Antibacterial, antifungal and antioxidant activities of honey collected from Timergara (Dir, Pakistan)

Muhammad Zahoor, Sumaira Naz and Muhammad Sangeen
Department of Chemistry University of Malakand, Chakdara Dir (Lower), KPK, Pakistan

Abstract: In this study honeys of *Acacia modesta, Prunus persica, Zizyphus sativa and Isodon rogosus* plants were tested against two Gram-positive bacterial strains (*Staphylococcus aureus and Bacillus cereus*), two Gram-negative bacterial strains (*Klebsilla pneumonia and Escherichia coli*) and two fungal strains (*Alternaria alternata and Trichoderma harzianum*) through Agar well diffusion method. The tested honeys showed high antimicrobial activities to the tested bacterial and fungal strains. All the tested honeys were more active against Gram-negative bacterial strains than the Gram-positive bacterial strains. They showed lower activity against the tested fungal strains as compared to all the tested bacterial strains. The given honeys showed free radical scavenging activity also.

Keywords: Honey, antioxidant, antibacterial, antifungal.

INTRODUCTION

Nature has been a source of medicinal agents and an impressive number of drugs have been isolated from natural sources and used as a medicine. Honey is a gaining acceptance and is used as an antibacterial agent for the treatment of ulcers, bed-sores, and for other surface infections resulting from burns and wounds (Allen *et al.*, 1991; Zumi and Luto, 1989). In many cases it has been used with good results on infections which were not treated by standard antibiotics and antiseptic therapy. Its useful effects in rapidly clearing up infection and promoting healing is not surprising in light of the large number of research findings on its antibacterial activity (Molan, 1992). Aristotle, 350 BC, and Dioscorides, (Gunther, 1934) recommended that honey collected in specific regions in a particular seasons from different floral sources can be used for the treatment of different minor diseases. Such types of considerations have been continued into present time folk medicine, the strawberry-tree *(Arbutus unedo)* honey of Sardinia have been reported as valuable for its therapeutic characteristics (Floris and Prota, 1989) while in India lotus *(Nelumbiumsceciosum)* honey has been found be a remedy for eye diseases (Fotidar, 1945).

As the pathogens develop resistant, the effect of the antibiotics is diminished. These types of bacterial resistance to the antimicrobial agent possess a very serious danger to public health and for all kinds of antibiotics the frequencies of resistance are increasing. Infectious diseases are the primarily heart that account for death worldwide. In the clinical efficacy of many synthetic antibiotics is being throated by the emergency of a serious problem which can be defined as multi-Drug resistant pathogens. Multi-Drug resistance in both human and plants pathogenic microorganisms has developed due to the indiscriminate usage of commercial antimicrobial drugs that have widely applied in the treatment of infectious diseases. The numbers of multi-drug resistant microbial strains are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheter, organ transplantation and ongoing epidemics of HIV infection (Patel *et al*., 2010; Bang *et al*., 2003; Molan, 2006).

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of actions because there has been an alarming increase in the incidence of new and re-emerging diseasse of undesirable side effects of certain antibiotics as well as the increasing development of resistance to the antibiotic in current clinical use (Cowan, 1999). Therefore, scientists have tried to discover new antimicrobial substances. More than 50% of all drugs in clinical use are originated from plants products (Eldeen *et al.*, 2005). In addition in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore there is need to introduce new infection-fighting agents capable of controlling microbial infections (Turkmen *et al*., 2005).

Keeping in view the importance of natural sources in drug formulation the present study was aimed to determine the antibacterial, antifungal and antioxidant activities of four different honeys collected from Timergara, Dir, Pakistan.

EXPERIMENTAL

Erythromycin and fluconazole were used as a control drugs and was purchased from Oxoid Ltd., Basingstoke,

