International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 06-08-2021, Accepted: 20-08-2021, Published: 23-09-2021
Volume 6, Issue 5, 2021, Page No. 186-189

Identification of antimicrobial potencies of *Floribundaria walkari* (Ren. & card): A moss Plant

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Abstract
The present investigation deals with Antimicrobial analysis on *Floribundariawalkari* a moss plant. The whole part of the plants were screened for antimicrobial evaluation. The antimicrobial activities tested using various extracts *viz*.. Ethyl acetate, chloroform, ethanol and aqueous extracts against nine bacterial and seven fungal strains. The antibacterial studies revealed that the most susceptible microorganisms to *Escherichia coli* and *Pseudomonas aeruginosa* and fungal activity the ethonolic extracts showed good antifungal activities against the test ed fungi. *Floribundaria wlkari* could be exploited in the Infectious management of various bacterial and fungal diseases.

Keywords: *Floribundaria walkari*, methanol extract, antimicrobial activity

Introduction
The dominant nature of mosses among the Bryophytes is due to their greater number in species and greater structural and morphological complexities. The term 'Bryophyte' is derived from the Greek word '*bryon'* meaning a moss. Mosses are considered to have the largest number of species among green plants, next to angiosperms. They are the simplest and the most primitive of the land plants. They do not have a well developed conductive tissue system. They were thought to be of little economic value. However they are the pioneers to have colonized terrestrial habitats from the aquatic environment. Their adaptation to a terrestrial mode of life is partial as water is indispensable in one stage or other in their life cycle and hence they are called amphibians of the plant kingdom. They have a remarkable capacity to absorb water on doing which they turn fresh in no time. This has given them the name 'resurrection plants'. They are more common in humid areas and during rainy season, they become abundant within a short period so as to occupy large areas but usually do not form a very conspicuous part of the vegetation.

Despite the richness and wide diversity of moss flora in our country, less attention has been devoted to bryophyte research. There exists a situation by which botanists or teachers are often unaware of the most common bryophytes seen around them. The present investigation intends to fill this gap in research with regard to Moss flora in the Kolli Hills, of the Eastern Ghats of Tamil Nadu.

Material and Methods
Material
The Plant material were collected from kolli hills and the plant were identified and authenticated in by Dr. S. Sahayasathish, Centre for cryptogamic studies, St. Joseph's College, Tiruchirappalli-620002.

Plant description
***Floribundariawalkeri* (Ren. & Card.) Broth.**
Floribundariawalkeri(Ren.& Card.) Broth.in Engl. &Prantl, Nat. Pflanzenfam. 1(3):822 (1906); Bruehl in Rec. Bot. Surv. India, 13(1):73 (1931); Wadhwa in M. V. M. Patrika, 4: 92 (1969); R. S. Chopra, Tax. Indian Moss., 346 (1975); Gangulee, Mosses E. India, 2(5): 1306 (1976); *Papillaria walker* Renauld & Cardot in Bull. Soc. Roy. Bot. Belgique, 34:70(1896); Type: India (W. Bengal), Edentale Plantation near Kurseong, Nov. 1893, *Walker s. n.* (Herb. Cardot PC).
Creeping, yellowish brown, very delicate, slender, branched pinnately, up to 2 cm long. Leaves arranged in distant, feather like, concave, ovatelanceolate, apex narrow acuminata, base narrowed margin flat, smooth, costa very weak, scarcely detectable, auriculate, leaf up to 2 mm long and 0.5 mm wide at base, narrow towards the top. Leaf cells narrow, linear, incrassate, seriate multipapillate. Sporophyte on short lateral shoots with perichaetial leaves.Seta erect, capsule cylindrical, operculum conical ((Plate 36).
Habitat: Corticolous in evergreen forests.
Specimen examined: Koilur (1000 m), 04-07-2004, RHTM 052; Semmedu (1220 m), 05-08-2004, RHTM 063; Settur (1200 m), 05-08-2004, RHTM 070; Solakkadu (1200 m), 06-02-2005, RHTM 078.

Methodology
Antimicrobial activities

Disc-diffusion Test (Maruzzella and Henry, 1958)
The disc diffusion method provides a simple and reliable test in routine clinical microbiology in order to find out the effect of a particular substance on specific bacterium. This method consists of impregnating small circular discs of standard filter paper with given amount of chosen concentration of substance. The discs are placed on the

plates of culture medium previously spread with bacterial inoculums to be tested. After incubation the degree of sensitivity is determined by measuring the incubation zone produced by diffusion of the antibiotic substances from the discs of the surrounding medium.

Preparation of discs

Discs usually consisted of absorbent paper impregnated with the compound (plant extract). It is most convenient to use Whatman № 1 filter paper for preparing the discs. Dry discs of 6 mm diameter were prepared from Whatman № 1 filter paper and sterilized in an autoclave. These dry discs were used for the assay.

