly in the last hundred years. Neat honey is sterile and inhibits the growth of both gram positive and gram negative organism (Jeddar *et al.*, 1985). Honey is also an excellent adjuvant for acceleration of wound healing (Beraman *et al.*, 1983) and is widely accepted in folk medicine. Scientific evaluation of its ability to accelerate the wound healing and antibacterial effects are still insufficient. Literature survey revealed that the scientists are engaged to establish the mechanism of wound healing and antibacterial effects of honey. In line to the connection, we want to establish the antimicrobial activity of the honey. Therefore, in this research work different brands of unifloral honey such as honey from Litchi (*Litchi shinensis*), Kadom (*Anthocephalus cadmba*), Til (*Seasamum indicum*), Plum (*Zizyphus mauritiana*) and Mustard (*Brassica campestris*) flowers were tested for their antibacterial activity *in vitro* and *in vivo* at different dilution and solvent extraction against five reported bacteria e.g., *Shigella dysenteriae, Salmonella typhi, Escherichia coli, Bacillus megaterium* and *Staphylococcus aureus*.

Materials and Methods

Materials: Five unifloral honey such as honey from Litchi (*Litchi shinensis*), Kadom (*Anthocephalus cadmba*), Til (*Seasamum indicum*), Plum (*Zizyphus mauritiana*) and Mustard (*Brassica campestris*) flowers were collected from BCSIR Laboratory, Rajshahi, Bangladesh.

Production of Unifloral Honey: The honey, produced in honeycomb from nectar and pollen collected by bees from particular kinds of flower, is said to be the unifloral honey. The honey bee- box was placed at the center of the cultivated land of particular kinds of flowers. The bee's flying range for collection of nectar and pollen were equivalent to the radius of the land. At that time there was no other crops surrounding the experimental plot. Thus the nectar and pollen were collected from that flowers to obtain unifloral honey (Mottalib, 2001).

Antibacterial screening:

Primary assay: The agar diffusion technique (Bauer *et al.*, 1966) was employed to conduct antimicrobial testing. Five pathogenic bacteria were selected for the test, three of them were gram negative and others were gram positive. The test organisms (Table 1) were collected from the Department of Microbiology, University of Dhaka, Bangladesh.

Table 1: List of pathogenic bacteria

Test Organisms	Strain No.
Shigella dysenteriae	AL-35587
Salmonella typhi	CRL
Escherichia coli	FPFC-1407
Staphylococcus aureus	ATCC-259233
Bacillus megaterium	QL-38

Secondary assay: The lowest concentration of antimicrobial agents requires to inhibiting the growth of the organism *in vitro* is referred to the minimum inhibitory concentration (MIC). The serial dilution technique (Hammond *et al.*, 1978) was followed using nutrient broth medium to determine the MIC value of the chemotherapeutic agent.

Solubility of the Antibacterial principle of honey in organic Solvents:

The honey was treated with a number of organic solvents of various polarities and the antibacterial activities were compared with the zone of inhibition thus produced. For this purpose petroleum ether (C_2H_5-O-C_2H_5), ethyl acetate ($CH_3COOC_2H_5$) and chloroform ($CHCl_3$) are used for extraction. The 10ml of honey was taken in a separating funnel and treated with an equal quantity of each of the organic solvents. The organic layer was collected and tested for antibacterial activity against five test organisms by disc-diffusion method (Bauer *et al.*, 1966). Tetracycline disc (30μg) was used as a standard for comparison of the antibacterial activity.

Extraction of Antimicrobial compounds from the honey: The 10ml of honey was taken separately in two separating funnels. These were extracted with $CHCl_3$ (3×10 ml). The $CHCl_3$ fraction thus obtained was evaporated under reduced pressure in a rotavapour at 45^{0}C, until a solid mass was obtained. Thus antimicrobial principles were obtained.