**Corresponding author:* e-mail: mohammadzahoorus@yahoo.com

Hampshire, England. Honeys of *Acacia modesta, Prunus persica, Zizyphus sativa* and *Isodon rogosus* were obtained from local markets at Timergara, Dir, Pakistan. Two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus* and two Gram-negative, *E. coli* and *Klebsilla pneumonia*; and two fungal species *Alternaria alternata* and *Trichoderma harzianum* were used in this study. All these bacterial and fungal strains were obtained from the Department of Chemistry (Biochemistry) University of Malakand, Khyber Pakhthunkhwa Pakistan. One liter Nutrient agar aqueous solution was made by dissolving 28 g of Nutrient agar in distilled water and makes 1000 ml solution and the pH was adjusted to 7.0. The media and glass wears (Petri dishes, test tubes, cotton swabs, distilled water) needed for further use were sterilized by autoclave at 15psi at 121 °C for 20 minutes. Powders of the antibiotic Erythromycin (purity 100%) were accurately weighted and dissolved in sterile distilled water to give appropriate dilutions of about 10, 50 and100 mg/ml to yield the required concentrations. The stock solutions were stored at -20°C. The honey obtained from the flowers of *Acacia modesta, Prunus persica, Zizyphus sativa* and *Isodon rogosus* were subjected to antimicrobial activity by using the agar well diffusion method. The inoculation was carried out in a laminar air-flow. Briefly 25ml quantities of nutrient agar were plated into the Petri dishes and allowed them to cool and solidified for 40 minutes. After solidification of the media the bacterial and fungal strains were inoculated by swabbing method. Wells of 6mm in diameter and 4cm apart were made in the culture media by using sterilized cork borer to make four uniform wells in each plate. A drop of molten nutrient agar was used to seal the bases of each well. These wells were filled with 50µl of honey by using micropipette and were allowed to diffuse for 40 minutes. The antimicrobial activities were determined after 24 hours of incubation at 37°C in incubator. The antimicrobial activities were measured from the diameter of the inhibition zone form by the honey around the well. The zone diameter of inhibition produced by the honey after measuring was compared with the inhibition zone produced by standard antibiotic (Erythromycin). Each sample was used in triplicate for the determination of antimicrobial activity.

To prepare stock solution of DPPH, 0.039gm of DPPH was accurately weighted and dissolved in 100ml distilled methanol. The stock solution was covered with aluminum foil and kept in dark place to protect it from light. 0.0125gm of each honey sample were accurately weighted and dissolved separately in 25ml distilled methanol to obtain the required concentration (0.0125gm/25 ml). These solutions were stored as honey stock solutions. One solution was of 5ml pure distilled methanol + 0ml of honey stock solution, this solution was used as a control solution. Solutions of different concentration of honey samples were prepared by using dilution formula. Added 1 ml of stock solution of DPPH into the diluted solutions and control solution, these all solutions were kept in dark place for 30 minutes then their absorbance was measured at 517nm wave length with the help of spectrophotometer. The percentage radical scavenging activity (% RSA) was measured from their absorbance by using the following formula:

%RSA= (control absorbance - honey stock solution absorbance) (control absorbance) x 100

RESULTS

Antimicrobial activities of the selected honey samples against Gram Positive bacteria

Honeys obtained from the flowers of *Acacia modesta, Prunus persica, Zizyphus sativa* and *Isodon rogosus* were used for the antibacterial activities against two different strains of Gram positive bacterial strains (*Staphylococcus aureus and Bacillus cereus*). Erythromycin was used as a standard drug for comparative inhibitory effect.

The mean value of zone diameter of inhibition of honey sample of the flowers of *Acacia modesta, Prunus persica, Zizyphus sativa, Isodon rogosus plants* were determined against *Staphylococcus aureus* (3.2mm, 7.6mm, 5.3mm, 3.6mm) and the diameter of zone of inhibition of Erythromycin was 11.4 mm. The mean of three values is given in the tables 1 to 4. The antibacterial activities of the selected honeys are graphically shown in figs. 1 to 4.

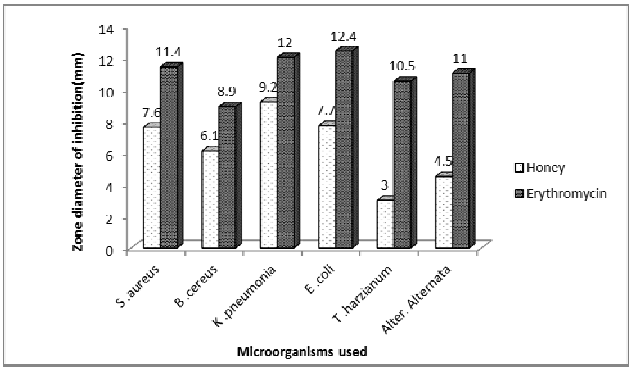

Fig. 1: Antimicrobial activity of *Prunus persica* honey

The diameter of zone of inhibition of honey sample of the flowers of *Acacia modesta, Prunus persica, Zizyphus sativa and Isodon rogosus* were also determined against *Bacillus cereus* (2.7mm, 6.2mm, 6.1mm, 4.3mm) and the zone diameter of inhibition of standard antibiotic *Erythromycin* was 8.9 mm. The data collected is given in the form of mean of three values in the tables 1 to 4.

