Journal of Medicinal Plants Research Vol. 6(15), pp. 2962-2970, 23 April, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1349
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Antimicrobial and antioxidant activities of *Mimosaceae* plants; *Acacia modesta* Wall (Phulai), *Prosopis cineraria* (Linn.) and *Prosopis juliflora* (Swartz)

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Accepted 30 January 2012

In the present study the methanolic leaf extracts of three plant species of family *Mimosaceae* viz., *Acacia modesta* Wall (Phulai), *Prosopis cineraria* (Linn.) Druce and *Prosopis juliflora* (Swartz) DC. were used to evaluate their antibacterial antifungal and antioxidant activity. Simple maceration method was used for the preparation of plant extracts. The extracts were tested against four strains of bacteria (*Bacillus subtilis, Escherichia coli*, *Vibrio cholera* and *Enterobacter aerogenes*) and two strains of fungi (*Aspergillus niger* and *Aspergillus fumigatus*). At 15 mg/ml extract concentration the maximum inhibitory zones observed in *Acacia modesta, P. cineraria* and *P. juliflora* were 20, 18 and 25 mm, respectively. *P. cineraria* gave best response against *A. niger* and *A. fumigatus* by producing 15.38 and 8% inhibition, respectively. *P. juliflora* showed 7.69% inhibition against *A. niger.* While *A. modesta* showed 11.53% activity against *A. niger* and 0.8% against *A. fumigatus.* The Antioxidant activities of these medicinal plants also showed significant results. Maximum radical scavenging activity (%RSA) was observed in *P. cineraria and P. juliflora*, that is, 60.48 and 47.82%, respectively, as compared to *A. modesta* which gave minimum %RSA value of 41.42%.

Key words: Medicinal plants, *Mimosaceae* plants, methanol extracts, antibacterial, antifungal, antioxidant.

INTRODUCTION

In spite of the amazing development in synthetic organic chemistry in modern age, more than twenty-five percent of the approved medicines in developed countries are derived directly or indirectly from plants (Newman et al., 2000). Plants produce a large group of bioactive molecules; therefore they have been recognized as a rich source of medicines. Since prehistoric times, many types of bioactive molecules derived from plants were evolved as a chemical protection against diseases for safeguarding the human fitness (Farombi, 2003). In recent years, different types of medicinal plants have been checked for their potential against several microbes and cured for a variety of disease (Chandrasekaran and Venkatesalu, 2004). In this association, plants continue to be a rich supply of curative agents. The significant involvement of plants to the medicine production was promising as a large number of phytochemicals and biological studies all over the world (Kianbakht and Jahaniani, 2003).

Several diseases are prevented and treated by herbal preparations in the world. In non-industrialized countries, World Health Organization (WHO) has expected that nearly eighty percent of the world population is dependent on herbal medicines in their conventional medicinal system and 85% of this medicinal system involved the use of plant extracts as the fundamental requirements for human health (WHO, 2000).

Many contagious diseases have been treated with herbal medicines throughout the history of mankind. There is prime importance for the discovery of new

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antimicrobial compounds with various chemical structures and mode of action for treating new contagious diseases (Rojas et al., 2003). Medicinal plants are a rich source of antibacterial and antifungal agents. Plant based chemicals have minimum or no negative effects as compared to synthetic chemicals (Shariff et al., 2006).

Many herbal-based medicines are considered to have a range of biomedical efficiencies including treatment of inflammation, hyperlipemia, arteriosclerosis, osteoporosis, bone resorption, and some have been reported to have useful effect in cardiovascular diseases, immune deficiency, central nervous system disorders, and cancer (Jin et al., 2006; Radad et al., 2006). Ethnobotanical data showed that preparations from different parts of *Acacia* spp. have been applied for diabetes, gastrointestinal disorders and inflammatory diseases in the traditional medicine (Bhatt et al., 2003; Li et al., 2003). In the past few decades, a global increase in the occurrence of fungal diseases has been observed as well as development of resistance of some species of fungus against some fungicides used in medicinal practice. The most of the fungicides have a lot disadvantages in terms of toxicity, efficiency, price and their regular uses have developed the resistant strains. Therefore, there is a dire need of new fungicides having a broad range of structural classes and selective mode of action with less side effects (Abad et al., 2007).