Results and Discussion

Antibacterial effect of crude honey: The antibacterial activities of the crude honey were determined at a concentration of 6.75μg disc^{-1} against the tested organisms. The results are shown in Table 2. It was found that the entire test sample showed various positive response against all the tested organism. Fig. 1 showed the antibacterial activity of crude honey against pathogenic organism. The variation of the activity may be due to the different sources of the unifloral honey. Both honey and sugar are used with good effect as dressings for wounds and ulcers. The good control of infection is attributed to the high osmolarity, but honey can have the additional antibacterial activity because of its content of hydrogen peroxide (H_2O_2), lysozyme and other unidentified substances from certain floral sources.

Table 2: Antibacterial activity of the five brands of unifloral honey against the test pathogenic organisms

Test sample (Honey) 6.75μg disc^{-1}	Diameter of zone of inhibition (mm)				
	S. aureus	*S. dysenteriae*	*S. typhi*	*E. coli*	*B. megaterium*
Litchi	12	8	11	14	9
Mustard	27	22	9	16	9
Plum	24	14	11	18	10
Til	20	13	9	15	10
Kadom	20	10	9	19	12
Tetracycline (30 μg)	22	34	28	30	21

Antibacterial effect of diluted honey: The antibacterial activity of the honey diluted with 50% water (3.375μg disc^{-1}). It was found that the entire test sample showed positive response (Table 3). Fig. 2 showed the antibacterial activity of diluted honey against pathogenic organism.

Table 3: Antibacterial activity of the diluted honey (50% water) against the test pathogenic organisms

Test sample (Honey) 6.75μg disc^{-1}	Diameter of zone of inhibition (mm)				
	S. aureus	*S. dysenteriae*	*S. typhi*	*E. coli*	*B. megaterium*
Litchi	9	13	9	11	8
Mustard	19	14	10	10	8
Plum	25	17	9	14	10
Til	19	13	8	15	9
Kadom	20	21	8	10	11
Tetracycline (30 μg)	20	13	23	20	15

Table 4: MIC values of the test unifloral honey against five pathogenic organisms

	Minimum Inhibitory Concentration (μg ml^{-1})				
Test organisms	Litchi	Mustard	Plum	Til	Kadom
S. dysenteriae	85	168.75	166.125	168.75	167.5
S. typhi	85	42.18	166.125	168.75	41.75
E. coli,	170	84.37	166.125	168.75	83.75
S. aureus	42.5	42.18	83.62	84.37	41.75
B. megaterium	340	675	166.125	337.5	670

Table 5: The MIC of the Litchi honey against *Shigella dysenteriae*

No. of test tube	Content of honey (μg ml^{-1})	Inoculum added	Observation
1	680.00	10	N.G
2	340.00	10	N.G
3	170.00	10	N.G
4	85.00	10	N.G
5	42.50	10	G
6	21.25	10	G
7	10.62	10	G
8	5.31	10	G
9	2.65	10	G
Cs	680.00	00	N.G
Ci	00.00	10	G
Cm	00.00	00	N.G

Cs = Test tube with sample; Ci = Test tube with inoculum; Cm = Test tube with medium; G = Growth; N.G. = No growth; Number of cells = 10^7 cell ml^{-1}

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) of the unifloral honey was determined against five bacteria by serial dilution technique (Hammond *et al.*, 1978).

The minimum inhibitory concentration of the honey from Litchi, Mustard, Plum, Til and Kadom against *S. dysenteriae, S. typhi, E. coli, S. aureus* and *B. megaterium* are shown in Table 4. Among the tested sample of honey, Kadom showed most prominent minimum inhibitory concentration. Fig. 3 showed the MIC of test sample against pathogenic organism. Specific gravity of different brand of honey are: Litchi, Mustard, Plum, Til and Kadom honey are 1.36, 1.35, 1.33, 1.35 and 1.34, respectively (Motalib, 2001).

