Int J Pharm Bio Sci 2015 July; 6(3): (B) 1289 - 1294

Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

IN VITRO ANTIBACTERIAL ACTIVITY OF HIGH ALTITUDE BRYOPHYTES AGAINST DRUG RESISTANT BACTERIAL PATHOGENS

PRASANTH G. WILLIAMS[1], DULIP DANIELS A. E.[1], BRIJITH LAL N. D[2], BHAGYA M.[2] AND G. PRAKASH WILLIAMS [*2]

[1.] Research Department of Botany, Scott Christian College (Autonomous), Nagercoil-3
[2.] P.G.Department of Botany and Biotechnology, Bishop Moore College, Mavelikara- 690110;

ABSTRACT

Resistance to drugs is a global concern. There is a continuous and an urgent need to discover new antimicrobial compounds from medicinal plants. Plant products provide unlimited opportunities for newdrug leads because of the unmatched availability of chemical diversity. The lower plant forms like bryophytes are one of the potential sources against new and re-emerging drug resistant infectious diseases. In this study, the collecetdepiphytic bryophytes*Homaliodendronrectifolium*(Mitt.) Fleisch*, Aerobrydiumfilamentosum*(Hook.) Fleisch*, Garckeaphascoides*(Hook.) C.Muellfrom high altitude area were tested against drug resistant bacterial pathogens. They were extracted by the percolation method using various solvents like acetone, ethyl acetate and distilled water. The antimicrobial activity was evaluated against the different drug resistant pathogenic bacterial strains using agar well diffusion method. The bryophyte extracts showed remarkable inhibitory activity against bacterial pathogens. The extracts were further screened for phytochemical studies and they revealed the presence of alkaloids, flavonoids, phenol, proteins and amino acids, resins, saponins, steroids, tannins, xanthoproteins and sugars. The antimicrobial activities of bryophytes weredue to the presence of various secondary metabolites, which may enhance at the high altitudes.

KEYWORDS: Antibacterial activity, Bryophytes, drug resistance, high altitude bryophytes, phytochemical screening*etc*.

***Corresponding author**

G. PRAKASH WILLIAMS
***P*.G.Department of Botany and Biotechnology,**
Bishop Moore College, Mavelikara- 690110

INTRODUCTION

Drug resistance is one of the biggest challenges to face global public health at the beginning of the third millennium because it leads to the re-emergence of new diseases and many death[1]. Eventhough it was first described in 1961, methicillin-resistant strains of *Staphylococcus aureus*quickly proved to be ablepotentialpathogens[2,3]. During the last 30 years, the epidemiology of methicillin-resistant staphylococci has shifted from that of a primarily nosocomial organism acquired in tertiary care centers[4]. The development of novel antimicrobial agents with activity against pathogens that have become resistant to currently available agents is one tactics for combating resistant organisms[5]. Therefore the rapid propagation in antibiotic resistance and the increasing interest in natural products have placed medicinal plants back in the front lights as a reliable source for the discovery of active anti-microbial agents and possibly even novel classes of antibiotics[6]. The bryophytes are recognized as the basal or first diversity lineage of the land plants which includes morphologically and biochemically diverse groups like liverworts, hornworts and mosses[7,8]. They may expect interesting bioactivities[9,10,11]. Traditional medicinal use was started around 400 years back in China[7]. According to Umadevi*et al.*[12], plants growing at high altitudes are subjected to a variety of stressful environments and hence they may produce a spectrum of secondary metabolites. So in keeping view of all these this study was made on the antibacterial activity and phytochemical analysis of bryophytes against drug resistant bacterial pathogens. A scientific study on their antimicrobial potential against drug resistant microorganisms is not been reported much. The epiphytic mosses, *Homaliodendronrectifolium*(Mitt.) Fleisch*, Aerobrydiumfilamentosum*(Hook.)Fleisch*, Garckeaphascoides*(Hook.) C.Muellwere collected from the highest peak region of the Nilgirihills(blue mountains), the Doddabetta Peak, 4 km east southeast from Udhagamandalam, 11°24′10″N 76°44′14″E, which are a range of mountains forming a part of the Western Ghats. There are at least 24 peaks above 2,000 metres (6,600 ft) in the Nilgiriswhich make the southwestern edge of the Deccan Plateau. The Doddabetta Peak is the highest point with a height of 2,637 metres (8,652 ft) in the Nilgiris and the southern extent of the range.