In the recent years, there is rapid increase of attention in remedial potential of medicinal plants as antioxidants in limiting the tissue injury caused by free radicals (Pourmorad et al., 2006). Scientific facts suggest that under oxidative stress conditions, oxygen radicals such as superoxide anion (O_2-) hydroxyl radical (OH) and peroxyl radicals (H_2O_2) are formed in biological systems. These oxygen radicals are called Reactive Oxygen Species (ROS) and they can lead to oxidative damage to cellular components such as proteins, lipids and DNA. These oxygen radicals play important roles in degenerative processes such as aging (Ames et al., 1993). Antioxidants play an important function in limiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases (Sreelatha and Padma, 2009).

MATERIALS AND METHODS

The present research work was carried out in the Department of Plant Sciences, Quaid-i-Azam University and NARC Islamabad, Pakistan.

Plant materials

Plant materials were collected from District Shikarpur, Sindh, Pakistan. The plants were identified and voucher specimens were deposited in Herbarium of QAU, Islamabad.

Extraction

Fresh leaves of medicinal plants namely *Acacia modesta, Prosopis cineraria* and *Prosopis juliflora* were rinsed in distilled water and kept under shade till drying. Leaves of the plants were weighed separately through electric balance. Extractions from leaves were carried out by simple maceration process. The leaves were taken and ground in methanol using kitchen blender. This mixture was kept for two weeks at room temperature (25°C) in extraction bottle. After two weeks, mixture was filtered twice through Whatman-41 filter paper. Methanol was then completely evaporated by rotary evaporator to obtain pure extract.

Preparation of samples

The extract (15 mg) was dissolved in 10 ml of DMSO. From this stock solution, 15 mg/10 ml was again diluted, thus eight different concentrations of the extract were prepared viz., 15, 12.5, 10, 7.5, 5, 3, 2 and 1 mg/ml. The solutions of DOX (Doxycycline) as a standard antibiotic were also prepared. Standard antibiotics and pure DMSO were used for positive and negative control.

Media for bacterial growth

Nutrient broth medium (Merck) was used for the growth of bacteria and nutrient agar medium (Merck) was used to perform antibacterial assay. Nutrient broth medium was prepared by dissolving 0.4 g/50 ml of distilled water for the growth of bacterial inoculums and nutrient agar medium was prepared by dissolving 2.3 g/100 ml of distilled water and pH was adjusted at 7.0 and then autoclaved.

Bacterial strains used

Four strains of bacteria were used. One was gram positive, that is, *Bacillus subtilis* and three were gram negative; *Escherichia coli*, *Vibrio cholera* and *Enterobacter aerogenes.* These microorganisms were maintained on nutrient agar medium at 4°C.

Preparation of Inocula

Twenty-four hours old culture of selected bacterial strains was centrifuged and pellets were mixed with physiological normal saline solution until a Mcfarland turbidity standard was obtained. Then this inoculum was used for seeding the nutrient agar.

Preparation of seeded agar plates

Nutrient agar medium was prepared by suspending nutrient agar (MERCK) 2.3 g in 100 ml of distilled water. pH of the medium was maintained at 7 and then autoclaving was done. It was cooled at 45°C. Then it was seeded with 10 ml of prepared inocula to have 10^6 CFU per ml. Petri plates were prepared by pouring 75 ml of seeded nutrient agar and allowed to solidify. Six wells per plate were made with sterile cork borer (5 mm).

Pouring of test solutions; Incubation and measurement of zone of inhibitions

Using micropipette, 100 µl of test solutions were poured in respective wells. These plates were incubated at 37°C. After 24 h of incubation; the diameter of the clear zones of inhibitions were measured by a ruler. Antibacterial activity of 8 dilutions of each plant extract was determined against four bacterial strains.

Table 1. Zones of inhibition (mm) after 24 h showing antibacterial activity of *Acacia modesta* against different bacterial strains.

