

*Full Length Research Paper*

# Assessment of the antimicrobial properties of fractions obtained from bryophytes

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Water, ethanol and acetone extracts were obtained from 10 selected bryophytes (seven mosses and three liverworts) viz: *Radula flacida*, *Cyatodium africanum*, *Frullania spongiosa*, *Thuidium gratum*, *Ectropothecium aeruginosum*, *Sematophyllum caespitosum*, *Stereophyllum radiculosum*, *Babula lamberenensis*, *Campilopus aspericuspis* and *Calymperes erosum*. A total of 24 fractions of various colors were obtained through a column chromatography. The antimicrobial efficacy of these fractions was assessed against *Candida albicans*, *Staphylococcus aureus*, *Penicillium notatum* and *Mucor rouxii*. Fractions obtained from *R. flacida* suppressed the growth of *C. albicans*, *S. aureus* and *M. rouxii*. *Cyatodium africanum* was also effective against *M. rouxii*. None of the fractions of these plants could antagonize the growth of *P. notatum*. The results obtained from this study indicate that effective antimicrobial compounds can be sourced from *R. flacida* and *C. africanum*.

**Key words:** Water, ethanol, acetone extracts, bryophytes, antimicrobial efficacy.

## INTRODUCTION

The search for effective antimicrobial agents had increased of recent. The major reason for this is the increasing resistance posed by microorganisms to commercial antibiotics. Plants such as bryophytes are known to contain phytochemicals that can serve as effective antimicrobial agents, some of these useful bryophytes compounds include; oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds, phenyl quinines, aromatic and phenolic substances, but much work has not been done to link medicinal effects with specific bryophytes species (William et al., 2005). Bryophytes species produce a broad range of antibiotics and the degree of antibiotic activity in a given species may depend on the age of the gametophyte (Subramoniam and Subhishi, 2005). The active ingredients responsible for antimicrobial effects have been isolated and identified from several bryophytes, such as Polygodial from *Porella*, Norpiguisonone from *Conocephalum conicum* and Lunularin from *Lunularia cruciata*. Some liverworts also have some

anti-leukemia activity, for example Diplophyllin was isolated from *Diplophyllum albicans* and *Diplophyllum taxifolium*, which have significant activity against human epidermoid carcinoma (Saxena and Harinder, 2004). Some of the medicinal species recognized are; Hepaticae (*Frullania tamarisci*, *Reboulia hemispherica*, *Conocephalum*, *Conocephalum supradecompositum* and *Marchantia polymorpha*), Musci (*Sphagnum girgensolinii*, *Sphagnum magellanicum*, *Sphagnum Palustre*, *Sphagnum teres*, *Rhodobryum giganteum* and *Rhodobryum roseum*). *Conocephalum conicum*, *Mnium undulatum* and *Leptodictyum riparium* have strong antibacterial activity against pathogenic bacteria species. Similarly, *Rhynchostegium riparioides* extract has good antibacterial activity against Gram negative bacteria, in particular *Escherichia coli*, *Proteus mirabilis*, *Enterobacter cloacae* and *Pseudomonas aeruginosa* (Basile et al., 1998). Petroleum-ether extracts of *Barbula* and *Timmia* species have antibacterial activity against both Gram-positive and Gram-negative bacteria (Subramoniam and Subhishi, 2005).

One danger in using bryophytes is that the same compounds that may have antibiotic properties may also

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be toxic or allergenic, or be associated with such compounds, thus many antibiotics have been isolated from bryophytes, but few have been developed for medical use, despite their demonstrated effectiveness. Nigeria in West Africa, is a home to varieties of these bryophytes and some of the common species in Nigeria include; *Jaegerina scariosa* (Lor.) Arz., *Octoblepharum albidum* Hedw. *Racopilum africanum* MITT., *Barbula lambarenensis* C. Mull and *Philonotis hastata* (Akande, 1992). However, there is little or no information on the antimicrobial effects of these African species of bryophyte plants. The present study is therefore meant to assess the antimicrobial properties of some of these plants on some indicator microorganisms.

## MATERIALS AND METHODS

### Sample collection

Some bryophytes were collected from the wall of buildings, some from the floor in the wild, some from the bark of trees, including the bark of palm trees, some from the river bank and others from the bark of fallen trees and logs in Ado – Ekiti, Ekiti State, Nigeria. All these collections were kept in herbarium packets and brought to the herbarium unit, Department of plant science, Ekiti State University, Ado – Ekiti, Ekiti State, Nigeria for proper observation and identification. These samples were then collected fresh from the field and used for the experiment. A total of 15 samples of bryophytes namely; *Calymperes erosum*, *Barbula lamberenensis*, *Radula flacida*, *Sematophyllum caespitosum*, *Stereophyllum radiculosum*, *Thuidium gratum*, *Ectropothecium aeruginosum*, *Cyatodium africanum*, *Campylopus aspericuspis*, *Frullania spongiosa*, *Polytricum commune*, *Marchantia polymorpha*, *Rhodobryum giganteum*, *Lapholejeunea subfusca* and *Pohlia cruda* were collected from the field, but only 10 species were used for the subsequent experiment based on their abundance and proximity.

