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STUDIES ON THE ANTIMICROBIAL ACTIVITY OF HONEY

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ABSTRACT

Ten samples of crude and processed honey were used to determine antimicrobial activity against twenty five species of pathogenic and non-pathogenic bacteria, commonly encountered in human infections. The antifungal activity was checked against ten parasitic and saprophytic fungi. Most of the samples of honey used in the study showed broad spectrum antibacterial and promising antifungal activity.

Introduction

The *Holy Quran,* the heritage of Islamic Sciences, has time and again emphasized the medicinal virtue of honey. The last scripture enlists honey as a miraculous food in a separate surah, "AI-Nahl (The Bee)".[11]

And they Lord commands the bee, saying:

"Make thee houses in the mountains and in the trees and in what they (people) build-up, then eat of all the fruits and walk thou the ways of thy Lord made easy. He produces from her inside a drink of various hues, in which there is healing for mankind. Most surely in this there is a sign for people who think".

Honey is the most primitive nourishing and healing agent. It is a thick, syrupy, translucent, pale yellow or yellowish brown liquid deposited in the honey comb by the bee, *Apis mellifera* Linn. (Family: Apidae). It has a characteristics odour and a sweet, faintly acidic taste. It contains: invert sugar (62-83%), sucrose (0-8%), dextrin (0.26-7%) together with water and traces of other nutrients.[7] Honey is levorotatory and is acidic to litmus paper.[5] It is a unique mixture having fat soluble as well as water soluble vitamins.[8]

During the 16th and 17th centuries it was recommended as a cure for almost every thing.[2] It is a simple, effective and inexpensive healer of wounds. It has been used in the treatment of infected wounds in the Department of Obstetrics and Gynaecology at the Kilimanjaro Christian Medical Centre with excellent results.[1]

Honey also serves as a substitute for glucose in oral rehydration solutions containing electrolytes. The correct dilution of honey and the presence of electrolytes in the oral solution must be maintained.[9]

In Soviet Union, honey is commonly used as a base for ointments and has very successfully been applied in surgical dressings for open wounds and burns to avoid septic infections. It is also used for many minor ailments of animals.[10]

In the pharmaceutical preparations it is used as a sweetening, flavouring and demulcent agent. It is used as an excepient for preparing pills and masses and as a flavouring agent in gargles, linctuses, and cough mixtures.[18]

Materials and Methods

10 samples of locally manufactured, imported and crude samples of honey were procured from the market and from the hives (Table 1) in order to determine their antimicrobial activities.

In the present study 25 species of common pathogenic and non- pathogenic bacteria and 10 species of fungi were used. Eleven bacteria were Gram positive and four-teen were Gram negative (Tables 2, 3). Among the fungi, nine species were pathogenic and only one specie was saprophytic and non-pathogenic (Table 6).

A comparative study of antimicrobial activity has been carried out by the procedure described by Bhakuni et a1.[3] and Baqir et al.[2]. The sample exhibiting maximum antimicrobial activity was fractionated into partly purified alkaloidal and non-alkaloidal portions, in order to find which portion contained antimicrobial constituents.

Preparation of Test Samples:

10 mg of honey samples *were* taken in 50 ml sterile volumetric flask. The volume was made up with sterile distilled water for a dilution of 20% w/v.

Separation of Alkaloidal and Non-Alkaloidal Fractions of Honey:

Crude honey sample (F) which indicated promising antimicrobial activity, w fractionated into alkaloidal and non-alkaloidal fractions by chemical method so as to determine which portion possessed maximum antimicrobial activity.

20 mg of honey (sample F) was extracted with ethanol (EtOH) three times. The EtOH extracts was acidified with 10% HCI and extracted with CHCI3 to remove the non-alkaloidal portion. The acidic aqueous fraction was then basil˙led with NH_3 and extracted thoroughly with $CHCl_3$. The mass when examined on TLC plate showed several spots indicating several constituents which gave coloured reactions with Dragendroff reagent.

