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Antibacterial activity of natural honey against antibiotic-resistant bacteria

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Abstract: Natural honey is an ancient remedy in folk medicine around the world particularly where conventional and modern therapeutic agents fail. Here we report antibacterial potential of a number of unifloral and multifloral natural honey samples produced in Pakistan. Honey samples were collected from colonies of *Apis mallifera* and *Apis cerana* bees foraged on *Acacia modesta, Plactranthus* speices, *Ziziphus* species, *Trachyspermum coptcum, Citrus spp.* etc. The tested honey samples showed antibacterial activity against ATCC cultures of *Salmonella typhi, Shigella Sonneie, Vibrio furnissii, Yersinia pestis, Campaylobecter jejuni* and *Escherichia coli.* Minimum inhibitory concentration (MIC) values of honey samples were in the range of 15-18% (v/v) against. This median level potency of Pakistani honey is comparable to the antibacterial activity of Australian honeys and less than the Manuka honey from New Zealand. Interestingly, tested honey samples also proved active against antibiotic-resistant strains of *Vibrio cholera, Salmonella typhi, Shigella dynsenteriae* and *Compylobacter spp.* isolated from clinical fecal samples.

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INTRODUCTION

Natural honey is an exemplary supersaturated viscous solution of sugars, minerals, vitamins and proteins abstracted by bee of *Apis* species[1]. Among many *Apis* species, *Apis andreniformis, Apis cerana, Apis dorsata* and *Apis florae* are found to produce honey. The *Apis Mellifera* bees are widely used in apiaries for large scale natural honey production. Natural honey has been used an effective nutraceutical around the world since ancient time. Our researches scientifically supported the therapeutic applications of honey which have been reported in an array of publications[3-7]. We reported remarkable bioactivities of honey including antinociceptive[3], immuno-modulatory[4], nematicidal[5], blood glucose level modulatory[6] and human platelet aggregation inhibition[7] activities.

In folk medicine, honey has been considered for antimicrobial treatment[8]. Natural honey has been tested against several pathogenic bacteria involved in gastrointestinal tract infections, ulcers and wound infections etc[9-11]. We have tested antibacterial activity of several honey samples originated from diverse floral and geographic sources. During this study we included ATCC cultures as well as antibiotic resistant clinical bacterial isolates.

MATERIAL AND METHODS

Natural honey samples and bacterial cultures

Honey samples

Twenty two natural untreated honey samples were collected from different locations of interior Sindh and northern Pakistan. Five multifloral wild honey samples (codes; SW-1, sam, SF, An, MON) were produced by oriental hive bee (*Apis cerana*); 17 predominantly unifloral honey samples collected from colonies of European bee (*Apis mellifera*) foraging on *Acacia modesta* (codes; Kh1, AC-I, AC-II, AC-IV, and Ac-VI), *Trachyspermum* species (codes; Aj-1, Aj-2, Aj-3, Aj-4 and TRI-1), *Citrus* species (codes; CIT-1 and CIT-2), *Ziziphus* species. (codes; Ziz-1 and SIN) and *Plactranthus* species (codes; Sw2, Sw3 and Sw4) were used for experimentation.

ATCC bacterial cultures

During the present study, ATCC isolates of *S. aureus (*ATCC No. 25923), *Y. pestis (*ATCC No. 19428), *S. typhi (*ATCC No. 14028), *Vibrio furnissii (*ATCC No. 11218), *Shigella sonnei (*ATCC No. 25921), *E. coli (*ATCC No. 25922), *P. aerogenosa (*ATCC No. 27853) and *Campaylobecter jejuni (*ATCC No. 33291) were used.

Antimicrobial activity of natural honey

During the present study, antibacterial activities of honey samples were tested against several pathogenic bacteria. The methodology used for antimicrobial activity determination of honey is described in following sections.

Antibacterial activity assays using ATCC bacterial cultures

Antibacterial activity of different honey samples was determined using ATCC cultures of *S. aureus, Y. pestis, S. typhi, Vibrio furnissii , Shegella sonnei, E. coli* and *P. aerogenosa.* The agar well diffusion method[12] was used for antibacterial assays.

Briefly, fresh ATCC bacterial cultures were prepared in BHI (Brain Heart Infusion) broth for 6 hours at 37ºC in order to get growth match with 0.5 McFarland. The fresh ATCC cultures were used for growing bacterial lawns on Mueller Hinton Agar (MHA) by sterile Cotton swab. The wells of diameter 6.0 mm were made in agar plates using sterile borer and 50µl of different

concentration of natural honey samples were dispensed in the wells. The Mueller Hinton Agar plates were than incubated at 37ºC for 48 hours in aerobic condition. Antibacterial susceptibility of honey was determined on the basis of size and appearance of zone of inhibition. The results represented the average of three independent experiments.

Antibacterial activity assays using clinical isolates

Antibacterial susceptibility of honey sample was tested against a number isolates of *Salmonela, Vibrio, Compylobacter* and *Shigella* species purified from clinical fecal specimens. The antibacterial assays were done using agar well diffusion method as mentioned above[12].