### Extraction of biologically active substance

Water, ethanol and acetone extracts were obtained from the 10 selected bryophytes (seven mosses and three liverworts) viz; *R. flacida*, *C. africanum*, *F. spongiosa*, *T. gratum*, *Ectropothecium aeruginosum*, *Sematophyllum caespitosum*, *S. radiculosum*, *Babula lamberenensis*, *C. aspericuspis* and *C. erosum*. In a mortar with pestle, 2 g of each of the plant samples was separately ground in 5 ml of distilled water, until homogenous slurry was obtained. This slurry was sieved through a sterile muslin cloth to discard the residue. The supernatants were collected in sterile labeled test tubes and covered with aluminum foil paper. The procedure above was also repeated for ethanol and acetone extracts (Alanis et al., 2005). The supernatants were further separated in a column chromatogram which was made of cellulose powder. A total of 24 fractions of various colors were obtained through a column chromatography.

### Determination of antibacterial activity of the extracts

Sensitivity test was done by using agar diffusion of Kirby-Bauer's procedure for the evaluation of the antimicrobial activity of chemotherapeutic agents by using extracts that have already been filter separated in the chromatogram, sample (0.1 ml) was carefully

transferred into each well of already prepared plates of the test organisms which were grown in Mueller Hinton Agar (MHA, Oxoid) for 24 h at 37°C and they were labeled appropriately. The microbial load after this incubation period was determined. Sterile distilled water was added (0.1 ml) into the second holes and served as the control. The plates were incubated un-inverted at 37°C for 24 h. The zones of inhibition were measured with a ruler in millimeters, and the results were recorded. The minimum inhibitory concentration (MIC) was determined as the lowest concentration that completely inhibited macroscopic growth of the organisms.

### Standard antibiotic sensitivity test

About 0.1 ml of 18 h broth culture of each of the test organism was inoculated unto the surface of the set agar on different Petri dishes in triplicates. The inoculums were spread on the surface of the agar with the aid of sterile hockey stick. Standard antibiotic disc were aseptically placed at a central position on the surface of the agar plates using a sterile forceps, it was then pressed gently against the inoculated plates. The plates were then incubated in an inverted position at 37°C for 24 h. Zones of inhibition around the discs were measured.

## RESULTS

From the results obtained in this study, some of the test organisms were susceptible to the fractions obtained from these plants and the pattern of their susceptibility was in accordance with their minimum inhibitory concentrations, for instance those fractions with low value of MIC are more active than the fractions with higher value of MIC. A total of 24 fractions of various colors were obtained from extracts of the 10 bryophytes through a column chromatography. Only two out of 10 species of bryophytes tested that is, *C. africanum* and *R. flacida*, showed antimicrobial effect on some of the test microorganisms used.

In this study, ethanol extract of *R. flacida* suppressed the growth of *Staphylococcus aureus* and *micrococcus* spp in culture having 10.0 and 8.0 mm diameter zone of inhibition with minimum inhibitory concentrations of 20.50 and 24.50 µg/ml, respectively. Water extract from *R. flacida* also displayed antimicrobial effect against *Mucor rouxii* with 7.0 mm zone of inhibition and minimum inhibitory concentration of 26.50 µg/ml. Water extracts from *C. africanum* gave positive effect on *M. rouxii* also with 9.0 mm zone of inhibition and MIC of 22.60 µg/ml (Table 4). *S. aureus* showed the highest sensitivity to ethanol extract of *R. flacida*, then next to it is *M. rouxii*, which was sensitive to water and ethanol extracts of *C. africanum* and the least sensitivity occurred in water extract of *R. flacida* against *M. rouxii*. All the remaining tested microorganisms did not show any sensitivity to all the concentrations of *Calymperes erosum*, *Barbula lamberenensis*, *Sematophyllum caespitosum*, *Stereophyllum radiculosum*, *T. gratum*, *Ectropothecium aeruginosa*, *Campylopus aspericuspis*, *Frullania spongiosa*, *Polytricum commune*, *Marchantia polymorpha*,