Antimicrobial Assay:

The tests were run in triplicate. Petri plates were prepared with trypticase soy gar. 1 ml of the diluted culture was poured on each plate. Wells of 5 mm diameter (approximately) were cut with sterile cork borer in the inoculated agar. The wells were filled with test samples with the help of sterile droppers. Similarly in control plates wells were filled with sterile distilled water. The plates were incubated for 24 hours at 37°C. At the end of incubation period the inhibition zones were measured. The results *were* tabulated in Tables 2, 3, 4, 5.

Antifungal Assay:

The tests were run in triplicate. 2 gm of honey was mixed with 8 ml sterile molten Sabouraud dextrose agar and was poured into sterile petri plates. 1 ml of 10^{-2} dilution in strile 0.9% NaCl of the two-day fungal cultures were spotted on dried Sabouraud agar plates. The plates were incubated at 25°C for 5 days. The results were noted as heavy growth (H), scanty growth (S) and no growth (N). In ontrol plates no honey was incorporated in Sabouraud dextrose agar. The results *were* tabulated in Tables 6, 7.

Results and Discussion

The *Holy Quran* intimates honey as the healing for all kinds of diseases. Honey is a divine drug quoted in all the scriptures. The therapeutic value of honey was under-scored in various literatures. It occupied a prominent place in traditional medicine. It is used in all the systems of medicine for the treatment of a number of human ailments such as wound infection, diarrhoea, dehydration, paralysis particularly facial paralysis, amenorrhoea, dropsy, chest infection, jaudice, tuberculosis, urinary tract infections, carcinoma of vulva, deafening ear, fever, tetanus, boils, skin strains and skin infections.

The antimicrobial effect of honey has been reported by a number of workers (1, 5, 9, 10, 18, 25) but most of these reports are conflicting and are not substantiated by enough scientific proof. The present study was carried out so as to determine the antimicrobial effects of honey using prescribed scientific procedures.

The important conclusions which can be drawn from the present study can be summarized as such. Firstly, honey has broad spectrum antimicrobial and prominent antifungal activity as indicated by Tables 2-7. In our study seven species of Gram positive cocci and four species of Gram positive bacilli were used. With the exception of *Streptococcus faecalis,* all the Gram positive organisms were inhibited by samples A and B (Langanese and crude honey). However this specie was inhibited by samples E, F, G, H, I and J. Thus the present study is in confi[r]mation with previous reports of Cavanaugh et a1,[5] Warnecke et al.[24] and Obaseiki et al.[13] The control plates did not show any inhibition.

Secondly, studies on species of Corynebacterium (*C. dipfheriae, C. hoffmani, C. xerosis*) have not previously been reported. The present study reveals that all the three species were inhibited by honey samples A, *B, F,* H and J. Honey samples C and D (Marhaba, Hamdard) showed no activity against these organisms. In fact these two samples exhibited activity only against four Gram positive cocci i.e. *M. lysodeikticus, S. aureus, S. laths* and *S. pyogenes* (Table 2).

Similar results are reported with Gram negative bacteria (Table 3). Samples A, B, E, F, G and H very effectively inhibited the growth of nearly all Gram negative organisms used in the study with the exception of E. *coil* 97 which was resistant to most of the honey samples. As far as the Gram negative organisms are concerned the present reports conforms the findings of Obaseiki et al.[13] and Ibrahim.[12]

Thirdly, the present study seems to be fast report in literature with respect to *Vibrio cholerae.* As indicated in Table 3, all the samples of honey produced large zones of inhibition against this organism. This importance of this finding lies in the fact that honey can be used in oral rehydration solution for treating cholera patients.