The minimum inhibitory concentration (MIC) of the Litchi honey against *Shigella dysenteriae* is shown in Table 5.

Antibacterial screening of the extract: The antibacterial activity of the $CHCl_3$, $CH_3COOC_2H_5$ and C_2H_5-O-C_2H_5 extract were tested against *S. dysenteriae, S. typhi, E. coli, S. aureus* and *B. megaterium* and the results obtained were compared with that of standard antibiotic disc such as Erythromycin-15, Tetracycline-30 and Doxycycline-30.

The unifloral honey from Litchi, Mustard, Kadom, Plum and Til showed significant antibacterial activity against the test pathogenic bacteria. The test sample also showed antibacterial activity even diluted with distilled water. To determine the cause or responsible compound of honey for antibacterial activity, honey was extracted with petroleum ether (C_2H_2-O-C_2H_5), chloroform ($CHCl_3$) and ethyl acetate

Table 6: Antibacterial activity of the chloroform extract ($CHCl_3$) of different brands of unifloral honey and standard antibiotics

		Diameter of zone of inhibition (mm)				
Honey	Type of extraction	*S. typhi*	*E. coli*	*S. dysenteriae*	*S. aureus*	*B. megaterium*
Litchi	$CHCl_3$, $CH_3COOC_2H_5$ and C_2H_5-O-C_2H_5	9,10,10	8,9,8	8,8,10	8,8,9	8,8,9
		10,10,10	9,9,10	8,9,9	9,9,9	9,8,10
Mustard	$CHCl_3$, $CH_3COOC_2H_5$ and C_2H_5-O-C_2H_5	10,11,10	11,10,9	11,10,10	16,12,13	11,10,11
		14,11,11	13,9,9	12,11,9	18,14,16	10,12,14
Kadom	$CHCl_3$, $CH_3COOC_2H_5$ and C_2H_5-O-C_2H_5	9,9,9	10,9,10	9,9,9	14,9,12	9,10,12
		12,10,9	12,9,10	10,11,10	15,12,13	10,11,10
Til	$CHCl_3$, $CH_3COOC_2H_5$ and C_2H_5-O-C_2H_5	10,9,8	9,8,9	8,10,9	13,9,11	9,10,12
		11,11,9	10,11,9	10,10,10	16,11,10	10,11,9
Plum	$CHCl_3$, $CH_3COOC_2H_5$ and C_2H_5-O-C_2H_5	8,8,10	9,10,10	10,9,8	15,,8,11	11,,8,10
		9,10,10	12,9,11	9,13,8	13,10,11	11,9,10
Erythromycin-15		28	32	27	24	30
Doxycycline-30		27	33	24	20	29
Tetracycline-30		33	30	18	31	

