JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2008. Vol. **2**(2), p. 603-606

Antimicrobial Screening and Phytochemical Analysis of *Blumea oblique*

Aruna Bhagat[1], Aqueel Khan[1] and Gopal Gond[2]

[1]PGTD Biochemistry, RTM University Nagpur, L.I.T. Premises Nagpur - 440 033, India.
[3]Guru Nanak College of Science, Ballarpur - 442 701 (India)

(Received: 06 April 2008; accepted: 15 August 2008)

Whole plant of *Blumea oblique* was studied for antimicrobial properties and phytochemical analysis. Aqueous extraction of whole plant was done for preliminary antimicrobial screening. Soxhlet extracts of plant were made in petroleum-ether, chloroform, acetone and methanol and tested against microorganisms. Study found that the aqueous extracts and soxhlet methanolic extracts showed antimicrobial activity. Comparatively higher activity was observed in methanolic extracts. Agar well diffusion technique was used to find out antimicrobial activity of whole plant extracts. Minimum inhibitory concentration (MIC) results confirm bacteriosidal and bacteriostatic activity at low and high dilution rate. Phytochemical analysis and various confirmatory tests have revealed the presence of alkaloids, cardiac glycosides, steroids and flavonoids. The present study found that bacteriosidal and bacteriostatic activity in *Blumea oblique* thus, the presnt study suggests that this plant is used as an antimicrobial agent.

Key words: *Blumea oblique*, antimicrobial property, MIC, Agar well diffusion, phytochemical analysis.

The genus Blumea belongs to the family Asteraceae and is placed in tribe Inuleae. This genus consists of sixteen species out of which only eleven are found in India and seven are found in Pakistan. The plant is 3-45 cm tall; stem erect, much branched, and terete, covered with spreading pilose hairs. Leaves are 0.5-6x0.3-2.5 cm.

The sources of Indian medicine are derived from 'Rigveda' and 'Ayurveda'. They are mainly based on the use of drugs of plant origin. The ayurvedic system of medicine is mainly attributed the Charaka and Sushruta, who cited about 700 medicinal plants. The Muslim rulers introduced their traditional system of medicine in India and incorporated it in the native ayurvedic medicine. This mixture is known as Unani medicine or eastern medicine. Higher plants have been the source of medicinal agents since earliest times, and today they continue to play a dominant role in the primary health care of about 80 % of the world population.

* To whom all correspondence should be addressed.

The extract of *Blumea oblique* leaves is considered to be insect repellent. Many plants of genus blumea are used in folk medicine for the treatment of various ailments. The leaves of genus Blumea are considered to have stomachic, expectorant, antispasmodic and sudorific properties. The powder of roots of *B. balsanifera* is considered as a stomachic and that of the bark ground and mixed with whey is a valuable remedy for piles. Some of its preparation is used as tonic. Mostly the medicinal properties of the plants mainly antimicrobial due to the presence of secondary metabolites like tannins, alkaloids, Saponins, cardiac glucosides, steroid and flavonoids. In view of this genus blumea like *B. oblique* are studied for quality and quantity of these bioactive classes. Therefore, present paper deals with the screening of antimicrobial properties and phytochemical analysis of this species.

MATERIAL AND METHODS

Plant collection

The plant was collected from Vidarbha region. Whole plant were brought to the laboratory and identified by an expert taxonomist in RTM university and washed thoroughly under running tap water and 70 % alcohol to free them from dust and other contaminant particles.

Methods of extraction

Soxhlet extracts were prepared in petroleum ether, chloroform, acetone and methanol.

Aqueous extraction

The fresh whole plants were used to prepare aqueous extracts. 10 gms of plant material were crushed in a mortar and pestle and kept for extraction. It was then filtered and stored in an airtight glass bottles.

Soxhlet extraction

The whole plant dried, powdered and sieved through the muslin cloth. These powders were used to prepare extracts by soxhlet method, using 10 gm of fine powder placed in thimble. The extract was then stored in an airtight glass bottles.

Test microorganisms and media

Gram positive and gram negative microorganisms were used. [*E. coli* (NCIM-2064), *B. subtilis* (NCIM-2439), *S. aureus* (NICM-2079), P. vulgaris (NICM-2027), *K. pneumonia* (NCIM-2018), *P. aeruginosa* (NCIM-2036)]. Nutrient agar was the media used for culturing the microorganisms.

Inoculum preparation

A bacterial inoculum was prepared by inoculating a loop full of the test organism into a 3 ml sterile nutrient broth tube and incubated at 37ºc for over night. The turbidity was matched with 0.5 mc Farlands Nephelometer standard. Dilutions to the tube were done with sterile nutrient broth to get an all density corresponding to 6 x 108 CFU/ml.

Antibacterial susceptibility test

A suspension (0.1ml) of the test organism from the 18 hrs cultures were thoroughly mixed with 20 ml sterile miller Hintong agar maintained at 45 to 50ºC. The seeded M.H. agar is poured in pre sterilized Petri dishes. Set aside after solidification the seeded agar was punched with a sterilized cork borer (10mm) in order to obtain a well of 10 mm diameter in center of the Petri plate. 100 ul of the aqueous plant extract is loaded in to the well accurately with a micropipette. The Petri plate kept in refrigerator for 30 minutes and then at room temperature for 30 minutes, which facilitated diffusion of the plant extract. The Petri plates were then incubated at 37ºC for 24 hrs. The zone of inhibition was measured with Hi-media antibiotic zone scale (PW096).