MATERIALS AND METHODS

(i) Collection of bryophytes

The epiphytic mosses were collectedfrom the Doddabetta region of Nilgiri Hills,the Nilgiris District, Tamilnadu.The specimens were brought to the laboratory and identified in the Botany Laboratory and specimen was depositedon the herbarium, PG. Department of Bishop Moore College, Mavelikara (BMC/BBT-15-07; BMC/BBT-15-08; BMC/BBT-15- 09 respectively). Collected samples were identified by using “Gangulee’sMosess of Eastern India and adjacent regions”(1969-1980). The identification was mainly based on habit, morphology, leaf structure, presence or absence of costa, pattern of cells in base, middle and terminal portion, leaf margin, arrangement of chloroplast, distribution *etc.* Using this features collected,they were identified as *Homaliodendronrectifolium*(Mitt.) Fleisch*, Aerobrydiumfilamentosum* (Hook.) Fleisch*, Garckeaphascoides*(Hook.) C.Muell*.*

(ii) Extract preparation

The bryophytes collected were separately washed with distilled water to remove the adhering soil or extraneous dust particles. The shade dried plants were further ground into a fine powder. Organic solvents such as 95 % (v/v) acetone, 95 % (v/v) Ethyl acetate (Nice, Cochin) and distilled water were employed for the extraction of different bioactive principles. In this study, cold extraction (percolation) was done. Powdered plants (50 g) were extracted with 100 ml of respective solvents for 96 h at room temperature.The crude extract can be

prepared by filtering the extracts with Whatman filter paper No. 1 followed by evaporating the solvent in open air.Then the extract was collected and stored at 4^{o}C and checked for their antimicrobial property.

(iii) Testing for antibacterial activity

The antibiotic resistance pattern of bacterial pathogens were deduced from the standard interpretation chart. These resistant bacterial strains are procured from Microbial Type Culture Collection (MTCC, Chandigarh, India) were employed in the present study to investigate the antibacterial properties. The Gram negative organisms such as *Escherichia coli* (MTCC 585),*Klebsiellapneumoniae* (MTCC 3040), *Mycobacterium smegmatis* (MTCC 994),*Pseudomonas aeruginosa*(MTCC 7925),*Shigellaflexneri* (MTCC 1457),*Xanthomonascampestris* (MTCC 2286) and Gram positive organisms such as *Bacillus subtilis*(MTCC 428),*Staphylococcus aureus*(MTCC 3160)were used as the test pathogens. Nutrient agar and nutrient broth were used for storage and sub-culturing of the bacterial pathogens. Muller Hinton Agar was used for antibacterial assay.

(iv) Assay of antimicrobial activity

The antimicrobial activity was assessed using the Agar well diffusion assay[13]. All bacterial cultures were plated out on Nutrient agar plates and were incubated for 24 h at 37 ± 0.5^{o}C and colonies from this fresh culture were used for making suspension. Fresh inoculum of approximately 10^{6} CFU (colony forming units)/ml of tested microorganisms were used for the study. 100µl of the bacterial suspension was uniformly spread on sterile Muller Hinton Agar plates. After solidification of the agar, wells were made with a 6 mm sterile cork borer. The powdered bryophyte extracts were made with 1ml of 99% (v/v) DMSO (Dimethyl sulfoxide) and 100µl was poured into the wells. The plates were incubated for 24 h at 37 ± 0.5^{o}C and antibacterial activity of the plant extract was observed by measuring the diameter zone of inhibition in millimeters. Negative controls were made with DMSO alone and positive controls were made with the antibiotic Streptomycin (25 µg)at the center of the plate.

(v)Preliminary phytochemical analysis

The extracts of the collected bryophytes using different solvents were screened for the qualitative analysis of different classes of natural compounds, using the standard methodology of Sofowora[14]. The major pharmaceutically valuable phytochemical compounds like alkaloids, carboxylic acids, coumarins, flavonoids, phenol, proteins and amino acids, quinones, resins, saponins, sterols, tannins, xanthoproteinsand sugars from the collected bryophytes were investigated in the present study.

RESULTS

The bryophytes were identified as identified as *Homaliodendronrectifolium*(Mitt.) Fleisch*, Aerobrydiumfilamentosum*(Hook.)Fleisch*, Garckeaphascoides*(Hook.) C.Muelland authenticated from the literature (Table 1).The antibacterial activity of thebryophyte extracts are given in Table2. The distilled water extract of the bryophyte*Homaliodendronrectifolium* (Mitt.) Fleischreported the maximum antibacterial activity (24 mm) against*Klebsiellapneumoniae*(MTCC 3040) followed by the ethyl acetate extract of *Aerobrydiumfilamentosum*(Hook.) Fleischagainst*Escherichia coli* (MTCC 585) (23 mm). The result of this research highlights the activity of both the organic solvent and aqueous extracts.The results of phytochemical screening indicate the presence of some secondary metabolites. They may be responsible for the antibacterial activity of the bryophytes.*Homaliodendronrectifolium* (Mitt.) Fleischreported the presence of steroids and sugars (Table 3)