**Table 1.** Antimicrobial effect of water extracts from bryophytes.

Microorganism	Zone of inhibition (mm)									
	1	2	3	4	5	6	7	8	9	10
<i>Candida albicans</i>	0	0	0	0	0	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	0	0	0	0	0	0	0	0	0	0
<i>Staphylococcus aureus</i>	0	0	0	0	0	0	0	0	0	0
<i>Botrytis cinera</i>	0	0	0	0	0	0	0	0	0	0
<i>Rhizoctonia solani</i>	0	0	0	0	0	0	0	0	0	0
<i>Phythium debaryanum</i>	0	0	0	0	0	0	0	0	0	0
<i>Escherichia coli</i>	0	0	0	0	0	0	0	0	0	0
<i>Klebsiella spp</i>	0	0	0	0	0	0	0	0	0	0
<i>Bacillus cereus</i>	0	0	0	0	0	0	0	0	0	0
<i>Micrococcus spp</i>	0	0	0	0	0	0	0	0	0	0
<i>Proteus spp</i>	0	0	0	0	0	0	0	0	0	0
<i>Aspergillus flavus</i>	0	0	0	0	0	0	0	0	0	0
<i>Mucor rouxeii</i>	0	0	0	0	9.0	7.0	0	0	0	0
<i>Penicillium spp</i>	0	0	0	0	0	0	0	0	0	0
<i>Botryodiploida theobromae</i>	0	0	0	0	0	0	0	0	0	0

1, *Thuidium gratum*; 2, *Ectopothecium aerginosum*; 3, *Calymperes erosum*; 4, *Sematophyllum caespitosum*; 5, *Cyatodium africanum*; 6, *Radula flacida*; 7, *Barbula lamberenensis*; 8, *Steriophyllum radiculosum*; 9, *Camphylopus aspecicuspsis*; 10, *Frullania spongiosa*. Values are mean of replicates.

**Table 2.** Antimicrobial effect of Acetone extracts from bryophytes.

Microorganism	Zone of inhibition (mm)									
	1	2	3	4	5	6	7	8	9	10
<i>Candida albicans</i>	0	0	0	0	0	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	0	0	0	0	0	0	0	0	0	0
<i>Staphylococcus aureus</i>	0	0	0	0	0	0	0	0	0	0
<i>Botrytis cinera</i>	0	0	0	0	0	0	0	0	0	0
<i>Rhizoctonia solani</i>	0	0	0	0	0	0	0	0	0	0
<i>Phythium debaryanum</i>	0	0	0	0	0	0	0	0	0	0
<i>Escherichia coli</i>	0	0	0	0	0	0	0	0	0	0
<i>Klebsiella spp</i>	0	0	0	0	0	0	0	0	0	0
<i>Bacillus cereus</i>	0	0	0	0	0	0	0	0	0	0
<i>Micrococcus spp</i>	0	0	0	0	0	0	0	0	0	0
<i>Proteus spp</i>	0	0	0	0	0	0	0	0	0	0
<i>Aspergillus flavus</i>	0	0	0	0	0	0	0	0	0	0
<i>Mucor rouxeii</i>	0	0	0	0	0	0	0	0	0	0
<i>Penicillium spp</i>	0	0	0	0	0	0	0	0	0	0
<i>Botryodiploida theobromae</i>	0	0	0	0	0	0	0	0	0	0

1, *Thuidium gratum*; 2, *Ectopothecium aerginosum*; 3, *Calymperes erosum*; 4, *Sematophyllum caespitosum*; 5, *Cyatodium africanum*; 6, *Radula flacida*; 7, *Barbula lamberenensis*; 8, *Steriophyllum radiculosum*; 9, *Camphylopus aspecicuspsis*; 10, *Frullania spongiosa*. Values are mean of replicates.

*Rhodobryum giganteum*, *Lapholejeunea subfusca* and *Pohlia cruda*. The dimension of zones of inhibition measured in millimeter is shown in Tables 1 to 3 below.

For the antibiotic sensitivity assay (Table 5), the anti-

biotic that has the highest inhibitory effect on the growth of *S. aureus* is gentamicin, it has an inhibitory zone of 28.0 mm and for *Micrococcus spp.* is Pefloxacin with zone of inhibition of 30 mm, while *M. rouxii* has the

**Table 3.** Antimicrobial effect of ethanol extracts from bryophytes.

Microorganism	Zone of inhibition (mm)									
	1	2	3	4	5	6	7	8	9	10
<i>Candida albicans</i>	0	0	0	0	0	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	0	0	0	0	0	0	0	0	0	0
<i>Staphylococcus aureus</i>	0	0	0	0	0	10.0	0	0	0	0
<i>Botrytis cinera</i>	0	0	0	0	0	0	0	0	0	0
<i>Rhizoctonia solani</i>	0	0	0	0	0	0	0	0	0	0
<i>Phythium debaryanum</i>	0	0	0	0	0	0	0	0	0	0
<i>Escherichia coli</i>	0	0	0	0	0	0	0	0	0	0
<i>Klebsiella spp</i>	0	0	0	0	0	0	0	0	0	0
<i>Bacillus cereus</i>	0	0	0	0	0	0	0	0	0	0
<i>Micrococcus spp</i>	0	0	0	0	0	0.8	0	0	0	0
<i>Proteus spp</i>	0	0	0	0	0	0	0	0	0	0
<i>Aspergillus flavus</i>	0	0	0	0	0	0	0	0	0	0
<i>Mucor rouxeii</i>	0	0	0	0	0.9	0.8	0	0	0	0
<i>Penicillium spp</i>	0	0	0	0	0	0	0	0	0	0
<i>Botryodiplodia theobromae</i>	0	0	0	0	0	0	0	0	0	0