As far as fungi are concerned, only two reports could be traced (13, 14). These reports indicated that honey had promising activity against *Candida albicans* and *Aspergillus niger.* Present study seems to be the first report indicating activity against dermatophytes *(M. ferrogenium, T. longfeuseus, T. mentag*ro*phyte, T. semmie, T. ton*surance) and parasitic fungi *(Allescheria boydii)* and saprophytic *(Mucor* mucaralis). Including two species of *Aspergillus* i.e. *A. flavus, A. niger* and *C. albicans,* ten species of fungi were used. Honey seems to be much less effective on fungi than bacteria (table 6). The finding which is most interesting is that as compared to other honeys, Capilano honey (I) from Australia seems to be most effective against most of the species of fungi used in the study. This sample did not show promising activity against bacteria.

Among the different samples of honey used in the study sample F, the crude honey procured from a bee-hive of Karachi University, showed maximum activity. An attempt was made to fractionate this sample of honey into alkaloidal and non-alkaloidal fractions by chemical method. Both the alkaloidal and non-alkaloidal fractions of honey exhibited similar activity indicating the antimicrobial system was present in both the fractions of honey (Tables 4, 5, 7).

It has been suggested that three major system are responsible for the antimicrobial activity of honey i.e. inhibine, high osmotic pressure and acidity.[10]

Inhibine

The initial report on inhibine, an antibacterial substance in honey was presented by Cavanagh et al.[5] who stated that inhibine content of honey was reduced by sunlight. Thereafter several workers reported various physical and chemical properties of the substance, the exact constitution of which is still unknown.[13,17,19]

High osmotic pressure

Honey is basically a supersaturated solution of carbohydrates, the osmotic pressure of honey creates a very effective antimicrobial system.[4,5,6,10]

Acidity

The general range of honey pH is 3.2-4.5. This high degree of acidity prevents the growth of many bactrial forms.[10,22,25]

From the results of the present study the most important conclusion which can be drawn is that the antimicrobial activity is maximum in the crude honey. The three samples of fresh crude honey used in the study showed much better activity than the processed honeys. It can be suggested that during the processing the active components were partially inactivated.

And above all this humble study scientifically proves what ever is indicated in Surah "AI-Nahl".

"He produces from her inside a drink of various hues, in which there is healing for mankind. Most surely in this there is a sign for a people who think".[11]

Table 1
Honey samples

Name	Code
Langanese	A
Crude honey*	B
Marhaba	C
Hamdard	D
Pak. Golden Honey	E
Crude honey*	F
Pak. honey	G
Crude honey*	H
Capilano	I
Australian Honey	J

*Procured from hives.

Table 2
Zones of inhibition produced by different samples of honey against Gram positive bacteia

Name of organisms	Zones of inhibition in mm									
	*A	B	C	D	E	F	G	H	I	J
Bacillus subtilis	28	20	-	-	34	40	30	20	10	10
Corynebacterium diptheriae	20	20	-	-	-	20	-	20	24	28
Corynebacterium hoffmani	30	20	-	-	30	24	-	20	-	20
Corynebacterium xerosis	15	20	-	-	15	5	30	20	25	22
Micrococcus lysodeikticus	40	40	25	20	10	5	10	30	-	-
Staphylococcus aureus	20	30	35	25	24	30	27	30	-	-
Staphylococcus epidermidis	15	20	-	-	10	30	30	-	12	17
Staphylococcus saprophyticus	26	30	-	-	30	40	35	24	22	24
Streptococcus faecalis	-	-	-	-	30	25	30	-	17	20
Streptococcus lactis	20	20	18	18	10	25	20	-	-	-
Streptococcus pyogenes	30	35	22	24	-	30	-	32	15	-

*Honey samples A - J.
Negative sign (-) indicates no inhibition.