Antibacterial activity of the selected honeys sample against Gram-negative bacteria

The zone diameter of inhibition of honey sample of the flowers of *Acacia modesta, Prunus persica, Zizyphus sativa and Isodon rogosus* plants were determined against *Klebsilla pneumonia* (5.1mm, 9.1mm, 6.6mm, 6.8mm)

and the sszone diameter of inhibition of standard antibiotic *Erythromycin* was 12 mm. Data collected is given in the form of mean of three values in the tables 1 to 4.

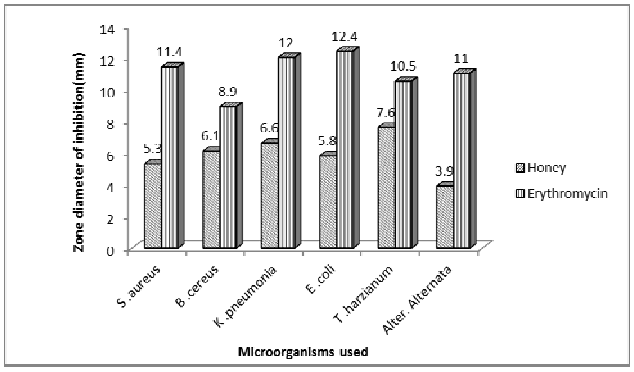

Fig. 2: Antimicrobial activity of *Zizyphus sativa* honey

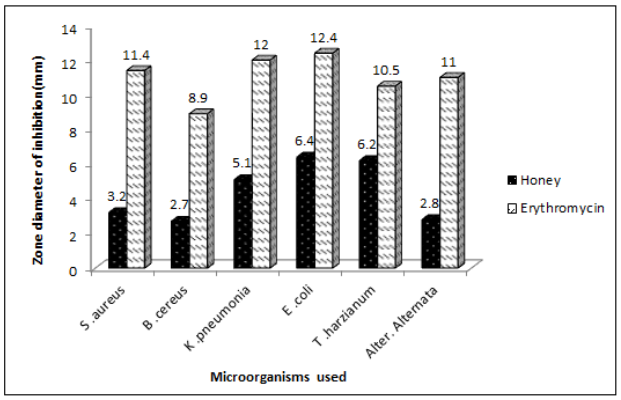

Fig. 3: Antimicrobial activity of *Acacia modesta* honey.

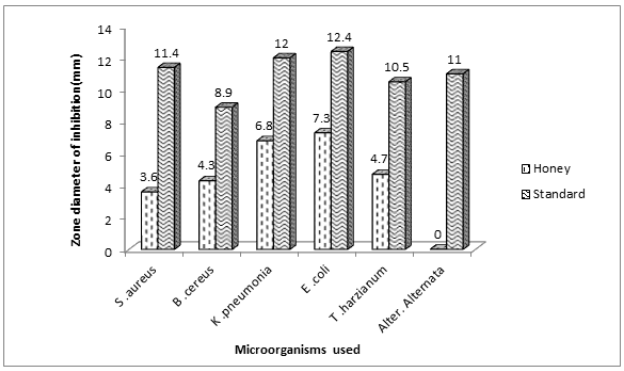

Fig. 4: Antimicrobial activity of *Isodon rogosus* honey

The zone diameter of inhibition of honey sample of the flowers of *Acacia modesta, Prunus persica, Zizyphus sativa and Isodon rogosus* plants have been determined against *Escherichia coli* (6.4mm, 7.7mm, 5.8mm, 7.3mm). While the zone diameter of inhibition of standard antibiotic *Erythromycin* was 12.4 mm. Data collected is given in the form of mean of three values in the tables 1 to 4. The antibacterial activities of the selected honeys are graphically shown in figs. 1 to 4.

