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COMPARISION OF INVITRO ANTIMICROBIAL ACTIVITY OF DIFFERENT CRUDE PAKISTANI HONEY SAMPLES AND COMMERCIALS ANTIBITOTICS AGAINST CLINICAL PATHOGENS

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ABSTRACT

Honey is a natural medicinal substance, has been applied since ancient times as a natural remedy of various aliments and diseases. It is valued to possess antimicrobial and anti-inflammatory activity. The object of the present study was to determine the invitro antimicrobial activity of different honeys samples belonging to Pakistani floral sources against certain clinical pathogens and to compared with commercial antibiotics. For the investigation of antimicrobial activity ten different floral sources *Apis mellifera* honey samples (*09 = Mix flora, 11= Gossypium species*, 13= *Phoenix dactylifera, 35= Salvadora persica*, 39= *Mangifera indica*, *46= Acacia, 56= Carissa opaca*, 58= *Helianthus annuus*, 59= *Zizyphus, 65= Medicago sativa*) were used, harvested during 2003 - 04 and 2004 - 05. The antimicrobial activity of honey samples were evaluated by ICLS (formerly NCCLS) reference Disc diffusion (Kirby-Bauer) method against recently isolated most common six Gram-positive (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus faecalis, Streptococcus pyogenes, Bacillus subtilis, Corynebacterium diptheriae*), eleven Gram-negative (*Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli, Shigella dysenteries, Salmonella typhi, Salmonella typhi para A, Salmonella typhi para B, Proteus mirabilis, Proteus vulgaris, Enterobacter species, Pseudomonas aerugenosa*) bacteria and one Fungus (*Candida albicans*),commonly encountered in human infections. Most of the samples of honey used in the study exhibited broad spectrum antimicrobial activity and promising antifungal activity. The results indicated that activity was present in all the honey samples. Comparison of crude honey samples with the existing commercial antibiotics was also carried out, in all cases the honey samples were found comparable to antibiotics used in the study.

KEY WARDS: Pakistani honey samples, antimicrobial activity, commercial antibiotics.

INTRODUCTION

Honey is a thick, sticky, translucent, pale yellow or yellowish brown substance of characteristic odor and a sweet faintly acidic test, formed from nectar and sweets collected from floral sources, modified and stored by the honey bee *Apis melliferia* Linn. in the honey comb. (The pharmaceutical codex, 1979).

The precise composition of honey primarily depends on the floral sources on which the bee forages but the main components are the same in all honeys. White (1975) reported that honey contain 181 substances. It includes fructose as a major component (38%) and glucose as a second prevalent constituent (31%) (Gheldof et al., 2002). It also includes minerals, Vitamin C, Vitamin E, and amino acid (Ball 2007) and Phenolic compounds (Wahdan, 1998; Jaganathan et al., 2009).

The application of honey as internal and external remedies is dates back to the history of medicine it self. In the ancient times, the Greek and Egyptians used unprocessed honey to inhibit microbial infections and in the treatment of wound management. (Zumla and Lulat, 1989; Alwaili and Noori, 2004). It is valued to possess antimicrobial, analgesic, anti inflammatory, haemostatic and healing enhancing properties (Wang et al., 2009).

A large number of honey samples evaluated for antimicrobial potential, and a large number of clinical pathogens have been reported to be inhibited by honey. Cavanagh et al. (1970) found that honeys not only inhibit the growth of *Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeruginosa, Proteus mirabililis,* and *Escherichia coli*, but actually killed them, the *Candida albicans, C. tropicalis*, and *C.stellatoidea* were also found sensitive to honey. Farouk (1988) found honey effective to several *Pseudomonas* and *Staphylococcus* strains then the antibiotic Gentamicin.

The effective use of honey in the treatment of such wounds not responding to antibiotic treatment has gained recognition from scientific community (Molan, 2000). There is reintroduction of the natural product into modern medical practice in Australia and Europe, the United State authority also gave clearance of Medi honey as a wound dressing Product in 2007 (Cutting, 2007).

Various physical and chemical properties of honey are useful in the treatment of wounds (Snow and Manley-Harris, 2004), the exact mechanism of honey in wound healing is still unknown, studies showed that it acts as a bactericidal rather than bacteriostatic (Wahdan, 1998; Blair, 2003). Thus researches focused on the antimicrobial activity of honey as a cause of wound healing (Zumla and Lulat, 1989; Efem, 1993; Adesunkanmi and Oyelami, 1994).

