Journal of Experimental Sciences Vol. 2, Issue 2, Pages 43-46 [2011]
www.jexpsciences.com

Regular Article

Studies on Antibacterial Activity of *Asterella angusta* (Steph.) Kachroo. against some Pathogenic Bacteria

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ABSTRACT: The *In vitro* antibacterial activity of whole thallus of *Asteralla angusta* and its fractions petroleum ether, benzene, acetone, methanol, ethanol, and aqueous extracts were tested against the growth of four human pathogenic gram negative bacteria namely *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonie* and two gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* using agar well diffusion technique. The plant showed significant antibacterial activity against all the organisms. The maximum antibacterial activity was observed in ethanolic extract against *Pseudomonas aeruginosa* and minimum activity was observed in petroleum ether extract against *Proteus mirabilis.* The phytochemical analysis of the extract indicated the presence of saponin, flavonoids, and carbohydrates. The inhibitory effect of the extract was compared with standard antibiotics, streptomycin.

Key words: *Asteralla angusta*, Phytochemical analysis, Antibacterial activity

Introduction

In recent times, the search for potent antibacterial agents has been shifted to plants. However, the major part of the search has focused mainly on higher plants (Oyagade 1998) while little attention is given to lower plants with possible antimicrobial properties. Most plants are medicinally useful in treating disease in the body and in most cases; the antimicrobial efficacy value attributed to some plants is beyond belief. Conservation estimates suggest that about 10% of all flowering plants on earth have at one time, been used by local communities throughout the world but only 1% have gained recognition by modern scientists. (Kafaru, 1994) Liverwort, mosses, hornwort are small low growing plants, which constitute the phylum bryophyte. They lack true stem, leaves and roots and modify their microclimate by conserving moisture, checking soil erosion on lilly slopes and serve as seed bed for forest cover. They are now increasingly used for diverse purpose including pollution control and as new source of pharmaceuticals (Saxena and Saxena, 2002) Liverworts and mosses have been tested and used as fuel in developed countries like Finland, Sweden, Ireland, Germany, Poland and the Soviet Union (Baker and Hawkinson, 1993). Chinese traditional medicine named 40 kinds of bryophytes used to treat cardiovascular diseases, tonsillitis, bronchitis, cystitis and skin infections. It has also been shown that an extract of *Giganteum* can increase arota blood transport by up to 30% in animals (Jorgnersteen, 1998).

Materials and Methods

Collection of plant material

Asterella angusta was collected from Achalgarh temple (Alt.1350 m) of Mount Abu district of Sirohi, Rajasthan, in the month of September 2006-07. The plants were identified and voucher specimens have been deposited in Bryology Laboratory, Dept. of Botany, Univ. College of science, Udaipur for future reference.

Phytochemical screening and extraction of plant material

Plant extracts were prepared by cold extraction method (Harborne, 1998). The plant material was carefully cleanup from attached litter and dead material under running tap water and finally with sterile distilled water. Air-dried and powdered (about 20 g) plant material of *Asterella angusta* was extracted by cold percolation in Petroleum ether, benzene, acetone, methanol, ethanol and about 200ml autoclaved water for aqueous extract. The extract were decanted, filtered with whatman No.1 filter paper and concentrated at reduced pressure below 40ºc through rota vapour and lyophilized (Buchi, Labconco, US) to obtain dry extract. The crude extracts were taken up for biological screening and also to observe the presence and absence of different phytochemical constituents.viz. Alkaloids (Dragendorff's test), saponins (foam formation), flavonoids (using magnesium (Mn) and dil.HCl), terpenes (Liebermann-Burchard's test) according to standard methods (Sofowora, 1982, Trease and Evans, 1987).

Test microorganisms

In the present study test microorganisms used for the antimicrobial screening were two gram negative human pathogenic bacteria *viz. Escherichia coli* (Castellani and Chalmers)MTCC-41, *Pseudomonas aeruginosa* (Schroter)MTCC-424, two gram positive bacteria *Bacillus subtilis* (Ehrenberg) Cohn. MTCC-441 and *Staphylococcus aureus* (Rosenbach) MTCC-740 were procured from Institute of Microbial Technology (IMTECH-CSIR), Chandigarh, India. *Klebsiella pneumoniae* (Schroeter 1886), *Proteus mirabilis* clinical isolates were obtained from Department of Pathology RNT Medical College. Udaipur, Rajasthan India.

Preperation of extract of different concentration

10 mg of crude extract was dissolved in 10 ml of N,N – Dimethyle formamide DMF (CDH, India) to prepare stock solution of 1000µg / ml. Extract of different concentration (1000µg/ml to 50 µg/ml) were prepared in aseptic condition. DMF as used concentration in the test did not interfere with the microbial growth.

Assay of antimicrobial activity of crude extract

Antibacterial activity was observed by Agar well assay method (Murray et al 1995).

