African Journal of Pharmacy and Pharmacology Vol. 6(46), pp. 3180-3183, 15 December, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP12.790
ISSN 1996-0816 © 2012 Academic Journals

Full Length Research Paper

Antimicrobial screening of *Albizia lebbeck* (l.) Benth. and *Acacia leucophloea* (Roxb.)

Shakir Ahmad Shahid[1]* and Najma Firdous[2]

[1]Department of Chemistry, University of Sargodha, Sargodha-40100, Pakistan.
[2]Department of Botany, Government College University Faisalabad-38040, Pakistan.

Accepted 3 October, 2012

***Albizzia lebbeck* Benth. and *Acacia leucophloea* (Roxb.) Willd. are two very important medicinal plants growing wild in various parts of Pakistan. Various parts of both species are used by indigenous people as antimicrobial therapy however no detailed study exists regarding this activity. Crude methanolic extracts of pods, seeds, flowers and roots of *Albizzia lebbeck* Benth. and *Acacia leucophloea* (Roxb.) Willd. were tested *in vitro* for their antibacterial and antifungal activities. Antibacterial study performed against six bacterial species of both gram positive and gram negative types viz., *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Proteus mirabilis* and *Bacillus subtilis,* when compared with gentamicin and gatifloxacin, indicated that investigated plants have potent activity against all tested microorganisms. The antifungal activity of these extracts was performed against six fungal strains viz., *Aspergilus parasiticus, Aspergilus Niger, Candida albicans, Aspergillus effusus, Fusarium solani* and *Saccharomyces cerevisiae* and compared with Itraconazole and AmphoteracinB. The extracts showed significant activity against all fungal strains. The order of antibacterial and antifunagl activity, expressed as minimum inhibitory concentration (MIC) observed for both plants was seed> pod> flower > roots for all bacterial and fungal strains tested. The results authenticate their traditional use and indicate promising potential of both species to be developed as antimicrobial agents. Further work is needed for isolation, structure elucidation and characterization of bioactive constituents responsible for this activity. These natural products isolated should be screened in vivo and in vitro for antimicrobial activity and may be developed as cheap alternatives to costly synthetic antimicrobial agents available in market.**

Key words: Antibacterial, antifungal, *Albizzia lebbeck*, *Acacia leucophloea*.

INTRODUCTION

Plant kingdom is a blessed and honoured source of various bioactive constituents that has been used by people of all civilizations throughout the world to cure various ailments from flu to cancer. Since earliest, man has tagged medicinal plants with classic pharmacological capacity in the light of their curative actions. It is generally accepted that plant based medicines are better than synthetic drugs as these are much safer for man and his environment. The ascendance of much-touted synthetic drugs over phytomedicines thrived due to their fast action. However, this skewed notion is now fading due to side effects. It is now conceded that plants based medicines, have more to do with healing than arsenal of synthetic drugs. Herbal medicines have stolen the show and are being credited due to their safety, efficacy and availability from indigenous sources. Pakistan with extensive floral biodiversity (species, genetic and habitat) distributed in various biomes unquestionably is among major countries in the use of herbal drugs and the practice of traditional medicine is widespread here (Hoareau and DaSilva, 1999). About 6000 flowering plants have been reported to occur in Pakistan of which about 400 to 600 are considered to be medicinally important (Shinwari and Gilani, 2003) and 1000 species

*Corresponding author. E-mail: shakirashahid@gmail.com.

have been recognized to possess phytochemical properties (Jabeen et al., 2009).

Albizzia lebbeck Benth. locally known as shirish and *Acacia leucophloea* (Roxb.) Willd. locally known as safed kikkar, both are multipurpose trees belonging to family Leguminosae found throughout Punjab province of Pakistan. Besides their diverse and broad spectrum ethnopharmacological uses, both plants possess antimicrobial, antioxidant, anti-inflammatory and anti-cancer potential. Previously, various authors have done considerable work on indigenous flora of Pakistan (Zia-Ul-Haq et al., 2009, 2010, 2011a, b, c, d, 2012a, b, c, d, e, f); however, major portion of this green wealth is still unexplored. We have screened crude methanolic extracts of pods, flowers and roots of both plants for antimicrobial activities. The study will provide a database for these important plants which have not been explored in depth so far.