Antimicrobial activity is attributed to the high osmalarity of honey, low pH, hydrogen peroxide, and phytochemicals. Honey is a supersaturated solution (17%water), the microorganisms cannot survive in honey, its hygroscopic action creates a moist environment by drawing lymph and other moisture to the wound surface thus dehydrates bacteria (Molan, 1999).The low pH level (3.2 – 4.5) of honey is sufficient to inhibit microbial growth (Pieper, 2009; Sharp, 2007).The enzymatic production of hydrogen peroxide is supposed to be an important cause of antimicrobial activity of honey, when honey comes in contact with wound fluids moist environment and antimicrobial hydrogen peroxide is produced successfully that act as a good disinfectant and kills the pathogenic bacteria, but remains well below the level that cause inflammatory effect to the surrounding tissue (Lusby et al., 2002; Sharp, 2007). The inhibine in the honey comprises many other non- peroxide organic substances (phytochemical). A wide range of phenolic compounds have been recovered in honeys for the antimicrobial activity consisting of cinnamic acids and their esters, benzoic acid and their esters, and flovonoids aglycones (Andrade et al., 1997 ; Jaganathan et al., 2009).

Various studies reported the effectiveness of honey in the treatment of wound, burn, and skin infections (Zumla and Lulat, 1989; Piper, 2009). Based on growing evidences of its efficacy and excellent record of safety, there is need for a product that combine antimicrobial activity with wound healing stimulating properties (Overgaaum and Kirpensterijin , 2005). Hence during the present study an attempt was made to investigate the antimicrobial activity of different Pakistani honey samples against clinical pathogens and compared with commercial antibiotics.

MATERIAL AND METHOD

Haney samples

A total numbers of ten crude *Apis mellifera* honey samples from different floral sources of Pakistan (*09 = Mix flora, 11= Gossypium species,* 13= *Phoenix dactylifera, 35= Salvadora persica* , 39= *Mangifera indica* ,46= *Acacia* ,56= *Carissa opaca,* 58= *Helianthus annuus,* 59= *Zizyphus* ,65= *Medicago sativa*) were used in this study, harvested during 2003 - 04 and

2004 - 05. The each honey sample was properly identified, established its purity according to the method of Fich's test (Hamdard Pharmacopeias of Eastern Medicine) and kept in sterile air tight glass at room temperature in the dark.

Clinical isolates

Freshly isolated most common six Gram-positive (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus faecalis, Streptococcus pyogenes, Bacillus subtilis, Corynebacterium diptheriae*), eleven Gram-negative (*Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli, Shigella dysenteries, Salmonella typhi, Salmonella typhi para A, Salmonella typhi para B, Proteus mirabilis, Proteus vulgaris, Enterobacter species, Pseudomonas aerugenosa*) bacteria and one Fungus (*Candida albicans*) were used in this study. Clinical isolates were obtained from Department of Pharmaceutics, Faculty of Pharmacy, and University of Karachi. The clinical isolates were identified on the bases of standard microbiological techniques, and maintained in laboratory on Mueller Hinton agar at 4 ^{o}C.

Commercial antibiotics

Amoxicillin – Clavulamic acid (AMC-30 ug/ml and AML – 10 ug/ml), Ceftazidim (CAZ - 30ug/ml), Cefixime (CFM- 05 ug /ml, Ciprofloxacin (CIP- 30 ug/ml) Erythromycin (E – 15 ug/ml), Vancomycin (VA - 10ug/ml), Tetracycline (TE- 30 ug/ml), Imipenum (IPM-10ug/ml), Doxycycline (DO- 30ug/ml), Oxytetracycline (OT-30ug/ml), Clarithromycin (CLR – 15ug/ml), Moxifloxacin (MXF- 5ug/ml), Nystatin (NT – 100 UT) were used in this study.

Methods

Purity Test

The presence or absence of synthetic invert sugar in honey identified according to the Fiche's test: 10 ml honey solution (20gm/100ml water) is mixed with 5ml ether and left till the ether layer separated out. The ether layer is transferred to a porcelain dish to evaporate the ether. The resorcinol solution is prepared by dissolving 1 gm resorcinol in 100ml of HCl. The appearance of a cherry red, orange red or reddish brown color shows the presence of invert sugar. The pink colors which disappear after 30 second are permissible.

Preparation of Honey samples

7.5 mg of honey sample mixed with 2.5 ml distilled sterile water to prepare 75% w/v sample. To prepare the test disc of honey samples cut the filter paper disc and soaked in honey sample solution.

Preparation of inoculums

With the flame sterilized wire loop at least four or five similar appearing well isolated colonies on an agar plate culture were transferred by touching top of each colony to a tube containing 4 to 5 ml Mueller – Hinton broth medium, and incubate broth culture at 37^{o}C until it achieved the turbidity of the Mac Farland 0.5 Standards, it usually takes 2 to 3 hours.

Inoculation of susceptibility plates

With in 15 minutes on the achievement of required turbidity of the inoculums suspension dipped a cotton swab (sterile) into the inoculums suspension and inoculated dried Mueller – Hinton agar plates (at room temperature) by streaking the swab over the entire agar surface three time by rotating the plates in three direction, than place the test / antibiotic disc by flam sterilized forsceps. The susceptibility plates were placed at 37^{o}C in incubator for 16 – 18 hours (lotus Co model 215 incubators), in upside down position.