Table 2
Zones of inhibition produced by different samples of honey against Gram negative bacteria

Name of organisms	Zones of inhibition in mm									
	*A	B	C	D	E	F	G	H	I	J
Aerobacter aerogenes	30	30	-	-	20	30	35	-	15	17
Escherichia coli 76	20	20	18	17	10	20	15	20	19	19
Escherichia coli 97	-	-	-	-	20	40	50	-	-	-
Klebsiella pneumoniae	12	20	-	-	-	-	10	20	-	-
Proteus vulgaris	20	22	-	-	10	5	10	30	-	-
Pseudomonas aeruginosa	20	20	-	-	15	20	10	10	18	24
Salmonella typhi	30	50	30	25	35	30	50	30	22	20
Salmonella typhi para A	8	10	-	-	35	40	-	30	22	30
Salmonella typhi para B	14	12	-	-	25	30	25	16	18	25
Shigella boydii	35	40	-	-	20	30	32	24	22	27
Shigella dysenteriae	12	30	18	16	12	10	-	20	15	20
Shigella flexneri	10	20	-	-	10	40	30	10	25	20
Shigella sonnei	20	20	-	-	20	25	32	24	15	10
Vibrio cholerae	24	20	25	25	25	20	25	16	19	19

*Honey samples A - J.
Negative sign (-) indicates no inhibition.

Table 4
Zones of inhibition produced by alkaloidal and non-alkaloidal fractions of sample 'F' against Gram positive bacteria

Name of organisms	Zones of inhibition in mm	
	Alkaloidal	Non-alkaloidal
Bacillus subtilis	10	12
Corynebacterium diptheriae	15	22
Corynebacterium hoffmani	-	-
Corynebacterium xerosis	17	27
Micrococcus lysodeikticus	-	-
Staphylococcus aureus	20	17
Staphylococcus epidermidis	12	20
Staphylococcus saprophyticus	20	30
Streptococcus faecalis	20	20
Streptocccus lactis	8	10
Streptococcus pyogenes	12	12

Negative sign (-) indicates no inhibition.

Table 5
Zones of inhibition produced by alkaloidal and non-alkaloidal fractions of sample 'F' against Gram negative bacteria

Name of organisms	Zones of inhibition in mm	
	Alkaloidal	Non-alkaloidal
Aerobacter aerogenes	15	15
Escherichia coli 76	10	25
Escherichia coli 97	8	25
Klebsiella pneumoniae	8	12
Proteus vulgaris	18	28
Pseudomonas aeruginosa	-	-
Salmonella typhi	20	25
Salmonella typhi para A	-	-
Salmonella typhi para B	15	32
Shigella boydii	20	28
Shigella dysenteriae	12	12
Shigella flexneri	16	20
Shigella sonnei	12	20
Vibrio cholerae	10	27

Negative sign (-) indicates no inhibition.

Table 6
Pattern of Growth of Fungi on honey incorporated media

Name of organisms	Zones of inhibition in mm									
	*A	B	C	D	E	F	G	H	I	J
Allescheria boydii	H	H	H	H	H	H	H	H	N	H
Aspergillus flavus	H	H	H	H	H	H	H	H	S	H
Aspergillus niger	N	S	H	H	H	N	H	H	S	S
Candida albicans	N	N	H	H	H	N	H	H	N	N
Microsporum ferrogenium	S	S	H	H	H	S	H	H	N	N
Mucor mucaralis	H	H	H	H	H	H	H	H	H	H
Tricophyton longifeusius	H	H	H	H	H	H	H	H	S	H
Tricophyton mentagrophytes	N	N	H	H	H	S	H	H	N	N
Tricophyton semmie	S	S	H	H	H	S	H	H	S	S
Tricophyton tonsurance	H	H	H	H	H	H	H	H	N	N

*Honey samples A-J
H - Heavy Growth
S - Scanty Growth
N - No Growth

Table 7
Pattern of Growth of Fungi on media incorporated with alkaloidal and non-alkaloidal fractions of honey

Name of organisms	Zones of inhibition in mm	
	Alkaloidal	Non-alkaloidal
Allescheria boydii	S	H
Aspergillus flavus	H	H
Aspergillus niger	H	H
Candida albicans	S	N
Microsporum ferrogenium	N	H
Mucor mucaralis	H	H
Tricophyton longifeuseus	S	H
Tricophyton mentagrophytes	H	S
Tricophyton semmie	H	H
Tricophyton tonsurance	H	H

H - Heavy Growth
S - Scanty Growth
N - No Growth

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