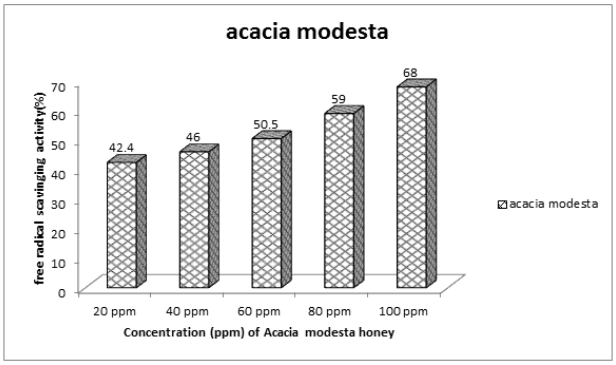

Fig. 5: Free radical scavenging activity of *Acacia modesta honey*

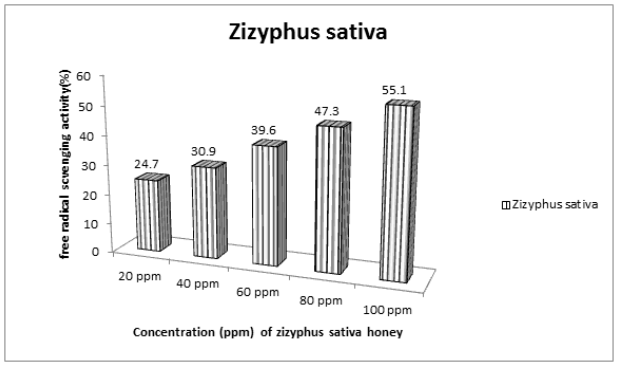

Fig. 6: Free radical scavenging activity of the honeys of *Zizyphus sativa*

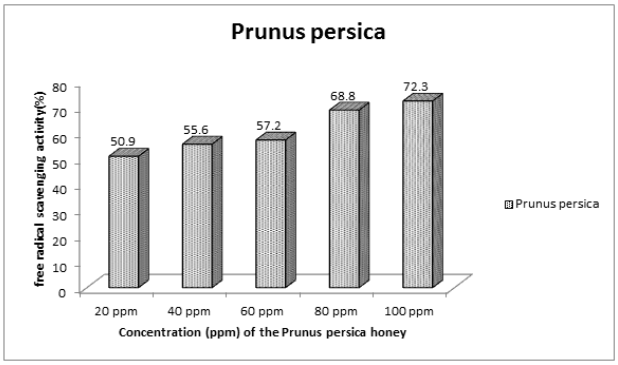

Fig. 7: Free radical scavenging activity of the honey of *Prunus persica*

Antifungal activity of the honey

The fungus species used in this study showed susceptibility to these selected honeys. The honey of *Prunus persica* plant shows much antifungal activity by measuring their zone diameter of inhibition (4.5mm) to *Alternaria alternata* as compared to *Trichoderma harzianum* (3mm). The honey of *Acacia modesta* and *Zizyphus sativa* show greater antifungal activity against *Trichoderma harzianum* than *Alternaria alternata* as given in the tables 1 to 4. The antifungal activities of the selected honeys are graphically shown in figs. 1 to 4. Fluconazole was used as standard.

Table 1: Antimicrobial activity of the honey of *Prunus persica*

Microorganism used	Mean diameter of zone of inhibition (mm) of honey	Diameter of zone of inhibition (mm) of Standard
Staphylococcus aureus	7.6	11.4
Bacillus cereus	6.2	8.9
Klebsilla pneumonia	9.1	12
Escherichia coli	7.7	12.4
Trichoderma harzianum	3	10.5
Alternaria alternata	4.5	11

Table 2: Antimicrobial activity of the honey of *Zizyphus sativa*

Microorganism used	Mean diameter of zone of inhibition (mm) of honey	Diameter of zone of inhibition (mm) of Standard
Staphylococcus aureus	5.3	11.4
Bacillus cereus	6.1	8.9
Klebsilla pneumonia	6.6	12
Escherichia coli	5.8	12.4
Trichoderma harzianum	7.6	10.5
Alternaria alternata	3.9	11

Table 3: Antimicrobial activity of the honey of *Acacia modesta*

Microorganism used	Mean diameter of zone of inhibition (mm) of honey	Diameter of zone of inhibition (mm) of Standard
Staphylococcus aureus	3.2	11.4
Bacillus cereus	2.7	8.9
Klebsilla pneumonia	5.1	12
Escherichia coli	6.4	12.4
Trichoderma harzianum	6.2	10.5
Alternaria alternata	2.8	11

Table 4: Antimicrobial activity of the honey of *Isodon rogosus*

Microorganism used	Mean diameter of zone of inhibition (mm) of honey	Diameter of zone of inhibition (mm) of Standard
Staphylococcus aureus	3.6	11.4
Bacillus cereus	4.3	8.9
Klebsilla pneumonia	6.8	12
Escherichia coli	7.3	12.4
Trichoderma harzianum	4.7	10.5
Alternaria alternata	00	11