Measurement of inhibition zone diameter

After incubation period zone of growth were appeared a round the discs. The diameter of zone of inhibition including the diameter of the discs was measured to the nearest whole millimeter using sliding caliper viewing from the back of the Petri plates using a bright source of treatment light. The results were tabulated in table. The tests were run three times with each honey sample, and 5 time with each antibiotics.

Data analysis

For each honey sample triplicate determination and for commercial antibiotics five experimental determinations were performed and values expressed as Mean and standard error, were calculated by using software MS Excel 2003. Data were analyzed by analysis of variance using SPSS 17 (2008) and separation of means by Duncan's multiple range tests at a probability level of $P < 0.05$.

RESULT AND DISCUSSION

The comprehensive invitro antibacterial and antifungal activity of Ten crude honey samples against freshly isolated common Gram positive, Gram negative bacteria and fungus (yeast) were performed by CLSI (Formally NCCLS) reference disc diffusion (Kirby - Baure) method (Koneman et al., 1997). The activity was estimated by measuring the diameter of inhibition zone place where the growth of bacteria was inhibited due to the antimicrobial activity of honey. Any inhibition zone of diameter less than 7mm indicated that microorganisms were

resistant to honey sample, however inhibition zone of diameter larger than 11mm suggested that the microorganism was sensitive to honey sample (Agbagwa and Frank-Peterside, 2010). Subrahmanyam (2001) observed 100% inhibition of microorganisms on Mueller Hinton agar with honey by 30% (v/v) concentration. Ceyhan and Ugur (2001) investigated that 50% (w/v) concentration of honey showed the better zone of inhibition against most bacteria but less effective against fungi. Fangio et al. (2007) also proved that 50% (w/v) concentration of honey give better results against *Escherichia coli*. In the present work 75% (w/v) concentration of honey samples were used that is in accordance with Wahdan (1998) supported the high concentration of honey for significant results.

In the present study it has been found that all the honey samples of different floral sources of Pakistan exhibited significant zone of inhibition against all the microorganisms used. Thus activity was found in all investigated honey samples. (Table1, 2 and 3). This corroborates the work of Efem (1992) reported that honey has great antimicrobial activity against *Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Bacteroides fragilis,* except *Clostridium oedematiens* and *Pseudomonas aeruginosa.*

Staphylococcus aureus and *Staphylococcus epidermidis* used in the present study primarily responsible for supportive lesions in the human body such as boils, pimples, and closely associated with infections of wounds (cross infections) and also have been isolated from burn wounds (Subrahmanyam, 2001). All honey samples showed antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* with the zone of inhibitions ranging from (14.667 $\pm$ 0.333 to 35.667 $\pm$ 0.333mm) and (16 $\pm$ 0.882 to 37.333 $\pm$ 0.333 mm) diameter respectively. The largest inhibitory zones were observed with the sample number (56) the *Carissa opaca* floral source honey against *Staphylococcus aureus* and *Staphylococcus epidermidis*. These results are in confirmation with the study of Lusby et al. (2005) reported that *Staphylococcus aureus* and *Staphylococcus epidermidis* were inhibited by honey. These results are also in confirmation with pervious reports of (Efem, 1992; Adetuyi et al., 2009)

Streptococcus pyogenes and *Streptococcus faecalis* were also used in the study. *Streptococcus faecalis* primarily responsible for 95% of *Enterococcal* infections including the urinary tract, biliary tract, ulcers (bed sores), wounds infections. *Streptococcus pyogenes* are responsible for burn wound infections (Cheesbrough, 2000).In this study diameter of

inhibition zone of honey samples against *Streptococcus pyogenes* and other strain *Streptococcus faecalis* were measured ranging from (14.0 ± 0to 30.333 ± 0.333 mm) and (16.0± 0 to 20.667± 0.333 mm) respectively. The sample number (59) *Zizyphus* honey and sample number (09) mix flora honey displayed the highest zone of inhibition against *Streptococcus pyogenes* and *Streptococcus facealis* respectively. These present results are in confirmation with pervious reports Efem (1992), Cavanagh et al. (1970) noted the zone of inhibition of honey against *Streptococcus pyogens* and *Streptococcus faecalis*.

