Antimicrobial activities of three species of family mimosaceae

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Abstract: The antimicrobial activities of crude methanolic extract of leaves of *Acacia nilotica* L., *Albizia lebbeck* L. and *Mimosa himalayana* Gamble belonging to family mimosaceae were investigated in this research work. Antibacterial activity was studied by agar well diffusion method against one gram-positive *Bacillus subtilis* and three gram-negative *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia.* Crude extract of all plants showed best activity against gram-negative bacterial strains while minor inhibition zones were found against gram positive bacterial strains. Antifungal activity of crude plant extract was screened by agar tube dilution method against *Aspergillus nigar* and *Aspergillus flavus.* These results showed that these plants extracts have potential against bacterias, while against fungi their activity is not much effective.

Keywords: Antimicrobial activity, Sialkot, bacterial resistance, mimosaceae.

INTRODUCTION

A number of medicinal plants are gifted by nature to mankind and a notable number of modern drugs have been synthesized from these natural medicinal plants. These drugs are based on the indigenous medicinal information of plants. This natural source has been used to cure various diseases throughout the world. The global utilization of medicinal plants as herbal remedies and healthcare preparations like those, found in ancient literature, as the Vedas and the Bible, has been showed to the occasion of natural products with medicinal values. Actually, plants have great diversity of bioactive compounds and it is an indication which makes plants a prosperous source of different types of drugs (Farombi, 2003).

Mostly, the worst human diseases have been caused by bacteria. Significantly, the most prevalent pathogenic organisms are viruses, bacterias as well as fungi and parasitic worms (Ritter *et al*., 1996). Number of plants have been extracted and screened against these pathogenic organisms and showed prominent antimicrobial activities *In vitro* and *In vivo,* which justified that indigenous medicines was focused on characterization of antimicrobial activities of plants (Marinez *et al.,* 1996).

Plant extracts and photochemical compounds have been used for antimicrobial properties and have significant therapeutic properties. In recent years, various investigations have been conducted worldwide to confirm such efficiency. Various plants showed antimicrobial properties, which were similar to that of synthetic standard antimicrobial agents.

It has been observed that most of the microbes are resistant to antimicrobial drugs that donate morbidity, transience and amplified healthcare spent (Gums, 2002). A boost in the occurrence of forthcoming infectious diseases is hazard. Extracts and chemical compounds isolated from plants with known antimicrobial activities can be of great impact in the healthcare. Medicinal action of plants is defined to some chemical compounds that impart a specific physiological action on human body (Satish *et al*., 2008).

Present research investigation reveals the antimicrobial properties of leaves three highly medicinal plant species of family mimosaceae (*Acacia nilotica* L., *Albizia lebbeck* L. and *Mimosa himalayana* Gamble) from district Sialkot, Pakistan. In past Ali and Yagoub (2007) studied the antimicrobial activity of ethanolic and chloroform extracts of *Acacia nilotica* L. against five bacterial strains. They found that susceptibility of ethanol extract was higher than the chloroform extract. In present research work plant were extracted in methanol and the activity of methanolic extracts is evaluated. Srinivasan *et al*. (2001) screened fifty medicinal plants for their antimicrobial activity. Among tested plants *Albizia lebbeck* L. showed best activity against bacterial strains.

MATERIALS AND METHODS

Collection of plant material

Plants were collected from district Sialkot, Pakistan on July 2010. Plants were identified and voucher specimens were submitted in Herbarium of Quaid-i-Azam University, Islamabad, Pakistan. Voucher numbers allotted to *Acacia nilotica* L., *Albizia lebbeck* L. and *Mimosa himalayana* Gamble were 125401, 125403 and 125402 respectively.