Minimum inhibitory concentration

The determination of minimum inhibitory concentration was done by agar dilution method (NCC LS, 1990). Stock solution of 100 mg/ml of methanolic extract of selected plant was prepared in DMSO-Tris buffer (3:7) 100-300 ul. Of this stock solution was added to achieve final concentration 0.5 mg/ml, 1 mg/ml, 2 mg/ml, 5 mg/ml, 8 mg/ml, 10 mg/ml. and 15 mg/ml. These were poured in Petri dishes and allowed to solidify. The reverse side of the plate was divided into checker board blocks by glass marker to accommodate bacterial culture. A bacterial innoculum of all the test organisms was prepared as discussed in antibacterial testing section. All the plates including control plate without plant extract were spot inoculate with 10 ul. Of bacterial innoculum using sterile micropipette. The Petri

plates were incubated at 37ºc for 24 hours. The result was read as presence or absence of bacterial growth complete suppression of growth was required for an extract to be declared active. MIC was determined as the least concentration of extract inhibiting the growth of the test organisms.

RESULTS AND DISCUSSION

According to zone of inhibition, activity against test microorganisms showed in Table 1. In aqueous extract showed moderate activity against *E. coli.* The zone of inhibition was found to be 13 mm. Table showed in soxhlet extract activity against test microorganisms except petroleum ether. Overall, methanol extract showed better results than other soxhlet extract solvent. Amongst test microorganisms E.coli showed higher activity in methanol extract that is 19 mm.

Rest of the test microorganisms exhibited mild zones of inhibition

Minimum inhibitory concentration results were obtained by agar dilution method. Various concentrations made from stock solution and observed growth of test microorganisms results shown in Table 2. Antibacterial agents with low activity against an organism have high MIC value while highly active antibacterial agent gives a low MIC value. *E.coli* has low MIC value means this extracts highly active against *E.coli*. Low antibacterial activity found against *B. subtilis*.

Grade wise representation results on the phytochemical analysis of *B. oblique* showed in Table 3. All bioactive principle showed phytochemical results except Saponins. Cardiac glucosides indicate three plus phytochemical result as compare to Tannins one plus means Cardiac glucosides having rich phytochemical results.

Table 1. Zone of inhibition according to effects of extracts on the test microorganisms

Plant Parts	Extracts	Zone of Inhibition (mm)					
		E. coli	*B. subtilis*	*S.aureus*	*P. vulgaris*	*K. pneumonia*	*P. aeroginosa*
Whole Plant WholePlant	Aqueous	13	10	10	07	11	12
Soxhlet extract	Petroleum ether	-	-	-	-	-	-
	Chloroform	12	-	-	-	-	-
	Acetone	-	11	12	-	-	
	Methanol	19	14	17	13	15	15

Table 2. Minimum inhibitory concentration results among test microorganisms in methanolic extracts

Extracts	Test Microorganisms	MIC results in mg/ml.
Methanol	*E. coli*	1.0
	B. subtilis	10.0
	S. aureus	2.5
	P. vulgaris	5.0
	K. pneumonia	5.0
	P. aeroginosa	8.0

Table 3. Results of bioactive principles in *Blumea oblique* after phytochemical analysis

Bioactive principle	Phytochemical Results
Tannins	+
Alkaloids	++
Saponins	-
Cardiac glucosides	+++
Steroid	++
Flavonoids	+++

CONCLUSION

Overall Soxhlet methanol extracts gave better zones of inhibition against test microorganisms. *Blumea oblique* showed higher antimicrobial properties in methanol extract. The most susceptible organism was found to be *E. coli* followed by *S. aureus* and other. The ongoing results on the phytochemical analysis of *B. oblique* showed presence of bioactive principles indicating medicinal nature of the plant. The quality and quantity of these bioactive classes of secondary metabolites together are responsible for the medicinal properties of the plant. Thus the present study found that medicinal properties in *Blumea oblique* against several diseases and used as a drug.

REFERENCES

1. Westh H., Zinn C. S., Rosdahl V. T. An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. *Microb Drug Resist.,* 2004; **10**: 169- 176.
2. Bandow J.E., Brotz H. Leichert LIO *et al.* Proteomics approach to understanding antibiotic action. *Antimicrob Agent Chemother.,* 2003; **47**: 948-955.
3. Rojas R, Bustamante B, Bauer J *et al.* Antimicrobial activity of selected Peruvian medicinal plants. *J Ethnopharmacol.,* 2004; **88**: 199-204.
4. Benkeblia N, Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (*Allium sativum*). *Lebensm-Wiss u-Technol.,* 2004; **37**: 263-268.
5. Colombo ML.,Bosisio E., Pharmacological activities of *Chelldonium majus* L (Papaveraceae), *Phamacol Res.,* 1996; **33**: 127 -134.
6. lwu MW, Duncan AR, Okunji CO, New antimicrobials of plant origin. In: Janick J.ed Perspectives on new crops and new uses. Alexandria, VA: ASHS Press; 1999; 457-462.
7. Martins AP, Salgueiro L, Goncalves MJ *et al.* Essential oil composition and antimicrobial activity of three Zingiberaceae from S. Tome e Principle. *Planta Med.,* 2001; **67**: 580-584.
8. Krishnaraju AV, Rao TVN, Sundararju D *et al,* Assessment of bioactivity of Indian medicinal plants using Brine shrimp (*Artemia sallna*) lethality assay. *Int J Appl Sci Eng.,* 2005; **2**: 125- 134.
9. Balandrin MF, Kjocke AJ, Wurtele E et al. Natural plant chemicals; source of industrial and mechanical materials. *Science.* 1985; **228**: 1154-1160.