The aerobic spore forming *Bacillus subtilis* which is widely distributed in soil, dust, and decomposing matter and another Gram-positive bacilli *Corynebacterium diptheriae* were also included in the study. *Corynebacterium diptheriae* causes nasal, nasopharyngeal and tonsillar deptheria especially in young children, often there is marked odema of the neck, cutaneous (skin) diphtheria which usually develops when *Corynebacterium diptheriae* infects open wounds. In many developing countries there is high prevalence of skin diphtheria, especially in rural areas. In the present study the range of inhibition zone diameters of honey samples against *Bacillus subtilis* and *Corynebacterium diptheriae* were observed (12 ± 0 to 15.333 ± 0.667 mm) and (14± 0 to 28 ± 0.577 mm) respectively. The largest zones of inhibition were observed with sample number (56) *Carissa opaca* honey and sample number (46) *Acacia* honey against *Bacillus subtilis* and *Corynebacterium diptheriae* respectively. The present result is in confirmation with the study of Malika et al., (2004) reported that *Bacillus subtilis* is inhibited by honey, and inhibition of *Corynebacterian diptheriae* is in confirmation with the study of Sheikh et al. (1995).

Among Gram-negative *Klebsiella pneumoniae* isolated from burn wounds (Subrahmanyam, 2001) and other species *Klebsiella oxytoca* were also included in the study. All the samples of honey were found active in inhibiting these strains, the diameter range of inhibition zone (15 ± 0 to 20 ± 0 mm) and (13.333 ± 0.333 to 15.667 ± 0.577 mm) were observed against *Klebsiella pneumoniae* and *Klebsiella oxytoca* respectively. Present results are in confirmation with the study of Subrahmanyam. (2001) indicated the inhibition *Klebsiella pneumoniae* with honey sample. Alwaili and Noori (2004) also reported the antimicrobial activity of honey against *Klebsiella pneumoniae* and Cooper (1998) reported the activity of honey against *Klebsiella oxytoca*.

Shigella dysenteries causes bacillary dysentery in human also included in the study. All samples of honey were tested and found active in inhibiting *Shigella dysenteries,* the diameter

range of inhibition zone (13 ± 0 to 19 ± 0.577mm) was observed, and the sample number (09) Mix flora honey exhibited the highest zone of inhibition. This is in confirmation with previous reports of Sheikh et al. (1995) and Alwaili and Noori (2004) and Adetuyi et al. (2009).

Antibiotic resistance in *Salmonella typhi* and *Salmonella typhi para A* has emerged and reported in various parts of Asia (Butt et al., 2003), *Salmonella typhi* is the causative agent of typhoid fever, where as *Salmonella typhi para A* and *Salmonella para B* are responsible para typhoid fever, during the study all the honey samples tested were found very active in inhibiting all *Salmonella* strains (*Salmonella typhi, Salmonella para typhi A* and *Salmonella para typhi* B) and range of diameter of inhibition zones (25.5 ± 0.667 to 45. 667 ± 0.333 mm), (25.333 ± 0.333 to 44.333 ± 0.882mm), and (14± 0 to 38.666 ± 0.882 mm) were observed respectively. The sample number (56) *Carissa opaca* honey, sample number (39) *Mangifera indica* honey and sample number (46) *Acaccia* honey showed the highest zone of inhibition respectively. Present results of the study are in confirmation with the study of Sheikh et al. (1995) reported the zone of inhibition produced by different samples of honey against *Salmonella typhi, Salmonella para typhi A,* and *Salmonella para typhi B.* Hannan et al. (2009) reported that black seed and shain Pakistani honey have the potential to inhibit all typhoid *Salmonella* strains.

Escherichia coli causes surgical wound infection (Cheesbroug, 2000) and is also isolated from burn wounds (Subrahmanyam, 1991) is included in the study. It was found that all the honey samples tested were active in inhibiting the *Escherichia coli* and the range of diameter of inhibition zone (14 ± 0 to 30.666 ± 0.667 mm) is observed, the highest zone of inhibition is displaced by the sample number (46) *Accacia* honey and sample number (56) *Carissa opaca* honey. The present results are in confirmation with the study of Wahdan (1998) and Efem et al. (1992) reported the anti *Escherichia coli* activity of honey. Present results also agree with Lusby (2005) and Fangio et al. (2007) studies indicated that various honey samples have been shown to exhibit antimicrobial activity against *Escherichia coli*.

Proteus mirabilis and *Proteus vulgaris* are also examined in the present study. *Proteus mirabilis* is a common cause of urinary infection and is also responsible for abdominal and wound infection, and is often a secondary invader of ulcers, pressure sores, burns and damaged tissues, is isolated from surgical wound infection (Cheesbrough, 2000).and from burn wounds (Subrahmanyam 2001). In the present work of the antimicrobial activity of

Pakistani honey against *Proteus mirabilis* and *Proteus vulgaris* were determined by measuring inhibition zone diameter around the disc, that is (14.666 ± 0.333 to 30. 333 ± 0.333mm) and (14.666 ± 0.333 to 27.0 ± 0mm) were observed respectively, samples number (56) *Carissa opaca* honey and sample number (59) *Zizpyphus* honey displayed the highest zone of inhibition against *Proteus mirabilis* and *Proteus vulgaris* respectively. The present finding are in confirmation with previous reports of Cavanagh et al. (1970) and Adetuyi et al. (2009) indicated that honey samples exhibited antimicrobial activity against *Proteus mirabilis*. These results are also in confirmation with pervious reports of Subrahmanyam (2001) and Alwaili and Noori (2004) reported that both *Proteus mirabilis* and *Proteus vulgris* exhibited inhibition with honey samples.