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Antimicrobial activity

Extraction of plant material and preparation of samples

Plant material (leaves) was shad dried and then ground to powder form. 100 g of plant material was mixed in 1 litter of 80% methanol for 5 days. Methanol was used as a solvent as it is highly polar solvent and it has ability to dissolve maximum chemical constituents. To extract the maximum chemical constituents from plant material methanol was used as solvent. Extract was filtered through Whatman 41 filter paper and then subjected to rotary-evaporator (Brinkmann rotavapor, Model # R). Semisolid plant extract was placed in open air to evaporate the methanol and final solid extract was preserved in refrigerator at -8 ^{0}C for further process.

15 mg of plant extract was dissolved in 10 ml of DMSO to prepare stock solution. DMSO is an organic solvent, and it has no activity on bacterial or fungal strains. From this stock solution further dilution were made to get 12.50 mg/ml, 10 mg/ml and 5 mg/ml and 3 mg/ml concentrations of plant extract. Standard antibiotic doxycycline (DOX) was prepared to compare the results of plant extracts with standard antibiotic.

Media for bacteria

For the growth of bacterial strains, nutrient broth medium (Difco) was prepared by dissolving 0.4g/ 50ml of distilled water. Nutrient agar medium was (MERCK) prepared by dissolving 2.3g/ 100ml of distilled water, pH was adjusted as 7 and both mediums were autoclaved.

McFarland 0.5 Barium Sulphate Turbidity Standard

McFarland 0.5 Barium Sulphate Turbidity Standard was prepared by dissolving 0.5 ml 0.04 M Barium chloride to 99.5 ml 0.36 N sulphuric acid.

Bacterial strains

Four bacterial strains, one gram-positive *Bacillus subtilis* and three gram-negative *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia* were used to screen antibacterial activity.

Preparation of inocula

Twenty four hours old culture of bacterial strains in nutrient broth (Difco) was mixed in normal saline solution till the formation of McFarland turbidity standard [10^{-6} colony farming unit (CFU) ml^{-1}]. Nutrient agar was then seeded with this resulting solution.

Preparation of seeded agar plates and Incubation of test sample

Autoclaved nutrient agar was allowed to cool up to 50^{o}C and prepared inocula was added to it; petri plates were prepared by pouring 80 ml of seeded nutrient agar. Eight wells were made with the help of sterile cork borer (5mm) after solidification of agar medium.100 µl of test solution was poured in respective wells and these plates were incubated at 37^{o}C. Diameter of clear zones was measured after 24 hours.

Antifungal activity

Antifungal activity of crude plant extract of leaves was screened by agar tube dilution method (Choudhary *et al*., 1995). *Aspergillus nigar* and *Aspergillus flavus* were used as fungal strains. Test sample was prepared by dissolving 24mg plant extract in ml DMSO. Fungus media was prepared by dissolving 6.5g of sabouraud dextrose agar (MERCK) in 100 ml of distilled water and pH was adjusted as 5.6. Agar was poured in screwed capped test tubes and was autoclaved. Agar containing test tubes were allowed to cool up to 50 ^{0}C, added test sample in each tube and allowed to solidify in slanting position. After solidification, each slant was inoculated by 4 mm diameter piece of fungal strain. Positive control was adjusted by adding 20 µl of terbinafin and negative control slants were without test sample. These test tubes were incubated at 28 ^{0}C for seven days. Readings were measured in mm and growth inhibition was calculated with reference to negative control. Percentage inhibition was calculated by following formula:

$$\%\text{Inhibition of fungal growth} = 100\frac{[\text{Linear growth in test tubes (mm)}}{\text{Linear growth in control (mm)}]} \times 100$$

