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Biological screening of *Albizia lebbek* L. and *Mimosa himalayana* Gamble (Mimosaceae)

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Methanolic leaf extracts of two medicinal plant species of family Mimosaceae: *Albizia lebbek* L. and *Mimosa himalayana* Gamble, were used to evaluate their antibacterial and antifungal activity using agar diffusion method. Extractions from leaves of selected plants were carried out by simple maceration process. The methanolic extracts of these plants were tested against four strains of bacteria (one strain was gram positive that is, *Bacillus subtilis* and three were gram negative that is, *Ecscherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and two strains of fungi (*Aspergillus niger* and *Aspergillus flavus*). At 15 mg/ml extract concentration, the maximum inhibitory zones observed in *A. lebbek* L. *and M. himalayana* Gamble were12.5 and 27 mm, respectively. *A. lebbek* L. and *M. himalayana* gave response against *A. niger* by producing 36.2 and 0.86% inhibition, respectively. No antifungal activity was reported by *A. lebbeck* L. and *M. himalayana* Gamble against *A. flavus*.

Key words: Mimosaceae, antibacterial, antifungal, methanol extracts, medicinal plants.

INTRODUCTION

It is estimated that there are 250,000 to 500,500 species of plants on earth (Borris, 1996). A relatively small percentage (1 to 10%) of these is used as food by both humans and other animal species. It is possible that even more are used for medicinal purposes. Hippocrates (in the late fifth century B.C) mentioned 300 to 400 medicinal plants. An estimate suggests that about 13,000 plant species worldwide are known to have use as drugs. The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobials (Das et al., 1999). Most of these plants are being used for therapeutic purposes without specific knowledge of their active ingredients. In fact, Pakistani medicinal plants, for the purpose of drug development, are one of the least investigated sources of natural compounds (Satyavati et

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al., 1976).

About 80% of the developing world population depends on traditional medicines for their primary health needs and 85% of these traditional medicines involve the use of plant extracts. This means that about four billion people depend on natural products as their primary source of medication. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). It has been reported that the higher plants have shown to be a potential source for the new antimicrobial agents. Natural antimicrobials can be derived from plants, animal tissues or microorganisms. To determine the potential and promote the use of herbal medicine, it is essential to investigate plants that find place in folklore medicine (Nair et al., 2005).

The aim of the present work was to determine whether the selected plant species of family Mimosaceae have antibacterial and antifungal activities using an agar diffusion method.

MATERIALS AND METHODS

Study site

The present research work was carried out in the Department of Plant Sciences, Quaid-i-Azam University (QAU) Islamabad. Brief accounts of materials as well as procedures used are described.

Plant materials and their handling

Plant materials were collected from vicinity of Quaid-i-Azam University, Islamabad-Pakistan. The plants were identified in the Herbarium, Department of Plant Sciences QAU, Islamabad and voucher specimens were deposited. Fresh leaves of *Albizia lebbek* L. and *Mimosa himalayana* were picked, rinsed with distilled water and kept under shade to dry before being weighed.

Preparation of leaf extracts

Extracts were prepared from the air-dried leaves using simple maceration process. The leaves were grounded in methanol using kitchen blender. This mixture was kept in extraction bottles at room temperature (25°C) for two weeks. After two weeks, the mixtures were filtered twice, using Whatman-41 filter paper. Methanol was then evaporated completely using rotary evaporator to obtain the extracts. The extract (15 mg) was dissolved in 1 ml of dimethyl sulfoxide. This stock solution 15 mg/ml was again diluted, thus 8 concentrations of the extract were prepared that is, 15.0, 12.5, 10.0, 7.5, 5.0, 3.0, 2.0 and 1.00 mg/ml. Along with these solutions, the standard antibiotic (DOX) was also prepared at a concentration of 2 mg/ml.

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Antimicrobial activity

Antibacterial activity was carried out by agar diffusion method and antifungal activity was carried out by using agar tube dilution as reported by Choudhary et al. (1995). Micro organisms used in this study were *Bacillus subtilis Ecscherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia, Aspergillus niger* and *Aspergillus flavus*. Antifungal activity was determined by using following formula.