Table 1
Characterization of bryophytes collected from high altitudes

Characteristics	***Bryophytes***		
	Specimen 01	**Specimen 02**	**Specimen 03**
Habit	Branched	Robust	Unbranched
Costa	Present	Present	Present
Leaf Margin	Serrate	Entire	Entire
Apical cell arrangement	Rhomboid	Rhomboid	Elongated
Middle cell arrangement	Hexogonal	Rhomboid	Elongated
Basal cell arrangement	Elongated	Quadrate	Elongated
Chloroplast	Parietal	Parietal	Parietal
Distribution	Nilgiri hills	Nilgiri hills	Nilgiri hills
Identification of the species	***Homaliodendronrectifolium* (Mitt.) Fleisch**	***Aerobrydiumfilamentosum*(Hook.) Fleisch**	***Garckeaphascoides* (Hook.)C.Muell**

Table 2
Antibacterial activity of bryophytes against penicillin resistant microorganisms

Microorganisms	**Zone of inhibition (mm in dia.)**										
	***Homaliodendron rectifolium* (Mitt.) Fleisch**			***Aerobrydiumfilamentosum*(Hook.) Fleisch**			***Garckeaphascoides* (Hook.)C.Muell**			**Streptomycin (25 µg /disc) +ve control**	**DMSO (-ve control)**
	A	EA	DW	A	EA	DW	A	A	DW		
Escherichia coli (MTCC 585)	22	21	18	16	23	21	21	12	18	27	-
Klebsiellapneumoniae(MTCC 3040)	19	T	24	15	18	18	15	18	19	22	-
Mycobacterium smegmatis (MTCC 994)	15	14	19	18	15	17	16	T	16	25	-
Pseudomonas aeruginosa (MTCC 7925)	21	19	16	11	14	18	19	21	14	23	-
Shigellaflexneri(MTCC 1457)	12	16	15	22	19	19	19	14	19	24	-
Xanthomonas. campestris (MTCC 2286)	19	17	16	18	18	19	20	16	16	25	-
Bacillus subtilis(MTCC 441)	19	18	15	12	13	17	T	13	15	21	-
Staphylococcus aureus(MTCC 3160)	15	18	15	19	15	15	12	15	12	22	-

Values are the average of experiments in triplicates

A- Acetone, EA- Ethyl Acetate, DW- Distilled water, Streptomycin25 µg /disc=+ve control; DMSO- Dimethyl Sulfoxide= -veControl, T=Trace (≤ 7mm);-=No activity

Table 3
Phytochemical analysis of selected bryophytes

Phytochemical constituents	*Homaliodendronrectifolium* (Mitt.) Fleisch			*Aerobrydiumfilamentosum*(Hook.) Fleisch			*Garckeaphascoides* (Hook.)C.Muell		
	A	**EA**	**DW**	**A**	**EA**	**DW**	**A**	**EA**	**DW**
Colour of extracts	Green	Green	Pale Green	Green	Green	Pale Green	Green	Green	Pale Green
Alkaloids	+	+	-	+	-	+	+	+	-
Carboxylic acids	-	-	-	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-	-	-	-
Flavonoids	+	-	-	-	-	+	-	-	-
Phenol	-	-	-	-	-	-	-	-	-
Proteins&amino acids	-	+	-	-	-	-	+	-	+
Quinones	+	-	-	-	+	+	+	-	+
Resins	-	+	-	-	+	+	-	+	+
Saponins	-	-	-	-	-	-	-	-	-
Steroids	-	+	+	+	-	+	-	+	-
Tannins	-	-	-	-	+	-	-	-	-
Xanthoproteins	-	-	-	+	-	-	-	+	-
Sugars	+	+	+	+	+	+	+	+	+
Signs are the average of experiments in triplicates									

+=Present; T=Trace; -=Absent

DISCUSSIONS

The antimicrobial principles were either polar or non-polar and they were extracted only through the respective solvent medium[15,16]. The results of phytochemical screening indicate the presence of some secondary metabolites they may be responsible for the antibacterial activity of the bryophytes.*Homaliodendronrectifolium* (Mitt.) Fleischreported the presence of steroids and sugars. In general the bryophytesreveals the presence of alkaloides, flavonoides, proteins&amino acids, quinones, resins, steroides, tannins, xanthoproteins and sugars. Ahmed *et al.*[17] and Batish*et al.*[18] reported that the phytochemical compounds are responsible for the antimicrobial activity.Kumaraswamy and Sathish[19]reported that the activity may be due to the presence of various secondary metabolites. The antibacterial activity of the bryophytes collected from the high altitude areas are remarkable and results are comparable with standard commercial antibiotics. These may be due to the stressful environment where the plants are inhabited. It is already reported that the plants interact with stressful environments by physiological adaptation and altering the biochemical profile of plant tissues and producing a spectrum of secondary metabolites[12,20].