RESULTS

Antibacterial activity

Methanolic crude extract of leaves of plant species *Acacia nilotica* L., *Albizia lebbeck* L. and *Mimosa himalayana* Gamble belonging to family Mimosaceae were screened against four bacterial strains i.e. one gram-positive *Bacillus subtilis* and three gram-negative *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia.* Eight dilutions of crude extract were made in DMSO (Dimethyl sulfoxide). Crude methanolic extract of *Acacia nilotica* L. showed maximum antibacterial activity against *Pseudomonas aeruginosa* and *Klebsiella pneumonia* as compared to standard antibiotic doxycycline. Results are depicted in Figure 1. Minimum inhibitory concentration of *Acacia nilotica* L was found 100µg/ml against all the screened bacterial strains except *Pseudomonas aeruginosa* which exhibited 200µg/ml MIC. *Acacia nilotica* L. showed considerable antibacterial activities against all the bacterial strains. Results of antibacterial activities of crude extract of *Albizia lebbeck* L. are presented in Figure 2. It showed maximum inhibition against *Pseudomonas aeruginosa* and *Klebsiella pneumonia,* which showed best results compared to standard antibiotics (doxycycline). MIC was found 500µg/ml, 500µg/ml and 100µg/ml against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Bacillus subtilis* respectively. In Figure 3 the results of crude extracts of *Mimosa himalayana* Gamble are presented. Best antimicrobial activities were found against *Escherichia coli, Pseudomonas aeruginosa* and

Klebsiella pneumonia. All four bacterial strains exhibited 100µg/ml MIC.

Antifungal activity

Antifungal activity of *Acacia nilotica* L., *Albizia lebbeck* L. and *Mimosa Himalayana* Gamble was investigated against *Aspergillus nigar* and *Aspergillus flavus*. Results are depicted in table 1 and these results revealed that all plants have less antifungal activity. *Albizia lebbeck* L. showed 36.20% antifungal activity against *Aspergillus niger* while it showed no activity against *Aspergillus flavus*. Remaining plant species exhibited no prominent activity against both the fungal strains.

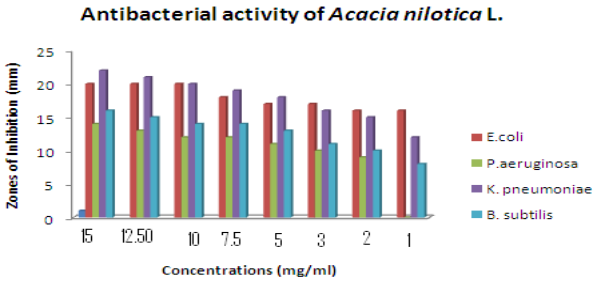

Fig. 1: Zones of inhibitions (mm) showing Antimicrobial activity of *Acacia nilotica* L. against *Escherichia coli* (E.coli), *Pseudomonas aeruginosa* (P. aeruginosa), *Klebsiella pneumonia* (K. pneumonia) and *Bacillus subtilis* (B. subtilis).

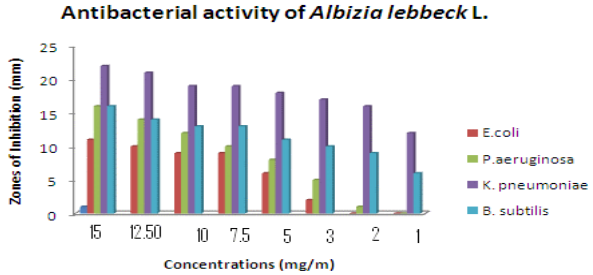

Fig. 2: Zones of inhibitions (mm) showing Antimicrobial activity of *Albizia lebbeck* L. against *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (P. aeruginosa), *Klebsiella pneumonia* (*K. pneumonia*) and *Bacillus subtilis* (*B. subtilis*).

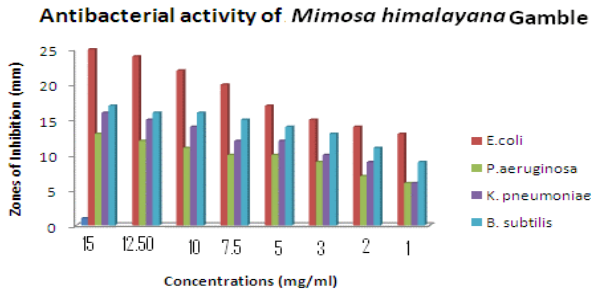

Fig. 3: Zones of inhibitions (mm) showing Antimicrobial activity of *Mimosa Himalayana* Gamble against *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumonia* (*K. pneumonia*) and *Bacillus subtilis* (*B. subtilis*).