Agar well assay method

In this method, 20 ml nutrient agar medium was poured in sterilized petri plates (100 X15 mm) and allowed to solidify at room temperature. 24 h broth culture of test bacteria was used as inoculum under sterile conditions. The freshly prepared 100µl or 0.1ml (1×10^6 cells/ml) of organisms was set to 0.5 optical density spread with a sterile L shaped bent glass rod. Using cork borer several wells of 6mm in diameter were punched. To each well 100µl extract was poured. The plates were incubated under optimum growth condition for various organisms. Inhibition zone was measured with zone scale of 1mm or more was considered positive inhibition.

Result and Discussion

Phytochemical analysis of extracts revealed the presence of saponin, flavonoids, and carbohydrates while alkaloids, terpenoids were absent (Table no.1). The antibacterial activity tested against six bacterial strains such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus mirabilis* by agar well assay method. The bioactivities measured in terms of zone of inhibition exhibited by the different extracts against the respective bacterial strains are summarized mentioned in following tables. All the extracts

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(petroleum ether, benzene, acetone, methanol, ethanol and aqueous) showed activity against the tested bacteria. The ethanolic extract showed maximum zone of inhibition against *Pseudomonas aeruginosa* (Table no.2, fig: 4) and minimum inhibition zone observed in petroleum ether extract of this plant against *Proteus mirabilis*.(Table.2,fig:6).

Table: 1 Phytochemical constituents of *Asterella angusta.*

Phytochemical test	**Result**
Saponin	+
Flavonoids	+
Alkaloides	-
Terpenoids	-
Carbohydrates	+

(+)Presence, (-) Absence

Table 2:- Results of antibacterial activity of *Asterella angusta* by agar well diffusion method against some pathogenic bacteria

Microorganisms	Extracts	Different Concentration of the Plant extracts (µg/ml)				
		65	125	250	500	1000
Bacillus subitilis	Petroleum ether	3.50	4.80	5.00	6.50	7.00
	Benzene	3.00	3.50	4.80	5.50	6.00
	Acetone	4.00	4.50	5.00	5.80	6.20
	Ethanol	10.2	11.2	13.5	16.5	18.0
	Methanol	6.80	7.20	8.50	10.5	12.0
	Aqueous	9.50	11.0	12.2	13.5	15.0
	Control	6.00	9.00	12.0	18.0	24.0
Staphylococcus aureus	Petroleum ether	3.60	4.00	5.50	6.80	8.00
	Benzene	4.50	5.80	7.20	9.00	10.5
	Acetone	4.80	6.00	6.50	7.00	8.20
	Ethanol	8.20	10.5	12.0	13.5	15.0
	Methanol	7.50	9.00	10.8	11.0	12.5
	Aqueous	5.50	6.00	7.50	8.00	8.00
	Control	8.00	10.0	15.0	19.0	22.0
Escherichia coli	Petroleum ether	4.00	4.80	6.50	7.00	7.50
	Benzene	5.50	6.00	6.80	7.20	8.50
	Acetone	6.20	6.80	7.00	7.50	8.90
	Ethanol	8.30	13.0	14.6	15.0	16.0
	Methanol	7.50	7.90	8.50	8.80	10.0
	Aqueous	6.80	7.00	7.20	7.50	7.90
	Control	7.00	10.0	15.0	19.0	23.0
Pseudomonas aeruginosa	Petroleum ether	2.50	2.50	3.60	5.50	7.00
	Benzene	3.00	4.50	6.50	7.20	8.60
	Acetone	3.20	5.50	8.60	10.5	12.0
	Ethanol	6.00	8.00	12.0	16.0	25.0
	Methanol	4.00	7.00	11.0	14.0	16.0
	Aqueous	2.00	3.50	5.00	7.00	10.0
	Control	7.00	9.00	14.0	17.0	20.0
Klebsiella pneumoniae	Petroleum ether	3.80	5.00	5.50	6.50	6.80
	Benzene	2.50	5.50	7.20	7.50	7.00
	Acetone	4.00	4.30	5.20	5.60	5.80
	Ethanol	5.50	5.90	6.20	6.80	7.30

Microorganisms	Extracts	Different Concentration of the Plant extracts (µg/ml)				
		65	125	250	500	1000
Proteus mirabilis	Methanol	5.60	5.90	6.50	7.50	8.00
	Aqueous	5.00	5.30	5.50	5.80	6.20
	Control	5.00	9.00	13.0	15.0	17.0
	Petroleum ether	1.50	2.50	3.80	4.50	5.30
	Benzene	3.50	5.20	5.60	5.90	6.20
	Acetone	4.00	6.00	9.00	10.5	13.2
	Ethanol	5.50	5.80	6.30	7.00	7.50
	Methanol	7.00	7.50	8.00	8.40	8.80
	Aqueous	4.40	4.90	5.50	5.80	6.30
	Control	5.00	8.00	12.0	16.0	19.0

Values of zone of Inhibition excluded cup borer diameter (6.00) in mm and are mean of three replicates