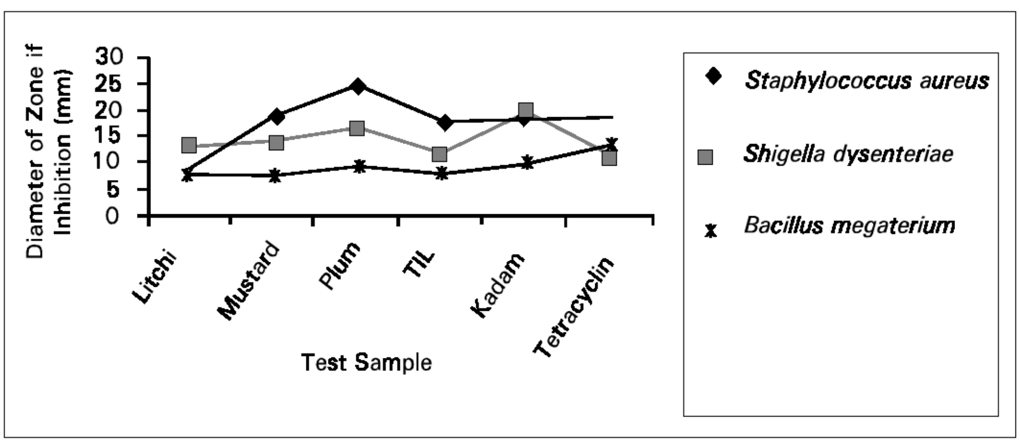

Fig .1: Antibacterial activity of crude honey against pathogenic organisms

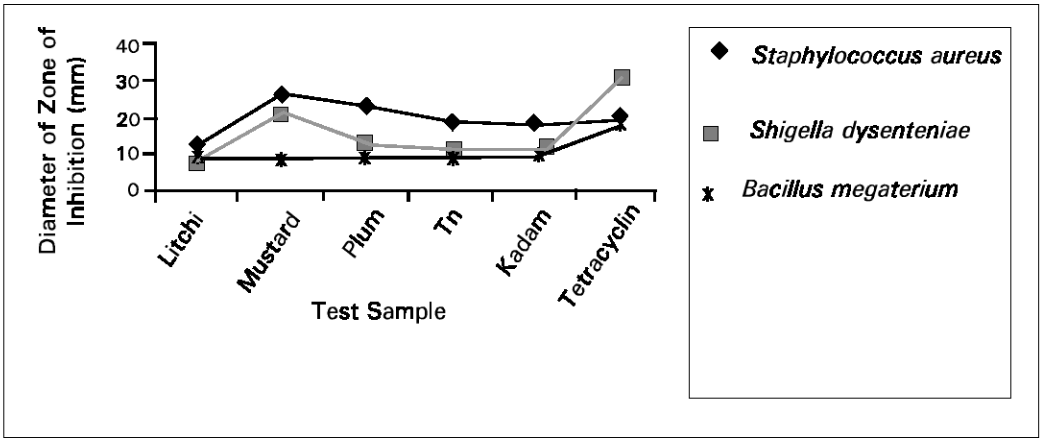

Fig. 2: Antibacterial activity of diluted honey (50 %) against pathogenic organisms

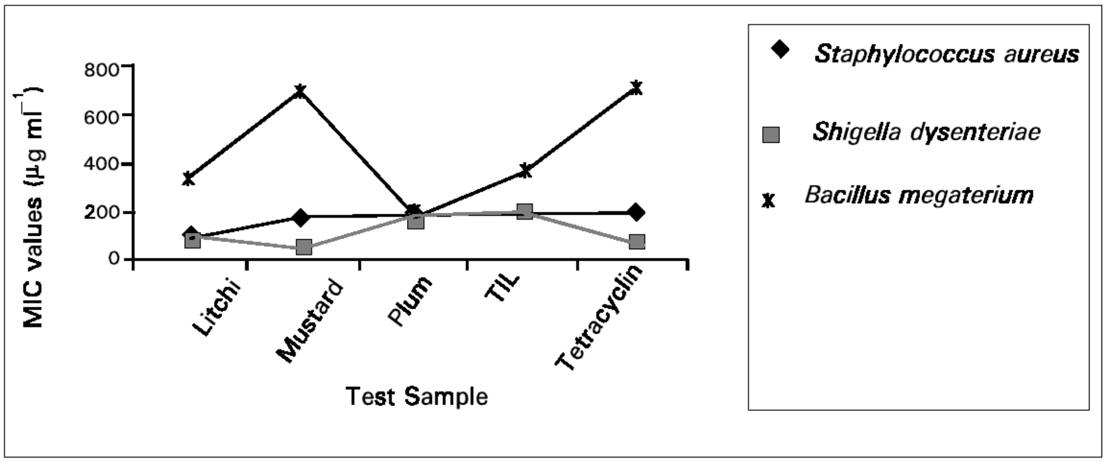

Fig. 3: MIC of the test sample against pathogenic organisms

($CH_3COOC_2H_5$), and were tested for antibacterial activity. In every case promising activity was observed. The research work is in progress.

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