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Antimicrobial Evaluation of *Buddleja asiatica* Lour. Leaves and Flowers Extract

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ABSTRACT

This study was designed to examine the *in vitro* antimicrobial activity of methanol extract of *Buddleja asiatica* Lour. leaves and flowers. The inhibitory effects were tested against 6 bacterial (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Bacillus mycoides*) and 2 fungal (*Aspergillus niger*, *Candida albicans*) strains using disc diffusion method. The results showed that the flowers extract exhibited good antimicrobial activity as compared to the leaves extract.

Key words: *Buddleja asiatica*, antimicrobial, disc diffusion method, zone of inhibition

INTRODUCTION

Medicinal plants contain various biologically active compounds which are known to possess antimicrobial properties (Cowan, 1999). These plant based antimicrobials have been documented as good source of medicines with enormous therapeutic potential. Many reports have confirmed the potential of these antimicrobials in the prevention of some infectious diseases with no side effects, as are often related with synthetic antimicrobials (Iwu *et al.*, 1999). They are also responsible for the medicinal plants to be used as food preservatives (Sunilson *et al.*, 2009). Developing countries still depend mainly on medicinal herbs due to their cheaper cost and their effectiveness in the treatment of various infectious diseases with lesser side effects (Butkhup and Samappito, 2011; Karim *et al.*, 2011). Traditional tropical herbs could serve as good source of new safe, biodegradable and renewable drugs for the treatment of fungal or related ailments (Musyimi and Ogur, 2008).

In recent times the critical area of primary health concern is the usual causative agents that are responsible for the incidence of new and re-emerging infectious diseases. They have now been increasingly resistant to some or most existing antibiotics due to development of resistant strains (Khanahmadi *et al.*, 2010; Bonjar, 2004). Therefore, the search for new antimicrobial compounds with novel action mechanisms becomes inevitable.

B. asiatica (Buddlejaceae), commonly known as butterfly bush has been used as an abortifacient and in the treatment of skin complaints (Chopra *et al.*, 1986; Anonymous, 1993). The present study was carried out to investigate the antimicrobial potential of crude methanolic extract of leaves and flowers of *B. asiatica* Lour. occurring in Kumaun Himalayas, India, against a wide range of pathogenic bacterial and fungal strains which have not been evaluated in the previous studies.

MATERIALS AND METHODS

Plant material: The leaves and flowers of the plant were collected in the month of February, 2009 from Nainital, India and authenticated by Botanical Survey of India, Dehradun. A voucher specimen (No. 112965) was deposited in the Herbarium Section at BSI, Dehradun, India.

The leaves and flowers of the plant were shade dried, powdered and extracted with methanol using Soxhlet apparatus. After extraction the filtrate was concentrated on a rotary evaporator under vacuum at 20°C till a residual mass was obtained.

Phytochemical screening: Phytochemical screening of methanolic extract of leaves and flowers of *B. asiatica*, showed the presence of triterpenoids, steroids, flavonoids and saponins (Harborne, 1984).

Microorganisms: Three gram positive, three gram negative and two fungi were used for antimicrobial activity studies. Gram positive bacteria were *S. aureus* (MTCC 3160), *B. subtilis* (MTCC 441) and *B. mycooides* (MTCC 645). Gram negative bacteria were *E. coli* (MTCC 406), *P. aeruginosa* (MTCC 424) and *P. vulgaris* (MTCC 426). Yeast like fungi used were *C. albicans* (MTCC 227) and *A. niger* (MTCC 404). Required microorganisms were procured from Institute of Microbial Technology, Chandigarh, India.

Determination of zone of inhibition: Antimicrobial tests of selected microorganisms were carried out by disc diffusion method (Murray *et al.*, 1995). Nutrient agar plates and potato dextrose agar plates were prepared for antibacterial and antifungal activity respectively. The test solutions of the methanol extract (4000, 2000, 1000, 500, 250 µg mL⁻¹) were prepared by dissolving the extract in dimethylsulphoxide (DMSO). The plates were cooled down at 20°C and then inoculated with bacterial and fungal cultures by spreading the inoculum over the entire agar surface. The filter paper discs (5 mm in diameter, Whatman filter paper 1) were individually impregnated with 0.1 mL of the test solutions which were subsequently placed on the surface of the inoculated petri dishes. Chloramphenicol (25 µg disc⁻¹), ampicillin (25 µg disc⁻¹) and fluconazole (25 µg mL⁻¹) were used as positive controls. As a negative control, a blank disc impregnated with DMSO (20%) was used. The test discs, standard discs and blank discs were placed in petridish with a particular microorganism. The petridishes were then incubated at 37°C for 24 h for bacterial growth and at 27°C for 48 h for the growth of yeast. After 24 h of incubation, the diameter was observed for zone of inhibition (measured in mm including the disc size). Each extract was analyzed in triplicate and observed values of ZOI were then expressed as average value.