Procedures for isolation and identification of pathogenic bacteria from fecal specimens

Isolation of *Vibrio, Salmonella, Shigella* and *Campylobacter* species from fecal specimens of diarrheal patients were carried out as follows.

The fecal specimens were streaked directly on the following media using sterilized cotton swab.

For *Vibrio, Salmonella* and *Shigella* bacteria: (a) MacConkey agar, (b) X.L.D agar, (c) T.T.G.A agar, (d) APW (Alkaline peptone water) and (e) Selinite Fecal broth followed by incubation at 37ºC overnight.
For Compylobacter: inoculated in CAMP medium (campylobacter agar) and incubated microaerobically at 42°C for 48 hours.

Next day the bacteria grown in Selinite Fecal broth were subcultured in Salmonela-Shigella (SS) agar and bacteria grown in APW broth were subclutured in TTGA agar followed by incubation at 37°C.

Non-lactose fermenting (NLF) colonies (Salmonela and Shigella) from MacConkey's agar and SS agar were picked and subjected to biochemical tests i.e. Urea, TSI and SIM tests. Oxidase test was carried out for gelatin hydrolyzing colonies on TTGA agar plates for identification of *V. cholerae.*

API 20E Screen tests were also used for identification of *Vibrio, Salmonella* and *Shigella bacteria***.** Serological assays for *Vibrio, Salmonella* and *Shigella* were done using slide agglutination technique.

Oxidase, Catalase and Hippurate tests were carried out for identification of Campylobacter species. Antibiotics susceptibility tests of clinical isolates of *Salmonella, Shigella* and *Vibrio cholera* were carried out using Amplicillin, Amoxicillin, Nalidixic acid, Ofloxacin, Chloramphenicol, Tetracycline, Cefixine, Cefpirome and Co-trimoxazole. The antibiotics Gentamycin, Erythromycin and Cephalothin were used for susceptibility tests of clinical isolates of Compylobacter species.

RESULTS AND DISCUSSION

Antibacterial activity of honey

Natural honey with antibacterial activity can be used for the treatment of a number of human ailments such as wound infections, diarrhea, dehydration etc. The antibacterial effect of honey has been reported by a number of researchers[13,14]. We determined the antibacterial activity of Pakistani honey against various bacteria of clinical significance in gastroenteritis and wound infections. Ten honey samples collected from *Apis mellifera* honeybee colonies foraged on four flora i.e. *Acacia modesta, Ziziphus spp., Citrus spp. and Plectranthus spp.* have been used in order to determine their effect on ATCC cultures of *Salmonella typhi, Shigella sonnei , Vibrio furnissii, Yersinia pestis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The antibacterial activity was determined by the method of macrodilution. The minimum inhibitory concentration (MIC) values of honey samples were in the range of 15-18% (v/v) against *Salmonella typhi, Shigella Sonneie, Vibrio furnissii, Yersinia pestis,* and *Escherichia coli* (Figure 1). This median level potency of Pakistani honey is comparable to the antibacterial activity of Australian honeys[15,16] and less than the Manuka honey from New Zealand[17,18]. Moreover, tested Pakistani honey samples did not show inhibitory activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

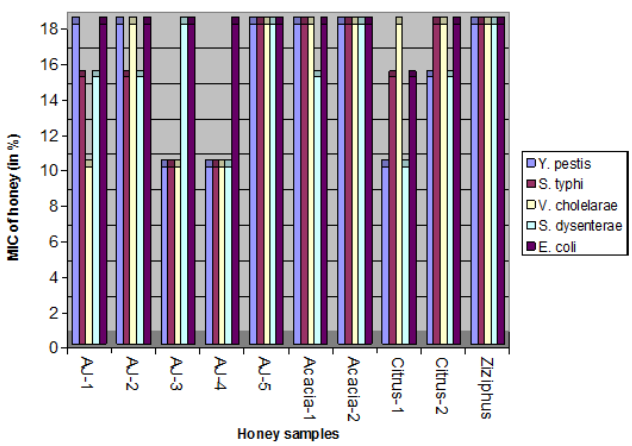

Figure 1: Antibacterial activities of different Pakistani honey samples against five pathogenic bacterial species.