Percentage inhibition of fungal growth = 100 - Linear growth in test (mm) / Linear growth in control (mm) × 100

RESULTS

Methanolic extracts of two Mimosaceae species that is, *A. lebbeck L.* and *M. himalayana* were tested against four strains of bacteria. Methanolic leaves extract of *A. lebbeck* L. at the concentrations of 15, 12.50 and 10.00 mg/ml exhibited 10 mm inhibition zones against the *E. coli*, whereas at the 7.50 mg/ml it showed the 9 mm inhibitory zone. Minimum inhibitory concentration (MIC) value was 5.00 mg/ml. *A. lebbeck* showed 15 mm inhibition zone at the concentration of 15 mg/ml, and at 12.50 mg/L it yielded 14 mm inhibition zone against *P. aeruginosa*. At 10.00 and 7.50 mg/ml, it showed 13 and 11 mm inhibitory zones, respectively. *A. lebbeck* extract yielded 21 mm inhibitory zones at the 15 and 12.50 mg/ml concentrations against the *K. pneumonia*. 10.00 and 7.50 mg/ml showed 20 mm inhibition of zone. Methanolic extract gave 19 mm clear inhibition zone at the 5.00 mg/ml concentration, whereas 3.00, 2.00 and 1.00 mg/ml gave 18 inhibittion zones against the *K. pneumonia.* Antibiotic DOX (doxycycline) gave 47 mm inhibition zone (Table 1). Methanolic extract of *M. himalayana* showed 27 mm inhibitory zone against *E. coli* at the 15 mg/ml concentration, whereas it showed 25 mm clear inhibition zone at 12.50 mg/ml concentration. Here MIC value was 1.00 mg/ml and it gave 13 mm inhibitory zone (Table 2, Figure 1).

Antifungal study of two Mimosaceae plants

This study was done to check antifungal activity of 2 species of Mimosaceae plants. Only one concentration of each plant extracts were prepared by dissolving 24 mg/ml in solvent dimethylsulfoxide (DMSO). The fungi used in this study were *A. niger* and *A. flavus.* After inoculation and incubation of the samples for about one week, antifungal assay gave the following results: *A. lebbeck* L. showed 36.20% and 74 mm growth inhibition; *M.*

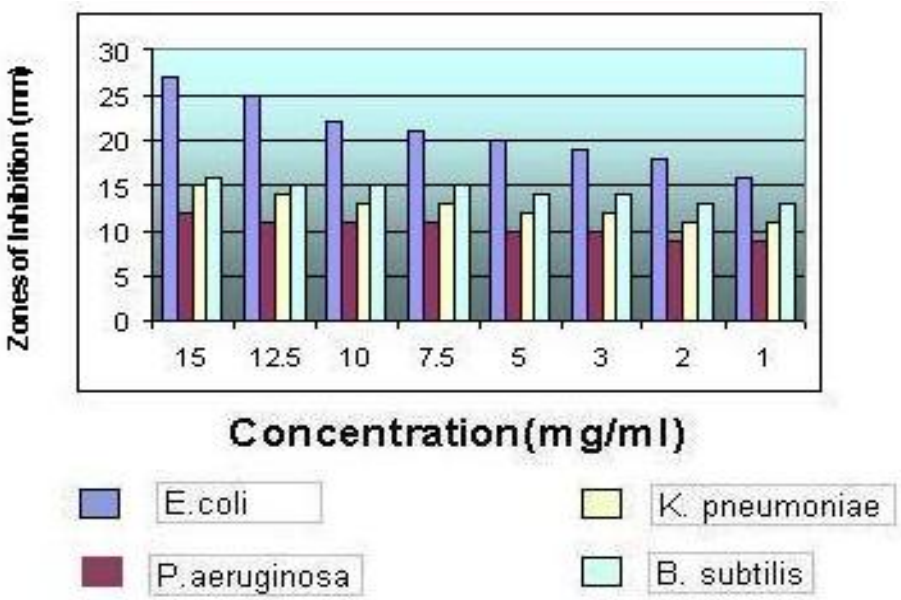

Figure 1. Zones of inhibition (mm) showing antibacterial activity of *M. himalayana*, against *E. coli, P., aeruginosa, K., pneumonia* and *B. subtilis.*

Table 1. Antibacterial activity of *A. lebbek* L. zones of inhibition (mm).

Concentration (mg/ml)	*E. coli* (mm)	*P. aeruginosa* (mm)	*K. pneumonia* (mm)	*B. subtilis* (mm)
15	10	15	21	14
12.5	10	14	21	14
10	10	13	20	14
7.5	9	11	20	13
5	8	9	19	12
3	0	0	18	11
2	0	0	18	11
1	0	0	18	11

Table 2. Antibacterial activity of *M. himalayana* L. zones of inhibition (mm).