1, *Thuidium gratum*; 2, *Ectropothecium aeruginosum*; 3, *Calymperes erosum*; 4, *Sematophyllum caespitosum*; 5, *Cyatodium africanum*; 6, *Radula flacida*; 7, *Barbula lamberenensis*; 8, *Sterophyllum radiculosum*; 9, *Camphylopus aspecicuspis*; 10, *Frullania spongiosa*. Values are mean of replicates.

**Table 4.** Minimum inhibitory concentrations of water and ethanol extracts of *Radula spp.* and *Cyatodium africanum* ( $\mu\text{g/ml}$ ).

Microorganism	<i>Radula flacida</i>		<i>Cyatodium africanum</i>	
	Water	80% ethanol	Water	80% ethanol
<i>S. aureus</i>	0	20.50	0	0
<i>Micrococcus spp.</i>	0	24.50	0	0
<i>Mucor rouxii</i>	26.50	0	22.60	22.60

**Table 5.** Inhibition zone (mm) of commercial antibiotics on indicator organisms.

Antibiotic	<i>S. aureus</i> (mm)	<i>Micrococcus spp.</i> (mm)
Septrin (30 $\mu\text{g}$ )	0.0	0.0
Chloramphenicol (30 $\mu\text{g}$ )	0.0	0.0
Sparfloxacin (10 $\mu\text{g}$ )	0.0	0.0
Ciprofloxin (10 $\mu\text{g}$ )	26.0	24.0
Amoxicillin (30 $\mu\text{g}$ )	0.0	0.0
Augimentin (30 $\mu\text{g}$ )	0.0	0.0
Gentamycin (10 $\mu\text{g}$ )	28.0	16.0
Pefloxacin (30 $\mu\text{g}$ )	27.0	30.0
Tarivid (10 $\mu\text{g}$ )	0.0	0.0
Streptomycin (30 $\mu\text{g}$ )	0.0	0.0

highest zone of inhibition in Gentamycin with 14 mm. Ketoconazole was used to inhibit fungal growth and the

result of the fungal biotest showed that *M. rouxii* has 14.0 mm zone of inhibition while *Penicillium spp.* was growing

without inhibition from the antibiotics used.

## DISCUSSION

The results of this study also show that the tested organisms were susceptible to different concentrations of water and ethanol extracts from *C. africanum* and *R. flacida*, which implies that they have some antimicrobial property. Other workers had earlier ascertained the antimicrobial properties of bryophytes (Oyagade, 1998; Jorgnersteen, 1998; Smith and Reynard, 1992). This implies that these two plants could be used as an alternative for the treatment of infections that could be caused by *S. aureus*, *Micrococcus* spp. and *M. rouxii*. *S. aureus*, was susceptible to 80% ethanol extract of *Radula* spp. with MIC of 20.50 µg/ml while *Mucor rouxii* was susceptible to water extract of *Radula flacida*, with MIC 26.50 µg/ml (Table 4). This result also agrees with the one obtained by Asakawa et al. (1982) who found that *R. flacida*, could inhibit the growth of *S. aureus* at concentrations of 20 to 30 mg/ml. Apart from these two bryophytes, *C. africanum* and *R. flacida*, all other bryophytes used in this study showed no antibacterial activity on the test organisms.

The variation in the susceptibilities of these organisms may be due to the permeability of their cell surface to the fractions obtained from these plants. The antimicrobial properties of the bryophytes observed in this study could be attributed to the phytochemical components, which include polyphenolic compounds such as 3-prenyl bibenzyl from *R. flacida*, and other chemicals like polygodial, norpiquisone and lunularin (Smith and Reynard, 1992). Madson and Paste (1952) in a study discovered antimicrobial substances from almost all plants groups and reported that aqueous extracts of some bryophytes such as *Sphagnum portoricense*, *S. antricum*, *Conocephalum conicum* and *Dinortiera hirsuts*, inhibited the growth of *Candida albicans*, *Pseudomonas aeruginosa* and *S. aureus*.

## Conclusion

The results obtained from this study indicate that fractions obtained from these two bryophytes, *C. africanum* and *R. flacida*, exhibit antimicrobial property on some pathogenic microorganisms. In the light of this, the two bryophytes viz; *C. africanum* and *R. flacida* that are effective can be recommended as source of antimicrobial agents in the treatment of some diseases caused by these etiological agents.

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