Procedure

Circular discs of 6 mm diameter were prepared from Whatman № 1 filter paper and sterilized in an autoclave. These paper discs were impregnated with test compounds (plant extract) in the respective solvents for overnight and placed on nutrient agar plates seeded with the test bacterium. The plates were incubated at 37 °C for 24 hr. After 24 hr the zone of inhibition around each disc was measured and the diameter was recorded. Gentamycin (10 mcg/disc) was used as the reference. A negative control was prepared using only the solvent used for extraction and kept for comparison. The tests were repeated 4 times to ensure reliability of the result.

Agar Well Diffusion Method (Perez *et al*., 1990)

Agar well diffusion method is also known as Hole Plate Diffusion Method (Brantner *et al*., 1993) or Cup Diffusion Method (Vikas Dhingra *et al*., 1999).

Principle

It is an important method for studying the inhibitory effect of any compound (plant extract or antibiotics) on the growth and multiplication of a particular bacterium. Here well or cups are made using a sterilized Cork borer on the seeded nutrient agar in a petridish to which the test compound is added. The treated petridisches are incubated at 37 °C for 24 hr. The inhibition zone formed around each well indicates the antimicrobial activity.

Procedure

Nutrient agar was used as the culture medium for this assay. The molten nutrient agar was dispensed in pre-sterilized petridisches (25 ml each) and allowed to cool. These agar plates were homogenously inoculated with the test bacterium previously suspended in tryptose broth (10^6 cells/ml).

The plates were allowed to solidify. After solidification holes/ wells (cups) of 6 mm diameter were punched into the agar with the help of flamed cork borer. Five wells were prepared for each plate. Of these five, three holes were filled with 0.2 ml of the plant extract and the fourth hole was filled with 0.2 ml of standard antibiotic solution (Gentamycin, 500 µg/ml) and the fifth hole was filled with blank (extracting solvent alone). The petridishes were incubated at 37 °C for 24 hr.

After this incubation period the diameter of the inhibition zone formed around each hole (well/cup) was measured and the values were recorded.

The antimicrobial activity was expressed as the ratio of the inhibition zone produced by the plant extract and the inhibition zone caused by the standard. Two sets of control were used.

One control was the organism control where standard antibiotic solution was used and the other control was the blank where only the extracting solvent was used. This was just to ensure the validity of the test. Testing was carried out for each bacterium in quadruplicates.

Disc Diffusion Method

a. *Floribundariawalkari*

The antibacterial activity of various extracts of *Floribundariawalkari* against the test bacteria by disc diffusion method has shown in the Table. It is observed from the result that the chloroform extract was found to have inhibitory effect against *Escherichia coli* and *Pseudomonas aeruginosa* and the zone of inhibition was from 1.5 and 2.0 mm respectively.

However ethyl acetate extract showed inhibitory effect against *Bacillus cereus* in addition to the above said two bacteria, with almost same diameter of inhibition zone.

Antimicrobial activity of *Floribundariawalkari* against various bacteria
(Disc diffusion method)

Table 1

Organism	Diameter of inhibition zone in mm (Mean*)				
	Ethyl acetate extract (30 µg/disc)	Chloroform extract (30 µg/disc)	Ethanolic extract (30 µg/ disc)	Aqueous extract (30 µg/disc)	Standard# antibiotic
Gram-positive bacteria:					
Bacillus cereus	–	–	1.5 ± 0.5	–	4.2
Streptococcus faecalis	–	–	–	–	5.0
Staphylococcus aureus	–	–	–	–	4.0
Gram-negative bacteria:					
Escherichia coli	–	1.5 ± 0.1	1.0±0.12	–	4.4
Proteus vulgaris	–	–	–	–	4.5
Enterobacteraerogenes	–	–	–	–	4.2
Salmonella typhi	–	–	–	–	2.4
Klebsiellapneumoniae	–	–	–	–	5.0
Pseudomonas aeruginosa	–	2.0 ± 0.21	2.2 ± 0.15	–	4.1

*: Mean of triplicate ±: Standard Deviation #: Gentamycin (Hi-media) –: Absence of measurable inhibitory action

Agar Well Diffusion Method

The various solvent extracts of this moss did not show any significance result against most of the tested bacteria. However the ethyl acetate extract produced little effect on *Escherichia coli* and *Pseudomonas aeruginosa* and the Diameter of inhibition zones were very less.

Antimicrobial activity of *Floribundariawalkari* against various bacteria (Agar Well diffusion method)

Table 2

Organism	Diameter of inhibition zone in mm (Mean*)				
	Ethyl acetate extract (30 µg/ml)	Chloroform extract (30 µg/ml)	Ethanolic extract (30 µg/ ml)	Aqueous extract (30µg/ml)	Standard# antibiotic
Gram-positive bacteria:					
Bacillus cereus	–	–	–	–	5.2
Streptococcus faecalis	–	–	–	–	4.0
Staphylococcus aureus	–	–	–	–	5.0
Gram-negative bacteria:					
Escherichia coli	–	–	2.0 ± 0.4	–	4.1
Proteus vulgaris	–	–	–	–	4.7
Enterobacteraerogenes	–	–	–	–	4.4
Salmonella typhi	–	–	–	–	4.8
Klebsiellapneumoniae	–	–	–	–	5.2
Pseudomonas aeruginosa	–	–	1.6 ± 0.2	–	5.1

*: Mean of triplicate ±: Standard Deviation #: Gentamycin (Hi-media) –: Absence of measurable inhibitory action

Antifungal activities

In antifungal activity *Floribundariawalkari*, did not show any inhibitory effect on the fungal organisms tested. However. The diameter of the inhibition zone is only lesser than the standard antibiotic.