Fig. 1: Antibacterial activity of *Asterella angusta* against *B. subtilis*

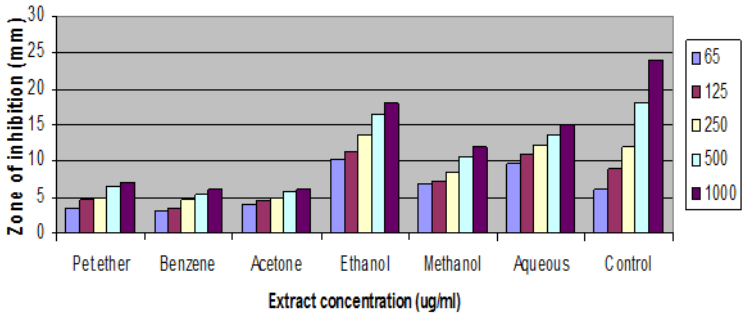


Fig. 2: Antibacterial activity of *Asterella angusta* against *S. aureus*

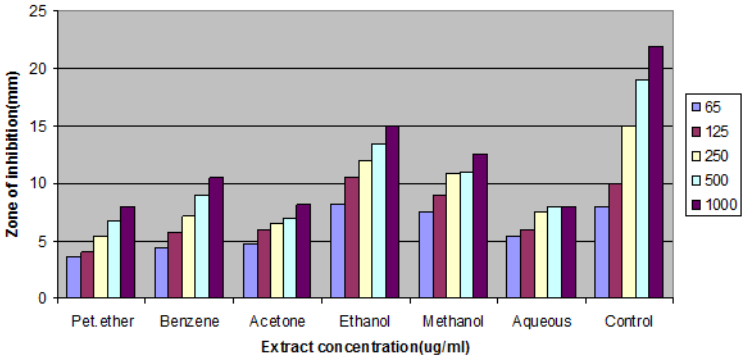


Fig. 3: Antibacterial activity of *Asterella angusta* against *E. coli*

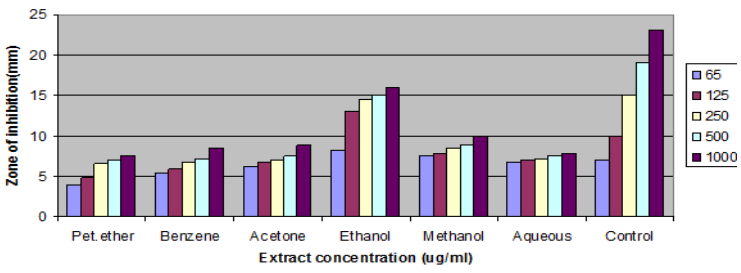


Fig. 4: Antibacterial activity of *Asterella angusta* against *P. aeruginosa*

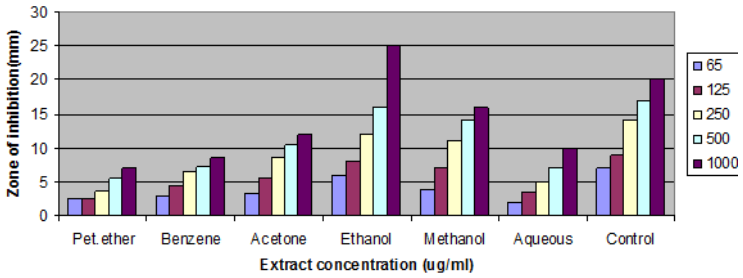


Fig. 5: Antibacterial activity of *Asterella angusta* against *K. pneumonia*

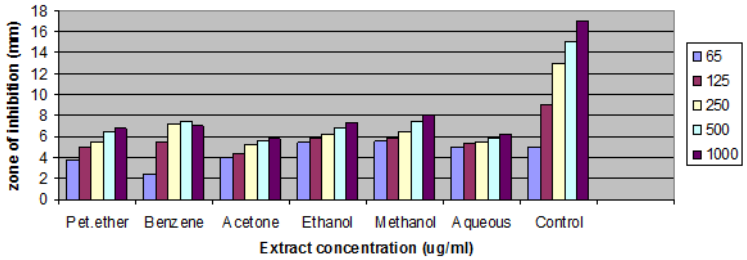
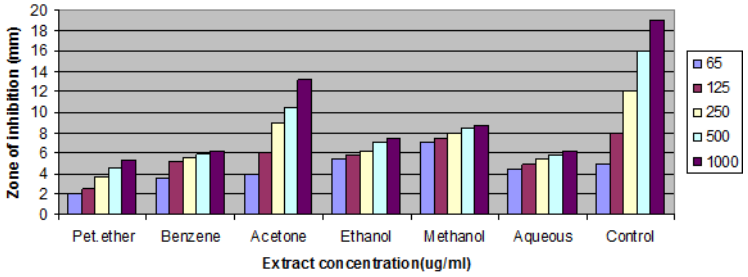


Fig. 6: Antibacterial activity of *Asterella angusta* against *P. mirabilis*



Acknowledgement

The author is thankful to Prof. B. L. Chaudhary, Supervisor, Prof. S. S. Katewa, Head Department of Botany, University College of Science, Mohan Lal Sukhadia University, Udaipur for their nice cooperation and help during research work. One of us (Rubina khanam), thankful to University Grant Commission, New Delhi, for providing financial support during the tenure of research work.

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