CONCLUSION

The results of antibacterial activity and phytochemical analysis of the high altitude bryophytes are considerable because it enables the identification of potential antimicrobials and other secondary metabolites present, which are act as source of innumerable therapeutic agents. Further research is highly warranted to find out the active principle responsible for antimicrobial activity and to elucidate the structure of particular compound.

ACKNOWLEDGEMENT

The authors are thankful to the Authorities of Scott Christian College, Nagercoiland BishopMoore College, Mavelikara for providing necessary facilities.

REFERENCES

1. WHO WHO Meetings – antimicrobial resistance, retrieved 22 February, 2011 fromhttp://whqlibdoc.who.int/hq/1995/WHO CDS BVI 95.7.pdf(1994)
2. Barber M. Methicillin-resistant staphylococci. J ClinPathol 14:385-93(1961)
3. Benner EJ, Kayser FH. Growing clinical significance of methicillin-resistant *Staphylococcus aureus*. Lancet 2:741-4(1968)
4. Chambers H.F.Penicillin-Binding Protein-Mediated Resistance in Pneumococci and Staphylococci. J Infect Dis. 179(Supplement 2): S353-S359 (1999)
5. Panghal M, Kaushal V,Yadav JP *In vitro* antimicrobial activity of ten medicinal plants against clinical isolates of oral cancer cases. Ann ClinMicrobiolAntimicrob. 10: 21(2011)
6. Kenneth VIR New antimicrobial agents for the treatment of bacterial infection in cancer patients. HematolOncol27: 107-114(2009)
7. Chaudhary BL and PremKumar. Antibacterial activity and prelimainary phytochemical screening of epiphytic moss *Stereophyllumligulatum*Jaeg. Int Journal of Pharma and Biosciences 2 (4) B 674-681(2011)
8. Forrest LL, Davis EC, Long DG, Crandall-Stotler BJ, Clark A, Hollingsworth ML. Unraveling the evolutionary history of the liverworts (Marchantiophyta):multiple taxa, genomes, analyses. Bryologist 109: 303-334(2006)
9. Dulger B, Yayintas OT, Gonuz. A Antimicrobial activity of some mosses fromTurkey. Fitoterapia76: 730-732(2005)
10. Chobot V, Kubicova L, Nabbout S, Jahodar L, Vytlacilova J. Antioxidant and free radical scavenging activities of five moss species. Fitoterapia 77: 598-600(2006)
11. Sabovljevic A, Sokovic M, Sabovljevic M, Grubisic D. Antimicrobial activity of *Bryumargenteum*.Fitoterapia77:144-145(2006)
12. Umadevi, M., K.P.Sampath Kumar, DebjitBhowmik, S. Duraivel TraditionallyUsed Anticancer Herbs In India. J Med Plants Studies1(3)56-74(2013)
13. Perez C, Paul M, Bazerque P. Antibiotic assay by agar well diffusion method. ActaBiolMed Exp.15: 113-115(1990)
14. Sofowora A (1993). Medicinal Plants and Traditional Medicine in Africa. 2nd Edn.Spectrum Books Limited, Ibadan, Nigeria, pp. 1-153.
15. Locher CP, Burch MT, Mower HF, Berestecky H, Davis H, Van Polel B, Lasure A, Vander Berghe DA, Vlieti-Nick AJ. Anti-microbial activity and anticomplement activity of extracts obtained from selected Hawaiian medicinal plants, JEthnopharmacol49: 23-32(1995).
16. Eloff J N Which extract should be used for the screening of antimicrobial components from plants? J Ethnopharmacol60: 1-8 (1998)
17 Ahmad I, Mehmood J, Mohammad F.Screening of some Indian medicinal plants for their antimicrobial properties. JEthnopharmacol62:183-193(1998)
18 Batish DR, Kohli RK, Singh HP and Saxena DB Studies on herbicidal activity of parthenin, a constituent of hysterophorus towards billgoat weed (*Ageratum conyzoides*). CurrSci 73: 369-371(1997)
19 Kumaraswamy MV, Satish S. Antioxidant and Anti-Lipoxygenase activity of *Thespesia lampas*Dalz&Gibs. AdvanBiol Res 2: 56-59(2008)
20 Sanjay Gairola, NoresahMohdShariff, Arvind Bhatt and Chandra Prakash Kala. Influence of climate change on production of secondary chemicals in high altitude medicinal plants: Issues needs immediate attention. J Med Plants Res: 4(18), pp. 1825-1829 (2010).