Antibacterial activity of honey against antibiotic-resistant clinical isolates

Moreover, tested honey samples proved active against antibiotic-resistant strains of *Vibrio cholera* and *Salmonella typhi* isolated from fecal samples of patients in Karachi, Pakistan (Tables 1 and 2). For this purpose, 10 *Vibrio cholera*, 12 *Salmonella typhi*, 10 *Shigella dynsenteriae* and 10 *Compylobacter* species clinical isolates were obtained from patients. These clinical isolates were identified and characterized using standard biochemical techniques. Antibiotics screening of *Vibrio cholerae* and *Salmonella typhi* isolates was carried out using nine (09) antibiotics. For *Vibrio cholerae*, Ampicillin, Chloramphenicol and Ofloxacin, Co-trimoxazole/ sulfomethaxazole and Tetracycline were used, where as for *Salmonella typhi*, Amoxicillin Clavulanate, cefixime, Ceftriaxone and Nalidixic acid were used in addition to five antibiotics mentioned above. All tested *Vibrio cholerae* isolates were resistant to Co-trimoxazole/sulfomethaxazole and one isolate was resistant to Ampicillin, Chloramphenicol, Ofloxacin. All *Vibrio cholerae* isolates were sensitive to tetracycline. Most of the *Salmonella typhi* isolates were resistant to ofloxacin, nalidixic acid and Co-trimoxazole/ sulfomethaxazole. These *Vibrio cholerae* and *Salmonella typhi* isolates were grown in agar plates containing 20% honey (5 honey samples tested; codes, SW1, SW2, SW3, SW4 and KH1). Growth of most of the clinical isolates of *Vibrio cholerae* and *Salmonella typhi* was inhibited by honey samples tested (Tables 1 and 2). Likewise, the sensitivity of *S. dynsenteriae* isolates were checked using 20% honey in agar plates (5 honey samples tested; codes, SW1, SW2, SW3, SW4 and KH1). The tested honey samples showed significant antibacterial activity against clinical *S. dynsenteriae* isolates (Table 3).

Table 1: Zone of inhibition produced by honey samples (SW1, SW2, SW3, SW4 and KHI) at 20% V/V concentration against clinical isolates of *Salmonella typhi.*

	Zone of inhibition in mm					
No.	Isolate code No.	SW1	SW2	SW3	SW4	KH1
1.	(3558)	0	0	0	0	20
2.	(3798)	18	19	20	17	18
3.	(3808)	19	20	22	20	15
4.	(3739)	16	20	20	21	19
5.	(3676)	21	20	21	21	19
6.	(3515)	19	18	20	17	18
7.	(3473)	19	20	21	0	18
8.	(3518)	17	18	20	20	18
9.	(3535)	16	0	0	0	0
10.	(3468)	19	0	0	0	0
11.	(3589)	20	21	22	19	20
12.	(5017)	20	18	17	19	21

Moreover, ten clinical isolates of *Compylaobacter* (seven *Compylobacter jejuni* and three *Compylobacter coli* isolates) were tested for their sensitivity to natural honey. Results showed that *Compylobacter jejuni* isolates were sensitive to *Plectranthus* honey samples (honey sample codes SW3 and SW4), whereas the growth of *Compylobacter coli* could not be inhibited by honey samples tested (table 4). These experiments indicated benefits of the use of honey among therapies in the treatment of bacterial gastroenteritis. The criteria for antibiotics susceptibility against *Salmonella typhi* and *vibrio cholerae* was adapted from NCCLS guidelines for antimicrobial susceptibility testing[19].

Table 2: Zone of inhibition produced by honey samples (SW1, SW2, SW3, Sw4 and KHI) at 20% V/V concentration against clinical isolates of *Vibrio cholera.*

	Zone of inhibition in mm					
No.	Isolate code No.	SW1	SW2	SW3	SW4	KH1
1.	(3833)	20	18	19	17	20
2.	(3819)	19	21	18	15	16
3.	(3838)	18	18	19	17	18
4	(3721)	20	19	20	18	21
5-	(3686)	17	0	0	0	0
6-	(3632)	20	18	19	17	20
7-	(3663)	19	22	16	20	20
8-	(3678)	20	16	18	22	16
9-	(3669)	18	19	21	17	19
10	(3653)	21	17	21	16	16

Table 3: Zone of inhibition produced by honey samples (SW1, SW2, SW3, Sw4 and KHI) at 20% V/V concentration against clinical isolates of *Shigella dysenteriae.*

	Zone of inhibitin in mm					
No.	Isolate code No.	SW1	SW2	SW3	SW4	KH1
1.	(141)	0	0	17	0	20
2.	(142)	25	22	25	31	24
3.	(143)	14	25	23	27	27
4.	(144)	22	15	30	23	22
5.	(145)	20	25	24	29	16
6.	(146)	23	20	22	14	27
7.	(147)	0	0	0	0	0
8.	(148)	20	20	18	26	25
9.	(149)	25	16	22	25	20
10.	(150)	0	0	16	25	0

Table 4: Zone of inhibition produced by honey samples (SW1, SW2, SW3, Sw4 and KHI) at 20% V/V concentration against clinical isolates of *campylobacter species.*

	Zone of inhibition in mm				
Campylobacter spp. Isolate	SW1	SW2	SW3	SW4	KH1
1- *Campylobacter jejuni*	0	0	40	35	0
2 *Campylobacter jejuni*	0	0	0	40	0
3 *Campylobacter jejuni*	0	0	35	37	0
4 *Campylobacter jejuni*	0	0	35	40	0
5- *Campylobacter jejuni*	0	0	0	0	0
6- *Campylobacter jejuni*	0	0	0	0	0
7 *Campylobacter jejuni*	0	0	32	34	0
8 *Campylobacter Coli*	0	0	0	0	0
9- *Campylobacter Coli*	0	0	0	0	0
10 *Campylobacter Coli*	0	0	0	0	0

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