During the study it was also noted that honey samples were active against *Enterobacter spp*. and diameter of inhibition zones (12 ± 0 to 16 ± 0.577mm) is measured. The highest zone of inhibition is displaced by sample number (39) *Manglfera* honey and sample number (56) *Carrisa opaca* honey. These results are in confirmation with the study of Alwaili and Noori (2004) evaluated the antimicrobial activity of natural honey against *Enterobacter cloacae.*

Pseudomonas aeruginosa is important cause of surgical wounds and burn wounds infections (Cheesbrough, 2000), is also responsible for urinary, respiratory and skin infections especially pressure sores and ulcers (often as invader). In the present study all crude honey samples were found active in inhibiting the *Pseudomonas aeruginosa* and the range of inhibition zone diameter (17. 666 ± 0.333 to30. 666 ± 0.333mm) is observed, the sample number (35) *Salvaora persica* honey displayed the highest zone of inhibition. These results are also in confirmation with Subrahmanyam (2003) reported the sensitivity of honey against multi drug resistant *Pseudomonas aeruginosa* from infected wounds. This study is in confirmation with previous reports of (Wahdan, 1998; Subrahmanyam, 2001; Efem et al., 1992 and Molan, 2000). These significant results showed that Pakistani floral sources honeys are effective against *Pseudomonas aeruginosa*, and compairable to Manuka honey from New Zealand (Molan,2000), United Kingdom Heather honey and Khadi Kraft Indian honey used against *Pseudomonas aeruginosa* (Mullai and Menon, 2007).

Prevalence of *Candida* infection is escalating world wide. In the present study the antifungal activity of Pakistani different floral source honey were also evaluated against *Candida albicans*. The range of inhibition zone diameter (10.0 ± 0 to 17 ± 0.577mm) was observed. Sample numbers (46, 56 and 59) Acacia, *Carissa opaca*, *Zizyphus* floral sources receptivity

showed the larger zone against *Candida albicans*. This antifungal activity of all tested honey samples towards *Candida albicans* in the present study is in confirmation with the study of Estevinho et al. (2011) reported that certain honeys have significant anti fungal activity against clinical isolates of *Candida albicans*. These results are also in confirmation with pervious reports of Cavanagh et al. (1970), Efem (1992), Alwaili and Noori (2004).

A comparison of crude honey samples of ten different floral sources of Pakistan was also carried out with fourteen commercial antibiotics against six Gram - Positive, eleven Gram - negative bacteria and one Fungus (yeast). It was found that Pakistani honey samples have a broad spectrum significant antibacterial and promising antifungal activity comparable to commercial antibiotics (Table1, 2, 3). The present results are in confirmation with the study of Farouk (1988) reported the efficacy of honey as an antimicrobial agents against *Staphylococcus* and *Pseudomonas aeruginosa* Strain than Gentamicin.

Honey is a most natural product so large variance was observed in its activity ranging from (10 $\pm$ 0 to 45. 667 $\pm$ 0.333mm) diameter of zone of inhibition against microorganism used. The present interesting finding of large variation in antimicrobial activity may be attributed to the difference existing in plants growing different floral areas of Pakistan. Plant derived factors are unique to each plant species that may be responsible for variation in antimicrobial activity (Molan, 1992). It is in confirmation with the previous reports of Sheikh et al. (1995) determines the diameter of zone of inhibition of crude honey samples in the range of 5 – 50mm for different clinical isolates. This is also in confirmation with the study of Iftikhar (2010) determined the diameter of inhibition zone of different Pakistani honey samples in the range of 15 – 30mm against different clinical isolates.