Extract concentrations (mg/ml)	*E. coli* (mm)	*E. aerogenes* (mm)	*V. cholera* (mm)	*B. subtilis* (mm)
1	08	11	06	10
2	09	12	10	11
3	09	12	11	12
5	10	14	12	13
7.5	11	15	13	13
10	12	15	13	15
12.5	13	16	14	18
15	13	18	18	20

Antifungal assay

The agar tube dilution method is used for antifungal activity of extract. *Aspergillus niger* and *Aspergillus fumigatus* strains were used. Each fungal strain was maintained on sabouraud dextrose agar medium at 4°C. Sabouraud dextrose agar (MERCK) was prepared by using 10 mg/l peptone complex, 40 mg/l glucose and 15 mg/l agar to grow fungus for inoculums preparation.

Preparation of samples

The samples for antifungal assay were prepared from initial stock of 15 mg of extract each sample per ml of DMSO. One sample of each extract was prepared, which were used for test. Slants without extract were used for negative control.

Fungal assay procedure

Media for fungus was prepared by dissolving 6.5 gm/100ml in distilled water pH was adjusted at 5.6. Test tubes were marked to 12 cm mark. The sabouraud dextrose agar (MERCK) dispensed as 4 ml volume into screw capped tubes or cotton plugged test tubes and were autoclaved at 121°C for 21 min. Only one concentration of each plant extracts, were prepared by dissolving 24 mg/ml in solvent DMSO (Dimethylsulfoxide). Tubes were allowed to cool to 50°C and non-solidified SDA was loaded with 100 µl of 24 mg/ml plant extracts were inserted by compound pipette from the stock solution. Tubes were then allowed to solidify in slanting position at room temperature.

One slant of the extract sample was prepared for each fungus species. The tubes containing solidified media and test compound were inoculated with 4 mm diameter piece of inoculum, taken from a seven days old culture of fungus. Negative control test tubes without extract were also inoculated. The test tubes were incubated at 28°C for 7 days. Cultures were examined twice weekly during the incubation. Reading was taken by measuring the linear length of fungus in slant by measuring growth (mm) and growth inhibition was calculated with reference to negative control. Percentage inhibition of fungal growth for each concentration of compound was determined by the following formula.

$$\text{Percentage inhibition of fungal growth} = \frac{100 - \text{Linear growth in test (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

Antioxidant activity (DPPH free radical scavenging activity)

The free radical scavenging activity (RSA) of metabolic extracts of *A. modesta, P. cineraria* and *P. juliflora* was measured in terms of hydrogen donating or radical scavenging ability using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). The test extracts were prepared in methanol therefore DPPH was also prepared in methanol. 3.96 mg (x 4) of DPPH was dissolved in 20 ml of methanol solvent to get a stock solution. With 0.5 ml of sample solution was added to 1 ml of DPPH solution separately. These solution mixtures were kept in dark for thirty minutes (incubation period) at room temperature. Thirty minutes later the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radicals scavenging activity.

Finally the radical scavenging activity calculated as percentage of DPPH discoloration using the equation;

$$\text{\% scavenging DPPH free radical} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

Antibacterial activity of Mimosaceae Plants; *Acacia modesta, Prosopis cineraria* and *Prosopis juliflora*

The methanolic leaves extract of *A. modesta* was tested against the four strains of bacteria viz., *B. subtilis, E. coli*, *V. cholera* and *E. aerogenes.* In case of *E. coli*, the inhibition zones were ranged from 8-13 mm at varying concentrations of leaves extract of *A. modesta*. The maximum inhibition zone was 13 mm at 15 mg/ml and minimum inhibition zone was recorded as 8 mm at 1 mg/ml concentration. The extract concentration of 12.5 mg/ml also exhibited the maximum inhibition zone of 13 mm as shown in Table 1 and Figure 1 (a) while standard antibiotic DOX (Doxycycline) (2 mg/ml concentration) show 22 mm zones of inhibition against *E. coli* (Table 4). In case of *E. aerogenes*, the inhibition zones were ranged from 11-18 mm at varying concentrations of leaves extract of *A. modesta* (Table 1 and Figure 1a) while standard antibiotic expressed 36 mm inhibition zones in