Antifungal activity of various extracts of *Floribundariawalkari* against various fungi (Disc diffusion method)

Table 3

Organism	Diameter of inhibition zone in mm (Mean*)				
	Ethyl acetate extract (30 µg/disc)	Chloroform extract (30 µg/disc)	Ethanolic extract (30 µg/ disc)	Aqueous extract (30 µg/disc)	Standard# antibiotic (30 µg/disc)
Aspergillus niger	–	–	1.8 ± 1.0	–	8.2
Aspergillus flavus	–	–	–	–	5.4
Aspergillusparasiticus	–	–	–	–	4.5
Aspergillus fumigatus	–	–	–	–	7.2
Candida albicans	–	–	–	–	8.4
Geotricum candidum	–	–	–	–	8.0
Microsporium gypseum	–	–	–	–	6.5

*: Mean of triplicate ±: Standard Deviation #: Nystatin –: Absence of measurable inhibitory action

Agar Well Diffusion Method

Antifungal activity of various solvent extracts of test mosses against various fungi by agar well diffusion method has revealed that only the ethanolic extract of all the mosses showed some notable effect, on *Aspergillusniger*, *Aspergillusflavus*and *Geotricumcandidum.*

Antifungal activity of various extracts of *Floribundariawalkari* against various fungi (Agar well diffusion method)

Table 4

Organism	Diameter of inhibition zone in mm (Mean*)				
	Ethyl acetate extract 10 mg/ml	Chloroform extract 10 mg/ml	Ethanolic extract 10 mg/ml	Aqueous extract 10 mg/ml	Standard# antibiotic 10 mg/ml
Aspergillusniger	–	–	4.0 ± 1.5	–	8.2
Aspergillusflavus	–	–	1.8 ± 1.4	–	5.9
Aspergillusparasiticus	–	–	–	–	6.5
Aspergillusfumigatus	–	–	–	–	5.0
Candida albicans	–	–	–	–	4.2
Geotricumcandidum	–	–	1.5 ± 0.6	–	8.2
Microsporiumgypseum	–	–	–	–	8.5

*: Mean of Triplicate ±: Standard Deviation #: Griseofulvin –: Absence of measurable inhibitory action

Summary and conclusion

The Bryophytes are abundant in the Indian sub-continent, there has been a neglect in research among these groups. As a result of the dearth of literature, mosses have been greatly neglected in India. There is a prime need to prepare a Bryophyte Flora of India. Much lacune exists in the study of the South Indian bryophytes when compared to the North Indian bryophytes. A reasonable method in developing a national database for Bryophytes is by adopting regional approaches of studying the smaller areas with deeper intensity. Keeping this in mind, in the present investigation an attempt was made to explore the richness of the Bryopsida (Moss) flora of the Kolli Hills in the Eastern

Ghats of Tamil Nadu. The study was carried out from years 2003 to 2006 covering different seasons.

The antimicrobial studies of the selected mosses on the test bacteria and fungi using various techniques such as disc diffusion method and agar well diffusion method clearly revealed that certain mosses possess significant antimicrobial property on certain bacteria and on a few fungi.

- The disc diffusion method also revealed that certain mosses found to have inhibitory effect on certain bacteria at higher concentrations. Here also ethanol extract of *Floribundariawalkar i*showed notable inhibitory effect on most of the gram-positive and gram-negative bacteria.
- The agar well diffusion method showed that *Floribundariawalkar i*has very strong inhibitory effect on *Escherichia coli* and *Pseudomonas aeruginosa.* The ethanolic extract of this moss produced inhibition zone which was slightly higher than the standard antibiotic used. This is a significant result, which indicates that *Floribundariawalkari* could be used to control *Escherichia coli.*
- Antifungal studies using streak method, disc diffusion method and agar well diffusion method showed that *Floribundariawalkari* possess antifungal properties. The ethanolic extract of these mosses produced significant result than the standard antifungal agent. The diameter of zone of inhibition was greatly higher than the standard antifungal agent.

To conclude, the *Floribundariawalkari* have shown a very high degree of inhibition especially in ethanolic extract. Hence these moss could be used for therapeutic purposes and the active compounds can be isolated from these mosses plants and used potentially for controlling the pathogenic microbes. Thus it is proved that this plant is potent enough to produce various therapeutically used phytocompounds.

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