NBU Journal of
Plant Sciences

Vol. 5, No.1 March 2011 p. 85-87
ISSN No. 0974 6927

Phytochemical screening and antibacterial activity of *Plagiochasma appendiculatum* Lehm. et Lindenb against pathogenic bacteria

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Abstract
The *in vitro* antibacterial activity of whole thallus of *Plagiochasma appendiculatum* 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 pneumoniae* and two gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* using agar well diffusion technique. The plant showed significant antibacterial activity against almost all the organisms. The maximum antibacterial activity was observed in methanolic extract against *Escherichia coli* and minimum activity was observed in petroleum ether extract against *Staphylococcus aureus*. The phytochemical analysis of the extract indicated the presence of saponin, flavonoids, and sesquiterpenes. The inhibitory effect of the extract was compared with standard antibiotics, streptomycin.

Keywords: Antibacterial activity, *Plagiochasma appendiculatum*, Agar well assay, crude extract

The search for plants with antimicrobial activity has gained increasing importance in recent years, due to a growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganism (Anhut, *et al* 1984). Numerous studies have been conducted with the extracts of various plants, screening antimicrobial compounds (Abu-Shanab *et al.* 2004; Basile, *et al.* 1988).In many developing countries, about 80% of available therapeutic substances are obtained from medicinal plants. Since the most infectious diseases are of a microbiological origin, with the advent of ever-increasing resistant bacterial strains, there has been a corresponding rise in the universal demand for natural antimicrobial therapeutics (Basile, *et al.* 1998).Bryophytes, which are phylogenitically placed between vascular plants and algae, from a unique division in the plant kingdom (Basile, *et al.* 1999).There exist more than 22,000 members of the Mosses (Bryophyta) in the world. This figure represents 5.5% of the 400,000 plant types spread throughout the world (Castaldo Cobianchi, *et al.* 1988). The simplest land plants,isoflavonoids,flavonoids,and bioflavonoid have been reported to be possible chemical barriers against microorganisms(Cowan, *et al.* 1999; Freitag *et al.* 1986; Hahn, *et al.* 1995; Harborne, *et al.* 1998; MatsuoA, *et al.* 1991).Terpenoids, phenolic and volatile constituents have also been investigated in some bryophytes (Castaldo Cobianchi, *et al.* 1988; Murray, *et al.* 1995).

The use of bryophytes as medical plants in China, Europe, and North America has been documented in the literature. Some species of *Fissidens* and *Polytrichum* were utilized as diuretic and hair growth stimulating drugs in China more than 400 years ago. North American Indians used *Bryum, Mnium, Philonotis spp.*, and *Polytrichm juniperinum* to heal burns, bruises, and wounds. In France, *Marchantia polymorpha* was used to enhance diuresis (Basile, *et al.* 1998).There is also evidence in the literature that confirms the antibiotic activity of bryophytes against fungi and prokaryotic cells (Saritas, *et al.* 2001).Among the biologically active compounds extracted from bryophytes, some has shown antitumoral activity (Salvat, *et al.* 2004).

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Materials and methods

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 *Plagiochasma appendiculatum* 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 40°c through rota vapour and lyophilized (Buchi, Laboonco, 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* (Hauser 1885) clinical isolates were obtained from Department of Pathology RNT Medical College. Udaipur, Rajasthan India.

Table: 1 Phytochemical constituents of *Plagiochasma appendiculatum*

Phytochemical test	Result
Saponin	+
Flavonoids	+
sesquiterpenes	+
Alkaloides	-
Steroids	-

(+)Presence, (-) Absence

Preparation of extract of different concentration

10 mg of crude extract was dissolved in 10 ml of N,N – Dimethyl 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

Table 2: Results of antibacterial activity of *Plagiochasma appendiculatum* by agar well diffusion method against some pathogenic bacteria

Microorganisms	Extracts	Different Concentration of the Plant extracts (µg/ml)				
		50	100	250	500	1000
Bacillus subtilis	Petroleum ether	3.00	5.00	6.50	7.00	8.80
	Benzene	2.50	5.00	8.00	10.0	13.0
	Acetone	2.80	6.50	7.00	12.0	16.0
	Ethanol	2.50	5.30	6.50	10.0	13.0
	Methanol	3.00	4.00	8.50	13.0	15.0
	Aqueous	2.50	4.50	5.00	8.00	10.5
	Control	6.00	9.00	12.0	18.0	24.0
Staphylococcus aureus	Petroleum ether	2.50	3.80	5.00	6.50	8.00
	Benzene	3.00	4.50	5.80	7.00	9.00
	Acetone	2.00	4.00	6.00	8.00	10.5
	Ethanol	3.00	5.00	7.00	9.00	12.0
	Methanol	3.50	5.50	8.10	12.0	15.5
	Aqueous	2.30	3.00	5.00	7.00	10.0
	Control	8.00	10.0	15.0	19.0	22.0
Escherichia coli	Petroleum ether	3.00	4.50	6.50	7.00	8.50
	Benzene	3.20	5.50	8.50	12.5	13.0
	Acetone	2.00	5.00	7.00	10.0	12.5
	Ethanol	4.00	7.00	9.50	11.0	15.0
	Methanol	6.50	9.00	15.0	18.0	20.0
	Aqueous	3.50	6.00	8.50	10.0	11.5
	Control	7.00	10.0	15.0	19.0	23.0
Pseudomonas aeruginosa	Petroleum ether	2.80	4.00	5.50	6.50	8.50
	Benzene	3.40	5.00	7.00	10.0	13.0
	Acetone	3.50	5.20	6.50	8.00	11.5
	Ethanol	4.00	5.50	7.30	10.2	12.0
	Methanol	5.00	8.00	11.0	13.0	16.0
	Aqueous	3.50	4.00	5.50	7.50	9.50
	Control	7.00	9.00	14.0	17.0	20.0
Klebsiella pneumoniae	Petroleum ether	2.00	2.20	5.00	6.50	8.00
	Benzene	2.00	2.50	6.00	7.00	10.0
	Acetone	3.50	5.20	5.50	6.50	8.00
	Ethanol	3.00	5.00	8.50	10.0	12.5
	Methanol	5.00	6.80	8.00	12.6	13.5
	Aqueous	2.50	3.80	5.00	8.50	10.3
	Control	5.00	9.00	13.0	15.0	17.0
Proteus mirabilis	Petroleum ether	1.50	2.00	2.30	5.00	6.20
	Benzene	2.00	5.00	6.50	8.00	11.5
	Acetone	3.50	6.00	8.00	9.50	12.0
	Ethanol	4.00	6.00	9.20	10.5	12.5
	Methanol	6.00	8.50	10.0	11.8	13.5
	Aqueous	1.80	2.20	3.50	4.20	4.50
	Control	5.00	8.00	12.0	16.0	19.0

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

Preliminary phytochemical screening of the plant showed the presence of saponin, and flavonoids, sesquiterpenes in *P. appendiculatum* while steroids and alkaloids were absent (Table1).The plant showed significant antibacterial activity against almost all the bacteria. Results of the present investigations reveal the antibacterial activity antibacterial nature of extract of different solvents of *Plagiochasma appendiculatum*. The plant was extracted using both aqueous and organic solvents (Table 2). Data obtained demonstrates the antibacterial activity of plant depends largely upon the types of solvent used for extraction. The plant showed significant antibacterial activity against almost all the organisms. The maximum antibacterial activity was observed in methanolic extract against *Escherichia coli* and minimum activity was observed in petroleum ether extract against *Staphylococcus aureus*.

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