RESULTS AND DISCUSSION

Antimicrobial potential of the extracts were evaluated by measuring the diameter of zones of inhibition (mm), including the diameter of disc and the results (mm of zone of inhibition) were expressed as average values. The zone of inhibition markedly decreased on decreasing the concentration of the extracts for all the strains used for study. The results of *B. asiatica* antibacterial evaluation showed that *B. subtilis*, *B. mycooides* and *P. vulgaris* were insensitive for both leaves and flowers extract. The best antibacterial activity was shown by flowers extract against *P. aeruginosa* while leaves extract showed good activity against *S. aureus*. In case of

Table 1: Antimicrobial activity of methanol extract of *Buddleja asiatica* L. leaves and flowers

Microbes	Zone of inhibition (mm)												
	Leaves ($\mu\text{g mL}^{-1}$)					Flowers ($\mu\text{g mL}^{-1}$)					Control		
	4000	2000	1000	500	250	4000	2000	1000	500	250	AM	CP	FZ
<i>S. aureus</i>	9	7	6	-	-	10	9	8	6	5	32	12	-
<i>B. mycoides</i>	-	-	-	-	-	-	-	-	-	-	25	25	-
<i>B. subtilis</i>	-	-	-	-	-	-	-	-	-	-	20	30	-
<i>P. vulgaris</i>	-	-	-	-	-	-	-	-	-	-	16	14	-
<i>E. coli</i>	7	6	5	-	-	12	10	9	7	6	21	23	-
<i>P. aeruginosa</i>	8	7	6	-	-	14	12	9	8	6	23	10	-
<i>C. albicans</i>	9	7	-	-	-	9	8	7	6	6	-	-	15
<i>A. niger</i>	-	-	-	-	-	13	11	9	8	7	-	-	13

AM: Ampicillin ($25 \mu\text{g disc}^{-1}$), CP: Chloramphenicol ($25 \mu\text{g disc}^{-1}$), FZ: Fluconazole ($25 \mu\text{g mL}^{-1}$)

antifungal activity, leaves extract was found inactive against *A. niger* while flowers extract showed very good activity. *C. albicans* was found sensitive for both the extracts. Antimicrobial activity of methanolic extract of leaves and flowers of *B. asiatica* could be due to the presence of triterpenoids, steroids, polyphenols, flavonoids (Taleb-Contini *et al.*, 2003; Jain *et al.*, 2001; Okoro *et al.*, 2010). The antibacterial activity of flavonoids and polyphenolic compounds might be due to their ability to complex with bacterial cell wall and therefore, inhibiting the microbial growth (Sivapriya *et al.*, 2011).

The result of screened plant extracts for antibacterial and antifungal activity is summarized in Table 1. The results clearly indicate that the antibacterial and antifungal activity vary with the type of tested microbes. This is based upon the fact that antimicrobial activity of plant extracts depends on the species of the plant and the type of tested microorganisms (Obeidat *et al.*, 2012). The present study could be used for further investigation on this plant to find the role in the total remedies of a wide range of microbial diseases of plants and animals. The studied plant flowers may be a good source of future drugs that could be used in the treatment of infections caused by these microbes.

CONCLUSION

From the results, it can be concluded that both the extracts were able to inhibit the growth of the investigated bacterial strains; the most susceptible bacteria was *P. aeruginosa* while resistant were *B. subtilis*, *B. mycoides* and *P. vulgaris*. Since *B. asiatica* flowers demonstrated good activity against the antibiotic low susceptibility pathogen, *P. aeruginosa*, the plant can be used in the treatment of infections caused by these microbes.

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