Table 5: DPPH anti scavenging activities (%) of the tested honeys

Honey sample	20 ppm	40 ppm	60ppm	80 ppm	100 ppm
Acacia modesta	42.4	46.0	50.5	59.0	68.0
Prunus persica	24.7	30.9	39.6	47.3	55.1
Zizyphus sativa	50.9	55.6	57.2	68.8	72.3
Isodon rogosus	40.8	45.3	55.6	60.7	69.8

Free radical scavenging activities of honey

The antioxidant activities of the selected honeys were determined at 517 nm wavelength using UV/Visible spectrophotometer. The results obtained are shown in table 5 and graphically in figs. 5 to 8.

DISCUSSION

In the present study four sample of honey were used to test their antibacterial, antifungal and antioxidant activities. Result obtained in this study revealed that the

tested honey of *Prunus persica, Zizyphus sativa, Acacia modesta and Isodon rogosus* possess potential antibacterial activities against two Gram positive bacterial strains (*Staphylococcus aureus and Bacillus cereus*) and two Gram-negative bacterial strains (*Klebsilla pneumonia* and *Escherichia coli*). All of these honeys showed significant antibacterial activity against *Klebsilla pneumonia* and *E. coli* as compared to the *Staphylococcus aureus* and *Bacillus cereus* but *Prunus persica* showed more significant antibacterial activity. The results showed that these two strains were more susceptible to the given honey (tables 1, 2, 3, 4). The least antibacterial activity was observed for *Acacia modesta* honey to *S. aureus* and *B. cereus* (table 3). The present study is in confirmation with previous reports (Cowan, 1999; Obaseiki *et al*., 1984; Obaseiki *et al.*, 1983).

The result obtained in this study also revealed that these tested honey also possess a potential antifungal activity against the two fungal strains (*Alternaria alternate and Trichoderma harzianum)*. The honey of *Prunus persica* plant showed more antifungal activity against *Alternaria alternata* than *Trichoderma harzianum* (table 1). Similarly honey of *Zizyphus sativa* showed more antifungal activity *to Trichoderma harzianum than Alternaria alternata* (table 2).

Erythromycin and fluconazole which were used as a standard in the current study showed activity against used bacterial and fungal strains. Erythromycin showed highest activity against *E. coli.* These standards showed significant zone of inhibition against all bacterial and fungal strains as compared to the tested honeys. Erythromycin and fluconazole are well refined industrial products therefore it showed high zones of inhibition.

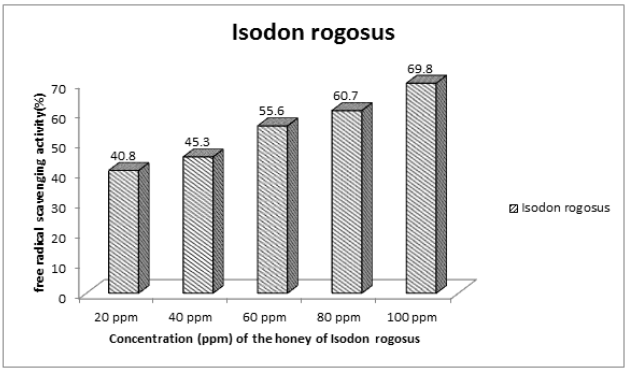

Fig. 8: Free radical scavenging activity of the honey of *Isodon rogosus.*

The antioxidant activities of honeys sample tested in the present study were determined by measuring their free radical scavenging activities (table 5). The honey of *Zizyphus sativa* plant have shown significant scavenging free radical activity, while the honey of *Prunus persica* plant have shown less significant antioxidant activity as compared to other honeys sample. The free radical scavenging activities of all the honeys increases with the increase in concentration (figs. 5 to 8).

CONCLUSION

It was concluded from the current investigation that the honeys samples selected for antimicrobial and antifungal activities have medicinal values and are resistant for the diseases caused by these pathogens. The selected honeys also have shown antioxidant activity. The present study verified the traditional use of honeys for various human ailments especially for various infectious diseases. Thus these honeys could be utilized as an alternative source of useful antimicrobial drugs. It is recommended that the above mentioned honeys are highly important on the basis of their medicinal values so, along with their further exploration for the mention strains, other strains should be also used in order to test out their significance.

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