MATERIALS AND METHODS

Plant material and preparation of crude extract

Pods, seeds, flowers and roots of *A. lebbeck* Benth. and *A. leucophloea* (Roxb.) Willd. were obtained from the Department of Agronomy, Bahauddin Zakariya University, Multan, Pakistan and authenticated by Dr. Shakeel Ahmad, Assistant Professor of same department. Plant material was cleaned and soaked in methanol for 15 days with occasional shaking. It was filtered through a muslin cloth and then through a filter paper. Filtrate was evaporated on under reduced pressure to a thick, semi-solid mass. This methanolic extract of each plant was used in current experiment.

Antibacterial bioassay

Soy agar Petri plates were prepared for testing the antibacterial activity of crude alcoholic extracts by disc diffusion method (Bauer et al., 1966; Baqir et al., 1985). Diluted culture (0.1 ml) was poured on each plate and the plates were dried for 30 min at 37°C. Disc of 8 mm diameter were used and soaked with different concentration of drug solutions and standard drugs Gentamicin 20 µg, and Gatifloxacin 20 µg were used as positive control with distilled water soaked disc as negative control.

The discs were placed on plates and incubated for 24 h at 37°C. At the end of incubation period, the inhibition zones were measured. The bacterial strains used are *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Proteus mirabilis* and *Bacillus subtilis.* Minimum inhibitory concentration (MIC) was measured as described previously (Zia-Ul-Haq et al., 2011c).

Antifungal bioassay

Antifungal activity was carried out using six fungal strains, *Aspergilus parasiticus, Aspergilus niger, Candida albicans, Aspergillus effusus, Fusarium solani* and *Saccharomyces cerevisiae.* The Petri plates of sabouraud dextrose agar (SDA) were prepared and 0.1 ml of diluted culture was poured on each plate as described earlier (Bauer et al., 1966; Baqir et al., 1985; Zia-Ul-Haq et al., 2011c). The discs of 8 mm diameter (approximate) were used. The plates were incubated for 24 h at 37°C. At the end of the incubation period, the inhibition zones were observed and MIC was measured. Itraconazole 2 mg and Amphoteracin B 2 mg were used as standard drugs.

RESULTS AND DISCUSSION

Phytomedicines return traces its origin to the fact that medical elite has been convinced that synthetic drugs could not conquest of infectious disease, and if they do, these are not without side effects. Anecdotal and hoary healing potential of medicinal plants are being unearthed throughout the world. Traditional medicinal knowledge is the culmination of a long, steady march of awareness of indigenous communities towards healing by herbs. Now, major part of the current remedial dollar and Euro is being spent on plants research. Recently, a “huge environmental antibiotic pressure,” resulting from the mass production, advertising, and usage of antibiotics, has led to the increase in resistance to these antibiotics (Baquero et al,, 1997). Further high cost, adulteration and increasing toxic side effects of these synthetic antibiotic drugs coupled with their inadequacy in diseases treatment (Shariff, 2001) has fueled research momentum on natural antibiotics from medicinal plants .

Crude methanolic extracts of pods, seeds, flowers and roots of *A. lebbeck* Benth. and *A. leucophloea* (Roxb.) were tested for their efficacy against some common bacterial and fungal pathogenic strains Table 1 and 2. Interestingly, same order of antibacterial activity was observed for both plant parts tested against both gram-positive and gram-negatives strains used. The order of antibacterial activity observed for both plants was seed > pod > flower > roots for all six bacterial strains tested. The results indicate that both plants are good source of antibacterial capacity against both gram-positive and gram-negative strains namely *E. coli, S. aureus, P. aeruginosa, S. typhi, P. mirabilis* and *B. subtilis*. The order of antifungal activity observed for pods, seeds, flowers and roots of *A. lebbeck* Benth. and *A. leucophloea* (Roxb.) was pod > seed > flower > roots against *A. parasiticus, A. niger, C. albicans, A. effusus, F. solani* and *S. cerevisiae*. The results indicate that both plants are good source of antifungal capacity against common fungal pathogens.