Table 1 Diameter Of Inhibition Zone (Mm) Of Different Floral Of Pakistani Honey Against Test Gram Positive Microorganism

Honey samples code / Antibiotic code	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Streptococcus faecalis*	*Streptococcus pyogenes*	*Bacillus subtilis*	*Corynebacterium diptheriae*
09	29. 6 ±0.882ij	24. 3± 0.333f	20. 7± 0.333i	21. 6± 0.333e	12 ± 0b	14 ± 0b
11	29.0 ± 1.527ghi	20. 0 ± 0.577d	14. 3± 0.333b	14 ± 0c	13± 0.333cd	15. 7 ±1.201c
13	18. 7 ± 0.333d	22.0 ± 1.155e	16 ± 0c	21.3 ± 0.577e	12.3 ± 0.333bc	25.6 ± 0.577f
35	29. 3± 0.667hi	22.0 ± 0e	20. 3 ± 0.333hi	23. 3 ± 0.667f	12 ± 0b	20.3 ± 0.881e
39	31. 7± 0.333j	33. 7 ± 0.666h	19 ± 0fghi	25.0 ± 0g	15.3 ± 0.667e	17.667 ± 0.577d
46	27. 7 ± 0.882ghi	26±0f	18 ± 0def	26. 6 ± 0.333h	13 ± 0bcd	30 ± 0h
56	35.7 ± 0.333k	37. 3 ± 0.333i	18. 3 ± 0.333defg	21. 7 ± 0.333e	15. 6 ± 0.333e	19.667 ± 0.333e
58	21. 3 ± 0.333e	20± 0d	17.00 ± 0cde	14 ± 0c	15.3 ± 0.667bcd	16 ± 0c
59	31 ± 0j	22 ±1.154e	19 ± 0fghi	30.333± 0.333i	14 ± 0e	28 ±0.577g
65	14.667±0.333c	16 ± 0.882f	17.25 ± 0.882cd	15± 0	13.0 ± 0e	18.667 ±0.577d
AMC 30	35.2 ± 0.862k	13.8± 0.374c	33 ± 0.836l	33.6 ±0.6j	18 ± 0.316cd	47.6 ±0.509l
AML 10	22.4± 0.42e	09.8 ± 0.2b	26 ± 0.447k	22 ± 0.547ef	12 ± 0.316bcd	26.4 ± 0.812fg
CAZ 30	12.6 ± 0.509b	7.8 ± 0.374a	07 ± 0a	19 ± 0.547d	12 ± 0.316f	08.4 ± 0.244a
CFM 05	7.2 ± 0.374a	8.0 ± 0a	07.2 ± 0a	07 ± 0a	07 ± 0b	07.8 ± 0.374a
CIP 30	21.2 ± 0.374e	31.8 ± 0.663g	19.8 ± 0.663g	39.4 ±0.6l	07± 0a	42.2 ± 0.583j
E 15	26 ± 0.316fh	30.2 ± 0.663g	07.2 ± 0.2a	37.4 ± 0.4k	27.0 ± 0.547a	44.0 ± 0.547k
IPM 10	52.2 ± 0.735l	48.8 ± 0.583k	52.8 ± 0.663m	53.4 ± 0.4m	26.6 ± 0.245h	56.2 ± 0.374m
TE 30	22.0 ± 0.632e	44.8 ± 0.489j	06.8 ± 0.2a	18.6 ± 0.245d	39 ± 0.316h	33.8 ± 0.374i
VA 30	33.8 ± 0.533k	34.4 ± 0.4h	24 ± 0.836g	22.6 ± 0.245ef	27.2 ± 0.374i	27.0 ± 0.632fg
DO 30	22.6 ± 0.4e	20.4 ± 0.589e	25.6 ± 0.678k	27.4 ± 0.4h	19.4 ± 0.4h	26.4 ± 0.678fg
OT 30	29.6 ± 0.748hij	33.8 ± 0.583h	18.6 ± 0.678h	14.2 ± 0.8c	17.4 ± 0.4g	20.0 ± 0.547e
CLR 15	22 ±0.336e	24.2 ± 0.8f	16 ± 0.547c	11.2 ± 0.735b	12.0±0b	27.2 ± 0.734fg
MXF 5	25.6±0.4f	24.2 ± 0.8f	20.2 ± 0.374h	21.8 ± 0.489ef	12.0±0b	34.0 ± 0.244i

09 = Mix flora, 11= Gossypium species, 13= *Phoenix dactylifera, 35= Salvadora persica* , 39= *Mangifera indica* ,46= *Acacia* ,56= *Carissa opaca,* 58= *Helianthus annuus,* 59= *Zizyphus* ,65= *Medicago sativa*..

Amoxicillin – Clavulamic acid (AMC-30 ug/ml and AML – 10 ug/ml), Ceftazidim (CAZ - 30ug/ml), Cefixime (CFM- 05 ug /ml, Ciprofloxacin (CIP- 30 ug/ml) Erythromycin (E – 15 ug/ml), Vancomycin (VA - 10ug/ml), Tetracycline (TE- 30 ug/ml), Imipenum (IPM- 10ug/ml)., Doxycycline (DO- 30ug/ml), Oxytetracycline (OT-30ug/ml), Clarithromycin (CLR – 15ug/ml), Moxifloxacin (MXF- 5ug/ml)

Table 2 Diameter Of Inhibition Zone (Mm) Of Different Floral Of Pakistani Honey Against Test Gram Negative Microorganism