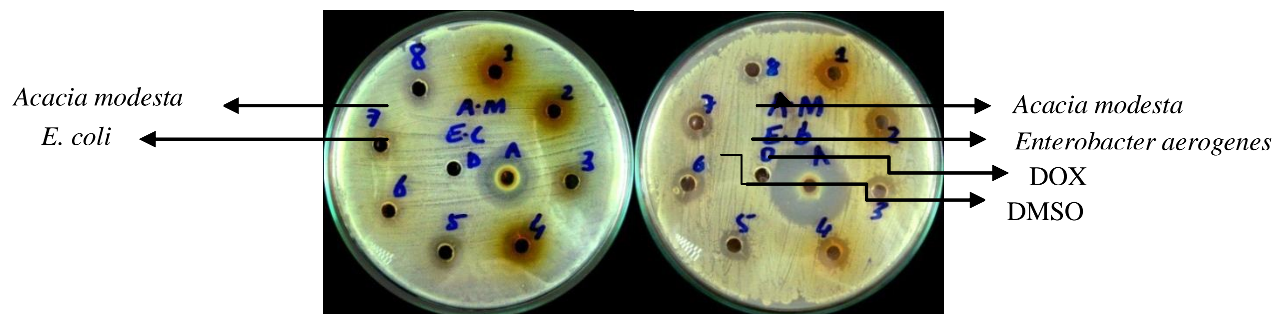

Figure 1a. Antibacterial activity of *Acacia modesta* against *Escherichia coli* and *Enterobacter aerogenes*.

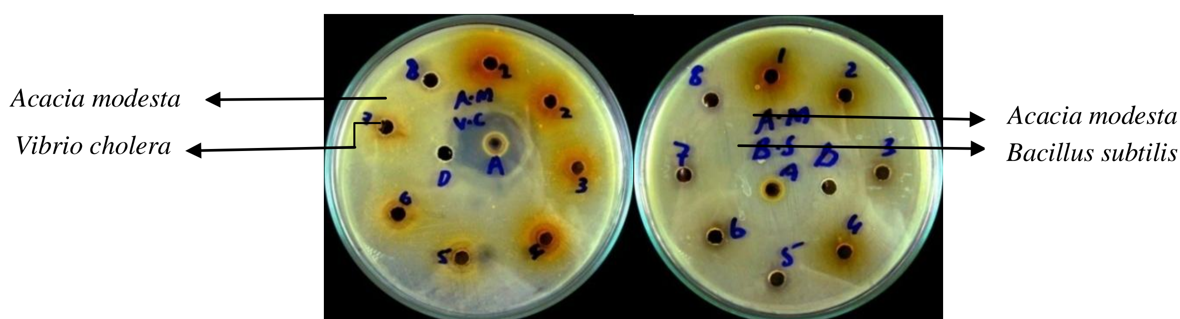

Figure 1b. Antibacterial activity of *Acacia modesta* against *Vibrio cholera* and *Bacillus subtilis*.

Table 2. Zones of inhibition (mm) after 24 h showing Antibacterial activity of *Prosopis cineraria* against different bacterial strains.

Extract concentration (mg/ml)	*E. coli* (mm)	*E. aerogenes* (mm)	*V. cholera* (mm)	*B. subtilis* (mm)
1	09	10	13	11
2	10	10	13	12
3	10	10	14	13
5	11	12	14	13
7.5	13	13	16	14
10	14	14	17	16
12.5	15	15	18	16
15	15	15	19	18

E. aerogenes (Table 4). In case of *V. cholera,* its inhibition zones were ranged from 6-18 mm (Table 1; Figure 1b) while 26 mm inhibition zone against *V. cholera* was measured by applying DOX. The inhibitory value was ranged from 10-20 mm against *B. subtilis* while antibacterial activity of antibiotic DOX showed 28 mm inhibitory zone in *B. subtilis* (Table 4). These results were confirmatory to the findings of Khalid et al. (2011) in which they used five different strains of bacteria. The same sort of antibacterial activities of *Acacia* plant were also determined by (El-Kamali and EL-Karim, 2009). *P. cineraria* leaf extracts were tested against the four strains of bacteria. In case of *E. coli,* the maximum inhibition was recorded as 15 mm at 12.5 mg/ml and 15 mg/ml extract concentrations. While minimum inhibitory concentration (MIC) value of 9 mm was recorded at 1 mg/ml concentration (Table 2 and Figure 2a). Minimum Inhibitory Concentration (MIC) is the lowest concentration of antimicrobial agent that inhibited visible growth of bacterial spots (Bosio et al., 2000) after an overnight