The bark of *A. lebbeck* has been previously shown to possess antimicrobial activities against *E. coli*, *S. typhi*, *P. aeruginosa*, *S. aureus*, *Bacillus cereus*, *Klebsiella aerogenes*, *Proteus vulgaris*, *Shigella boydii*, *Aspergillus fumigatus*, *Aspergillus flavus*, *A. niger*, *C. albicans* (Dabur et al., 2007) *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella dysenteria*, *Shigella flexneri*, *C. albicans*, *Candida tropicalis* and *Candida kruse* (Uma et al., 2009). *A. lebbeck* leaves showed potent activity against *E. coli*, *S. aureus*, *P. aeruginosa* and *B. cereus* (Rahul et al., 2010). Similarly, *A. leucophloea* bark also possesses antimicrobial activities against *B. subtilis, B.*

Table 1. Antibacterial bioassay.

Drug used	*E. coli*	*S. typhi*	*S. aureus*	*P. aerugenosa*	*P. mirabilis*	*B. subtilis*
A. lebbeck pods	>9 mg	>9 mg	>10 mg	>9 mg	>9 mg	>9 mg
A. lebbeck flowers	>10 mg	>9 mg	>10 mg	>10 mg	>9 mg	>8 mg
A. lebbeck seeds	>8 mg	>8 mg	>8 mg	>8 mg	>6 mg	>8 mg
A. lebbeck roots	>14 mg	>14 mg	>14 mg	>15 mg	>13 mg	>14 mg
A. leucophloea pods	>7 mg	>7 mg	>7 mg	>7 mg	>6 mg	>7 mg
A. leucophloea flowers	>9 mg	>9 mg	>9 mg	>9 mg	>9 mg	>8 mg
A. leucophloea seeds	>6 mg	>6 mg	>6 mg	>6 mg	>6 mg	>5 mg
A. leucophloea roots	>13 mg	>13 mg	>13 mg	>13 mg	>13 mg	>13 mg
Gentamicin 20 µg	19 ± 0.17	20 ± 0.16	18 ± 0.36	18 ± 0.21	19 ± 0.11	17 ± 0.65
Gatifloxacin 20 µg	19 ± 0.77	25 ± 0.68	20 ± 0.14	16 ± 0.07	23 ± 0.39	22 ± 0.29

Table 2. Antifungal bioassay.

Drug used	*S. cerevisiae*	*A. parasiticus*	*A. effusus*	*A. niger*	*C. albicans*	*F. solani*
A. lebbeck pods	>9 mg	>9 mg	>9 mg	>9 mg	>7 mg	>9 mg
A. lebbeck flowers	>12 mg	>9 mg	>14 mg	>13 mg	>11 mg	>11 mg
A. lebbeck seeds	>10 mg	>10 mg	>11 mg	>11 mg	>10 mg	>10 mg
A. lebbeck roots	>17 mg	>17 mg	>17 mg	>19 mg	>17 mg	>18 mg
A. leucophloea pods	>8 mg	>8 mg	>8 mg	>8 mg	>7 mg	>8 mg
A. leucophloea flowers	>12 mg	>12 mg	>12 mg	>12 mg	>11 mg	>9 mg
A. leucophloea seeds	>10 mg	>9 mg	>9 mg	>9 mg	>10 mg	>9 mg
A. leucophloea roots	>16 mg	>16 mg	>16 mg	>16 mg	>16 mg	>16 mg
Itraconazole 2mg	19 ± 0.67	16 ± 0.88	16 ± 0.34	13 ± 0.71	14 ± 0.66	12 ± 0.34
AmphoteracinB 2mg	14 ± 0.91	13 ± 0.71	12 ± 0.32	12 ± 0.63	14 ± 0.54	12 ± 0.44

pneumoniae, E. coli and *S. typhi* (Anjaneyulu et al., 2010). There was a dearth of information on antimicrobial activity of seeds, flowers and roots of both species. It is hoped that present data will cover this lacuna successfully.

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