Honey samples code / Antibiotics code	*Klebsiella pneumoniae*	*Klebsiella oxytoea*	*Escherichia coli*	*Shigella dysenteriae*	*Salmonella typhi*	*Salmonella typhi A*	*Salmonella typhi B*	*Proteus mirabilis*	*Proteus vulgaris*	*Enterobacter*	*Pseudomonas aeruginosa*
09	16. 3± 0.333bcd	14.333± 0.333fg	14 ± 0c	19 ± 0.577h	31.333 ± 1.202ef	25.333± 0.333c	14 ± 0b	14.666 ± 0.333b	16.667±0.333ef	14±0d	17.666±0.333cde
11	15 ± 0bc	14 ± 0efg	22± 0.577e	14 ± 0bcd	32 ± 1.155f	34 ± 0g	14 ± 0.577b	20.666± 0.333f	16± 0def	12±0c	18.333± 1.453def
13	16 ± 0bc	14 ± 0efg	18.333±0.666d	13 ± 0b	31.333 ± 0.333ef	38.33 ± 1.202j	14 ± 0b	18 ± 0de	14.666±0.333cd	14.333±0.882de	19± 0.333efg
35	16 ± 0bc	14 ± 0efg	14± 0c	14.333 ± 0.333cd	35.333 ± 0.333hi	39 ± 1.154j	34 ± 0.577j	23±1.732g	17±0.577f	14±0d	30.666± 0.333n
39	18.333 ± 0.577efg	15.667 ± 0.577h	25.333 ±0.667f	16 ± 0ef	35.667 ± 0.333hi	44.33 ± 0.882l	15.667 ± 0.333cd	27± 0i	17±0f	16± 0.577f	20.666± 0.333ghi
46	18.667 ± 0.577fg	14 ± 0efg	30.0±0gh	13 ± 0b	33.667 ± 0.882g	36.333 ± 0.667i	38.666± 0.882l	27.333± 0.667ij	24.666± 0.333h	14± 0d	24.333± 0.882kl
56	18 ± 0def	15.667 ± 0.333h	29.667±0.333gh	17 ± 0gf	45.667 ± 0.333j	42.667 ± 0.333k	36 ± 0k	30.333± 0.333k	19±0g	15.666± 0.333ef	20±.0.577fgh
58	15.667 ± 0.577bc	13.667 ± 0.333ef	18 ± 0d	13.33 ± 0.333bc	33 ± 0.333g	30.667 ± 1.202f	14 ± 0b	25.333±0.333h	17±0f	13 ± 0cd	18.666± 0.333def
59	20 ± 0g	15 ± 0gh	30.666 ± 0.667h	15 ±0cd	34.333 ± 0.333gh	39.333 ± 0.666j	34.333 ± 0.667j	30.333±0.333k	27±0i	14± 0d	24± 0.577k
65	15.667 ± 0.577bc	13.33 ± 0.333de	19 ± 1.547d	15 ± 0d	25.5 ± 0.667c	33± 0.577g	23.333 ± 0.333g	19 ± 1.155e	17±0f	13± 0cd	17.666± 0.333de
AMC 30	33.333 ±1.527j	16 ± 0h	18.6 ± 0.245d	34.4 ± 0.812j	30 ± 0.316e	28 ± 0.547d	48.4 ± 0.678m	24 ± 0.316g	28 ± 0.547ij	17.8 ± 0.735g	15.8 ± 0.969c
AML 10	24 ± 0.316h	12 .2 ± 0.2cd	0.8.2 ± 0.583d	13 ± 0b	19 ± 0.316b	08 ± 0 a	14.8 ± 0.489bc	7.8 ± 0.2a	19 ± 0.547g	08 ± 0a	21.4 ± 0.678hij
CAZ 30	33.8 ± 0.663j	21.4 ± 0.4k	07 ± 0a	17.4 ±0.245g	25 ± 0.547d	26 ± 0.632c	20.4 ± 0.245f	08 ±0a	27 ± 0.547i	7.8 ± 0.2a	22 ± 0.316ij
CFM 05	23.8 ± 0.374h	11.6 ± 0.4c	7.2 ± 0.2a	07 ± 0 a	18.6 ±	18.2 ± 0.374b	08 ± 0.316e	08 ± 0.316a	07.6 ±	8.2 ± 0.2a	27.2 ± 0.489m