Concentration (mg/ml)	*E. coli* (mm)	*P. aeruginosa* (mm)	*K. pneumonia* (mm)	*B. subtilis* (mm)
15	27	12	15	16
12.5	25	11	14	15
10	22	11	13	15
7.5	21	11	13	15
5	20	10	12	14
3	19	10	12	14
2	18	9	11	13
1	16	9	11	13

Table 3. *A. lebbek* L. against *A. niger* and *A. flavus.*

Fungi	LGC (mm)	LGT (mm)	% Inhibition
A. niger	116	74	36.20
A. flavus	122	122	No activity

LGC mm = linear growth in control (millimeter); LGT mm = linear growth in test (millimeter).

Table 4. *M. himalayana* against *A. niger* and *A. flavus.*

Fungi	LGC mm	LGT mm	% Inhibition
A. niger	116	115	0.86
A. flavus	122	122	No activity

LGC mm = linear growth in control (millimeter); LGT mm = linear growth in test (millimeter).

himalayana inhibited 0.86% and 115 mm growth against *A. niger*; *A. lebbeck* L. and *M. himalayana* did not show any activity against *A. flavus* as indicated in Tables 3 and 4.

DISCUSSION

Pakistan is rich in diversity of plants. People living in rural areas are interested in the use of plant-based drugs, because plant based drugs have no side effects and they are inexpensive. *A. lebbeck* L. and *M. himalayana* appear to have potential for testing as a plant of high medicinal values for various antimicrobial activities as well as other biological activities. These plants are abundantly found in Pakistan and easily accessible. In this study, MIC value of *A. lebbeck* L. ranged from 5.00 to 1.00 mg/ml for all bacteria, whereas *M. himalayana* MIC was 1.00 mg/ml. Srinivasan et al. (2001) showed that *A. lebbeck* L. has broadest spectra activity against all tested nine micro-organisms. *A. lebbeck* L. showed activity against *A. niger*.

Agyare et al. (2006) concluded that the *A. ferruginea* leaf and stem bark extracts contain tannins, sterols and saponins. They analyzed that the ethyl alcohol extract of *A. ferruginea* leaves and stem bark showed remarkable activity against all the test organisms. They showed that the extracts were less active against *P. aeruginosa* which is highly resistant to available orthodox antibiotics (Walker and Edwards, 1999). The petroleum ether extract was less active against organism. They described that the antimicrobial activity was more pronounced with leaf extract compared to stem bark.

In this study it was found that *A. lebbek* L. was active against all test microorganisms except *P. aeruginosa* below 5 mg/ml, whereas against fungus *A. flavus* it did not show any activity. Gandhirajan et al. (2009) found that the *M. pudica* methanolic extract exhibited anti-microbial activity against the tested microorganisms at three different concentrations of 50, 100 and 200 µg/disc. It gave 6 mm inhibition zone against *A. fumigatus* at the concentration of 50 µl and at the same concentration it gave 12 mm inhibition zone against *K. pneumonia*. They found that highest inhibition zone of 20 mm was observed against *K. pneumonia* at 200 µl.

In this work it was found that *Mimosa himalayana* showed highest inhibition zone of 27 mm against *E. coli* at 15 mg/ml concentration. It can be concluded from the study that *M. himalayana* showed significant activity against all test organism. Genest et al. (2008) found that none of extracts of *M. rubicaulis* was active against any of the test bacterial strains at test concentrations, while in this study *M. himalayana* gave lagre zone of 27 mm against *E. coli* and minimum of 9 mm against *P. aeruginosa* and it did not show any activity against *A. flavus*.

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 studies, it is concluded 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 (Gandhirajan et al., 2009).

The results of the present study provide a scientific validation for the popular use of the medicinal plants studied and serve as a guide which may help in selection of plants with antimicrobial activities for further phyto-chemical work on the isolation and the identification of the active compounds.

From this study a conclusion can be drawn that two Mimosaceae plants are efficient against all pathogens. It was observed that plants were highly effective even at low concentration. In this research work, it was shown that plants showed remarkable activity against gram negative bacteria. It was and even it is believed today that gram negative bacteria are more resistant than gram positive.

Conclusion

The herbal plants may be used as an alternative and potential and promising source of pharmaceutical agents against different pathogens. The results suggest the presence of high concentration of an active principle in all the extracts of the tested two plants which showed potential antimicrobial activity. There is need of developing drugs from plants as micro organisms are becoming resistant to antibiotics and creating health problems.

Conflict of Interest

Authors declare no conflict of interest.

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