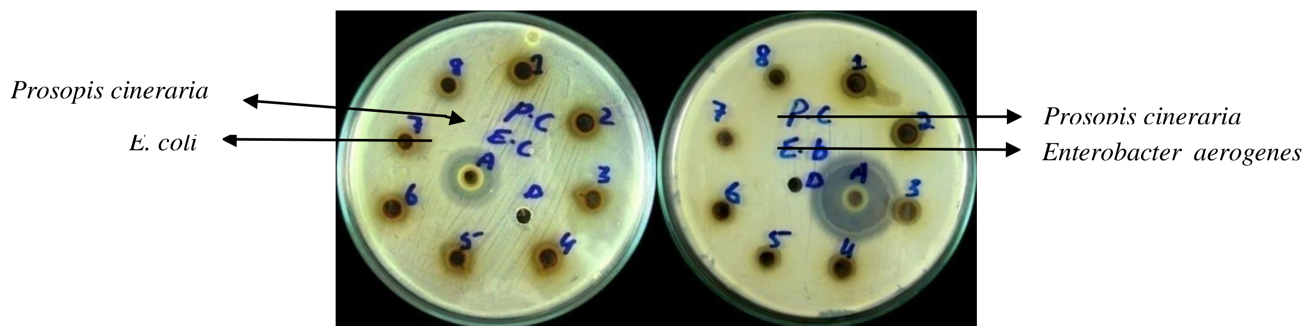

Figure 2a. Antibacterial activity of *Prosopis cineraria* against *Escherichia coli* and *Enterobacter aerogenes*.

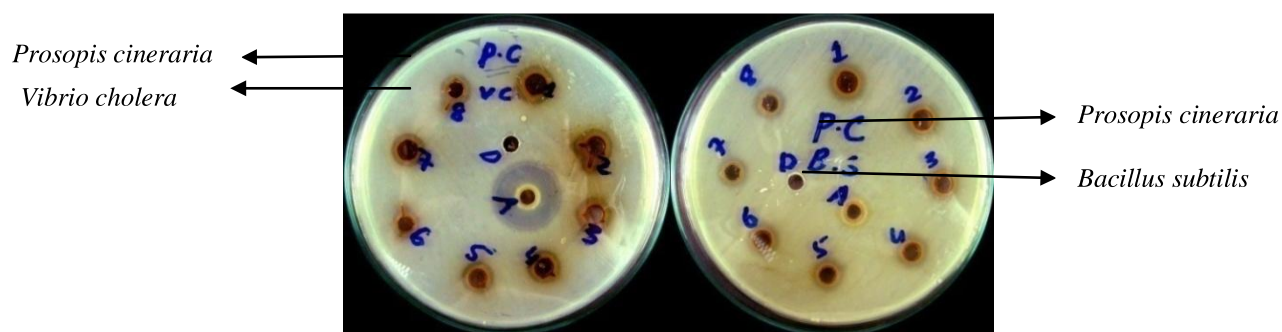

Figure 2b. Antibacterial activity of *Prosopis cineraria* against *Vibrio cholera* and *Bacillus subtilis*.

incubation, disregarding a few single colonies or a fine barely visible haze (Andrews, 2001). In case of *E. aerogenes*, the inhibition zones were ranged from 10-15 mm. The maximum inhibition zones were recorded as 15 mm at 15 mg/ml and 12.5 mg/ml concentration. While minimum inhibition zone was recorded as 10 mm at 1 mg/ml extract concentration (Table 2 and Figure 2a) while standard antibiotic expressed 36 mm inhibition zones in *E. aerogenes* (Table 4). In case of *V. cholera, P. cineraria* extract exhibited 18 and 19 mm inhibitory zones at 12.5 and 15 mg/ml concentrations, respectively (Table 2 and Figure 2b). Here MIC value (that is 1 mg/ml) showed 13 mm inhibition zone. Antibiotic DOX (Doxycycline) showed 26 mm inhibition zone against *V. cholera*. *P. cineraria* extract showed maximum inhibitory zone of 18 mm against *B. subtilis* at the concentration of 15 mg/ml while concentrations; 12.5 and 10 mg/ml showed 16 mm inhibitory zones. The 7.5 mg/ml concentration showed 14 mm inhibition zone. The two same inhibition zones of 13 mm were recorded at 5 and 3 mg/ml. Here MIC value (that is, 1 mg/ml) showed 11 mm inhibitory zone (Table 2 and Figure 2b). The antibacterial activities of *P. cineraria* plant against gram positive and gram negative bacteria were successfully evaluated by Velmurugan et al. (2010). Their uses as remedies for many infectious diseases as well as searches for additional substances in plants with antimicrobial activity were frequent (Lewis and Ausubel, 2006).