					0.245b				0.547a		
CIP 30	40.2 ±0.916k	26 ± 0.316i	07 ± 0a	34 ± 0.547j	21.4 ± 0. 4c	34.4±0.4g	34.4 ± 0.4j	32.2±0.663n	32.2 ± 0.663k	39.2 ± 0.583j	36 ± 0.447p
E 15	29.4 ± 0.4i	10 + 0b	8.2 ± 0.2b	34.2 ± 0.374j	08 ± 0a	7.8 ± 0.2a	10 ± 0.623a	08 ±0a	20 ± 0.316g	10 ± 0b	26.0 ± 0.216lm
IPM 10	42±0.632l	22.6 ± 0.245l	29.4 ± 0.4g	39.4 ± 0.678k	36 ± 0.633i	35.8 ±0.2i	54.4 ± 0.4n	28.4 ± 0.4j	29 ± 0.447j	30.2 ±0.663i	32.4 ± 0.6o
TE 30	14.6± 0.812ab	19.4 ± 0.812j	08 ± 0b	7.8 ± 0.2a	08.2 ± 0.2a	08 ± 0a	32 ± 0.316i	07 ± 0a	7.6 ± 0.245a	8.2 ± 0.2a	11.4 ± 0.6b
VA 30	13 ± 0.548a	07 ± 0a	08 ± 0b	6.8 ± 0.2a	08.2 ± 0.2a	7.8 ± 0.2a	22.8 ± 0.2g	08 ± 0a	8.2 ± 0.2a	08 ± 0a	9.6 ± 0.4a
DO 30	18 ± 0.316def	22.6 ±0.4l	23 ± 0.316e	22 ± 0.316i	33.6 ± 0.245g	33.6 ± 0.678g	29. 8 ± 0.489h	16 ± 0.316c	13.8 ± 0.583bc	15.6 ± 0.245cf	23.2 ± 0.583k
OT 30	14.8 ± 0.583b	14 ± 0efg	22.4 ± 0.4e	13.8 ±0.2bcd	25.2 ± 0.374d	30 ± 0.316ef	16.6 ± 0.4d	17 ± 0c	13.2 ± 0.2b	12 ±0c	13 ± 0.316b
CLR 15	19 ± 0.316 fg	18 ± 0.316i	15 ± 0c	16.8 ± 0.2fg	35.2 ± 0.2h	39.8 ± 0.374j	23 ± 0.316g	26. 8 ± 0.489i	28.2 ± 0.489j	28 ±0.948h	17.6 ± 0.509cd
MXF 5	16.8 ± 0.489cde	13.8 ± 0.2ef	15 ±0.316c	16 .8 ± 0.2h	25 ± 0.316d	28.8 ± 0.374de	14 ± 0.316h	18.6 ± 0.245e	15.4 ± 0.4d	14 ± 0d	17.2 ± 0.374cd

09 = Mix flora, 11= Gossypium species, 13= *Phoenix dactylifera, 35= Salvadora persica* , 39= *Mangifera indica* ,46= *Acacia* ,56= *Carissa opaca*, 58= *Helianthus annuus*, 59= *Zizyphus* ,65= *Medicago sativa*..

Amoxicillin – Clavulamic acid (AMC-30 ug/ml and AML – 10 ug/ml), Ceftazidim (CAZ - 30ug/ml), Cefixime (CFM- 05 ug /ml, Ciprofloxacin (CIP- 30 ug/ml) Erythromycin (E – 15 ug/ml), Vancomycin (VA - 10ug/ml), Tetracycline (TE- 30 ug/ml), Imipenum (IPM- 10ug/ml)., Doxycycline (DO- 30ug/ml), Oxytetracycline (OT-30ug/ml), Clarithromycin (CLR – 15ug/ml), Moxifloxacin (MXF- 5ug/ml)

Table 3 Diameter Of Inhibition Zone (Mm) Of Different Floral Of Pakistani Honey Against Test Fungus (Yeast)

Honey samples code / Antibiotics code	Candida albican
09	13±0cd
11	10± 0a
13	13.333 ± 0.333de
35	14 ± 0de
39	14.333±0.889e
46	17±0f
56	17± 0.577f
58	12± 0bc
59	17± 0f
65	11± 0ab
NT 100UT	20 ± 0.969g

09 = Mix flora, 11= Gossypium species, 13= *Phoenix dactylifera, 35= Salvadora persica* , 39= *Mangifera indica* ,46= *Acacia* ,56= *Carissa opaca,* 58= *Helianthus annuus,* 59= *Zizyphus* ,65= *Medicago sativa..*

Nystatin (NT – 100 UT)

CONCLUSION

Over all the present significant results exhibited by the crude honey samples in relation to clinical isolates used may be important as there is noticeable rise in difficult to treat skin and surgical wounds infection in relation with *Staphylococcus aureus* (Halcon and Milkus, 2004) and *Pseudomonas aeruginosa* (Semidtchen et al., 2003). Thus Pakistani honey samples from different floral sources showed variable activities against different microorganisms could be potentially applied as a alternative therapeutic agent, which may be economical and without the risk of side effect. Thus it is expected that the present work will be a mile stone in the antimicrobial treatment and will bring new ideas in the management of infected wounds, burn wounds and common skin infections caused by pathogen bacteria.

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