DISCUSSION

Pakistan has great diversity of plants. People living in rural areas prefer to cure themselves with plant based drugs, which have no side effects and are inexpensive. *Acacia nilotica* L., *Albizia lebbeck* L. and *Mimosa Himalayana* Gamble are commonly used by local people of study area; in various ailments these three plant species are frequently used. Due to their high medicinal values, these plants were selected for screening of antimicrobial activities. Antibacterial and antifungal activities of these plants were investigated in presenting research work.

In present time antimicrobial resistance is a burning issue. Synthesized drugs are not more effective against various pathogenic microbes, which are causing number of clinical infections in different areas (Hsuek *et al*., 2010). It was found that throughout the world, Asia Pacific has highest microbial resistance issues (Hawser *et al*., 2009). Main reason of this issue is frequent use of antibiotics against a minor problem of healthcare, due to which microbes became resistance to the antibiotics. So, it is necessary to find out the understanding of this emerging issue to minimize this problem in healthcare (Anderson and Keye, 2009).

Table 1: Antifungal activity of *Acacia nilotica* L., *Albizia lebbeck* L. and *Mimosa Himalayana* Gamble against *Aspergillus niger* and *Aspergillus flavus*

Names of Plants	Fungal strains used	L.G.C. (mm)	L.G.T. (mm)	% Inhibition
Acacia nilotica L.	*A.niger*	116	111	4.31%
	A. flavus	122	116	4.91%
Albizia lebbeck L.	*A.niger*	116	74	36.20%
	A. flavus	122	122	No activity
Mimosa Himalayana Gamble	*A.niger*	116	115	0.86%
	A. flavus	122	122	No activity

Aspergillus niger – *A. niger* and *Aspergillus flavus*- *A. flavus*
L.G.C. (mm) = Linear Growth in Control (millimeter), L.G.T. (mm) = Linear Growth in Test (millimeter)

Synthesized pharmaceutical drugs are not more effective now against pathogenic microbes as erythromycin resistance is a major problem (Mycek *et al.,* 2000). Now, pharmacists and microbiologists are trying to extract drugs from natural resources like plants. Pharmaceutical companies are now paying attention to synthesize new drugs from plants. Biological screening of plants in this regard is the first step to find out the antimicrobial potential in plants.

In this research work *Acacia nilotica* L., *Albizia lebbeck* L. and *Mimosa Himalayana* Gamble were found effective against *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia.* Four bacterial strains were used to screen the metanolic crude extracts of *Acacia nilotica* L., *Albizia lebbeck* L. and *Mimosa Himalayana* Gamble. All these plants showed best results against gram negative bacterial strains, *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia. Bacillus subtilis* that is a gram-positive strain showed somewhat resistance to crude extracts of plants. These results were found prosperous; however, more extensive screening of these plants can discover new potentially active drugs. Results of antibacterial activity of three plants are given in figs. 1-3.

Fungi are major dermatophytes. A number of skin diseases are the result of fungi. These fungi are also responsible for numerous plant pathogens. Antifungal activity of *Acacia nilotica* L., *Albizia lebbeck* L. and *Mimosa Himalayana* Gamble was studied and no considerable activity was found. A little bit activity was exhibited by all these plants. *Albizia lebbeck* L. exhibited 36.20% fungus inhibition against *Aspergillus niger* while it showed no activity against *Aspergillus flavus. Acacia nilotica* L. showed minor activity against both fungal strains. Results are presented in table 1.

This study work provides a scientific validation for highly medicinal plants, in having potential to be a good drug. This work is a base for the further photochemical analysis and their isolation and identification. Results revealed that all three plants of family Mimosaceae are effective against pathogens. These plants are effective even at low concentration. This work requires further pharmacological screening for the isolation and identification of active compounds.

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