The antibacterial activity of *P. juliflora* was tested against the four strains of bacteria. In case of *E. coli*, the inhibition zones were ranged from 17-24 mm at different concentrations of leaves extract. The maximum inhibition zone was 24 mm at 15 mg/ml and minimum inhibition zone was recorded as 17 mm at 1 mg/ml concentration. The extract concentration of 12.5 mg/ml also showed the maximum inhibition zone of 24 mm as shown in Table 3 and Figure 3a, while standard antibiotic DOX (Doxycycline) (2 mg/ml concentration) showed 22 mm zones of inhibition (Table 4). In case of *E. aerogenes*, the inhibition zones were ranged from 19-25 mm. The maximum inhibition zones were recorded as 24 and 25 mm at 12.5 and 15 mg/ml concentrations, respectively. While minimum inhibition zone was recorded as 19 mm at 1 mg/ml (Table 3 and Figure 3a) and standard antibiotic expressed 36 mm inhibition zones in *E. aerogenes* (Table 4).

In case of *V. cholera,* its inhibition zones were ranged from 8-16 mm. The maximum inhibition zones were measured as 14 and 16 mm at 12.5 and 15 mg/ml extract concentration, respectively. While 1 mg/ml concentration showed minimum inhibitory zone of 8 mm (Table 3 and Figure 3b) and 26 mm inhibition zone was measured by

Table 3. Zones of inhibition (mm) after 24 h showing Antibacterial activity of *Prosopis juliflora* against different bacterial strains.

Extract concentration (mg/ml)	*E. coli*(mm)	*E. aerogenes* (mm)	*V. cholera* (mm)	*B. subtilis* (mm)
1	17	19	08	09
2	18	19	10	10
3	19	21	11	10
5	20	22	11	10
7.5	22	22	11	11
10	23	23	13	12
12.5	24	24	14	14
15	24	25	16	17

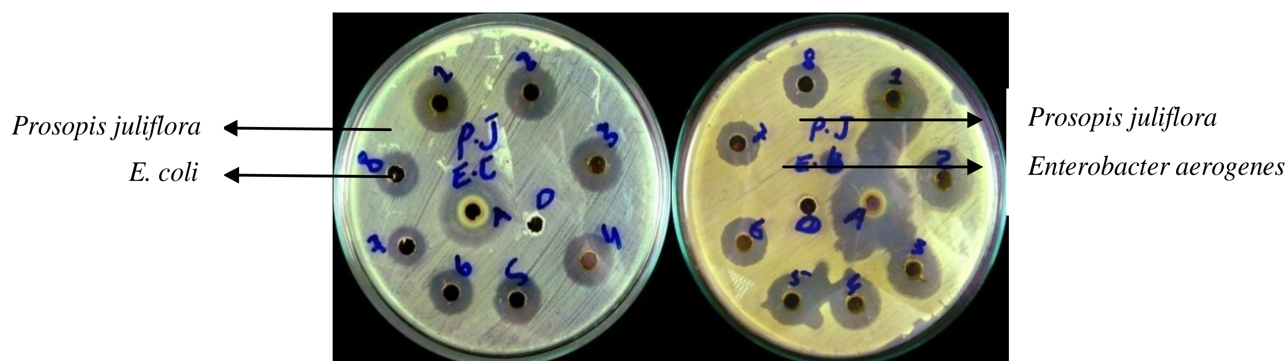

Figure 3a. Antibacterial activity of *Prosopis juliflora* against *Escherichia coli* and *Enterobacter aerogenes*.

Table 4. Zones of inhibition after 24 h showing Antibacterial activity of Standard antibiotic DOX (Doxycycline).

DOX (Doxycyclin) (2 mg/ml)	Bacterial strains	Zones of inhibition (mm)
2	*Escherichia coli*	22
2	*Enterobacter aerogenes*	36
2	*Vibrio cholera*	26
2	*Bacillus subtilis*	28

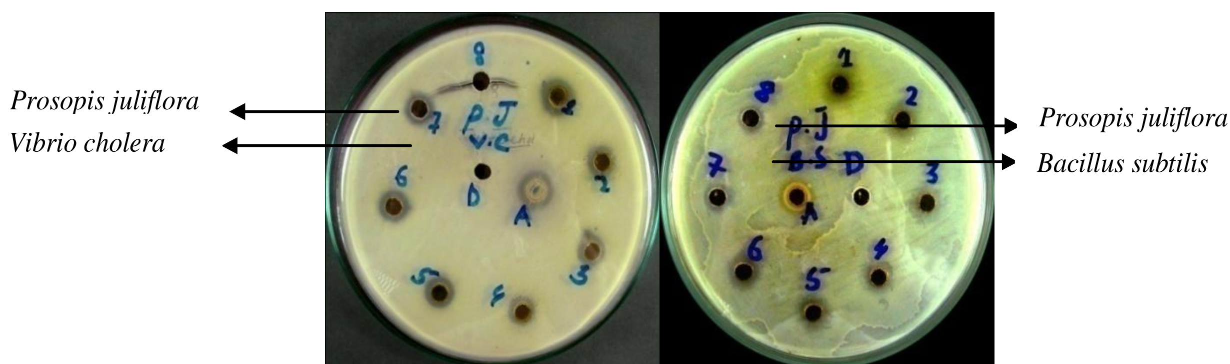

Figure 3b. Antibacterial activity of *Prosopis juliflora* against *Vibrio cholera* and *Bacillus subtilis*.
Note: The numbers on the plates showed treatments.

Table 5. Antifungal activity of *Acacia modesta* against *Aspergillus niger* and *Aspergillus fumigates*.

Fungi	Linear growth (Control) (mm)	Linear growth (Treatment) (mm)	% inhibition
A. niger	130	115	11.53
A. fumigates	125	100	0.80

Table 6. Antifungal activity of *Prosopis cineraria* against *Aspergillus niger* and *Aspergillus fumigates*.

Fungi	Linear growth (Control) (mm)	Linear growth (Treatment) (mm)	% inhibition
A. niger	130	120	15.38
A. fumigatus	125	110	8.00

Table 7. Antifungal activity of *Prosopis juliflora* against *Aspergillus niger* and *Aspergillus fumigates*.

Fungi	Linear growth (Control) (mm)	Linear growth (Treatment) (mm)	% inhibition
A. niger	130	110	7.69
A. fumigatus	125	105	4.00

applying DOX. Similarly, the response of *P. juliflora* leaves extracts were observed against *B. subtilis* (Figure 3b). The inhibitory value was ranged from 9-17 mm. The maximum inhibitory values were recorded as 14 and 17 mm at 12.5 and 15 mg/ml extract concentrations, respectively (Table 3). The antibacterial activity of antibiotic DOX showed 28 mm inhibitory zone in *B. subtilis* (Table 4). Antibacterial assay revealed that the leaf extract of *P. juliflora* showed good inhibitory activity against all the tested pathogens compared with standard antibiotics (Sathiya and Muthuchelian, 2008). Similar work was also performed by Singh and Swapnil, (2010). There were slight variations in the extract and antibiotics concentrations as compared to our results but it might be suggested that the inhibitory activities were found to be dose dependent.

Antifungal activity of Acacia modesta, Prosopis cineraria and Prosopis juliflora

The antifungal activities of three *Mimosaceae* plants viz., *A. modesta, P. cineraria* and *P. juliflora* were evaluated. The plant extracts were used as treatments. The control was used as a standard to compare the growth of inhibition. The control test tubes showed 130 mm linear growth of *A. niger* and 125 mm linear growth of *A. fumigatus*. These control antifungal results were compared with other treated test tubes.

A. modesta showed 115 mm (11.53%) growth inhibition against *A. niger* and 100 mm (0.8 %) growth inhibition against *A. fumigatus* (Table 5). While *P. cineraria* gave 120 mm (15.38 %) growth inhibition againt *A. niger* and 110 mm (8 %) inhibition zone against *A. fumigatus* (Table 6). In case of *P. juliflora;* it inhibited 110 mm (7.69 %) growth against *A. niger* and 105 mm (4 %) growth inhibition of *A. fumigatus* (Table 7). Hence the extracts of three *Mimosaceae* species, that is, *A. modesta, P. cineraria* and *P. juliflora* appeared to have potential for testing as a plant of high medicinal values for various antimicrobial activities.

From the above results it is evident that the *Mimosaceae* plants exhibited the inhibition zones against both types of fungi. These results are in confirmatory to the findings of Lakshmi et al. (2010) who reported that thirty-four species of plants were evaluated for antimicrobial activity against different plant pathogens using the agar diffusion method. The leaf extracts of *P. juliflora* showed significant inhibition zones against all types of tested pathogens. Koirala et al. (2005) concluded that various species of fungi including *Aspergillus* cause nutrient losses and hence reduce the quality of grains. Due to high toxic effects of chemical pesticides, many of them have been restricted by WHO, as they have non-target effects and cause environmental pollution (Barnard et al., 1997).

Antioxidant activity of *Mimosaceae* plants through DPPH

The free radical scavenging activity (%RSA) of plants

Table 8. Antioxidant activity of *Acacia modesta, Prosopis cineraria* and *Prosopis juliflora* through DPPH.

S/N	Plant name	Extract	Absorbance of reaction mixture (Mean)	% RSA
1	*Acacia modesta*	Methanol	0.5182 a	41.42
2	*Prosopis cineraria*	Methanol	0.3496 ab	60.48
3	*Prosopis juliflora*	Methanol	0.4616 b	47.82

LSD = 0.135

Table 9. One-way ANOVA for Antioxidant activity of *Acacia modesta, Prosopis cineraria* and *Prosopis juliflora* through DPPH.

Source	DF	SS	MS	F	P
Plant	2	0.04414	0.02207	21.6	0.0018
Error	6	0.00613	0.00102		
Total	8	0.05027			

Grand mean 0.4432; CV 7.21.

was determined through DPPH. Maximum radical scavenging activity, that is, 60.48% was achieved in *P. cineraria* followed by *P. juliflora* which produced 47.82% radical scavenging activity. The minimum value of % RSA was obtained in *A. modesta*, that is, 41.42% as shown in Table 8.

Analyses of variance showed that there were significant differences among the three plants for the absorbance of reaction mixture (0.5 ml of sample solution and 1 ml of DPPH solution) (Table 8 and Table 9). The maximum mean value for absorbance of reaction mixture was recorded in *A. modeta*, that is, 0.5182 followed by *P. juliflora* which gave 0.4616 mean absorbance of reaction mixture.

The minimum mean value of absorbance of reaction mixture, that is, 0.3496 was recorded in *P. cineraria* (Table 8). Lower absorbance of the reaction mixture indicated higher free radicals scavenging activity. So maximum antioxidant activity (that is, 60.48%) was obtained in *P. cineraria* as it has minimum value of absorbance of reaction mixture as shown in Table 8. While *P. juliflora* and *A. modesta* exhibited 47.82 and 41.42% antioxidant activity, respectively. The present research work clearly indicated that *P. cineraria* is highly efficient for antioxidant activity because it has lower absorbance of reaction mixture and more %RSA. From the results it is apparent that all three plants of *Mimosaceae* family have concentration free radical scavenging activity. A number of explanations can be given for the difference in biological activity reports of some common extracts against same or similar microorganism, but the first logic is dissimilarities in phytochemicals of similar plants growing at different geographical locations (Olila et al., 2001). From the above studies, it is clear that the traditional plants may represent new sources of anti-microbials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery (Gandhiraja et al., 2009).

Conclusion

From the present study it can be concluded that the three *Mimosaceae* plants were more efficient against all types of microbes. The plants were effective even at low concentrations. In this research work it was observed that plants showed remarkable activity against gram negative bacteria. It can also be concluded from the study that the inhibitory activities were found to be dose dependent.

ACKNOWLEDGEMENT

Authors are grateful to Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan for providing financial support for this project. Authors also thank to National Agricultural Research Centre (NARC) Islamabad for providing facilities during this research work.

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