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Original Article

Antimicrobial Activity of Selected Mosses on Obafemi Awolowo University Campus, Ile-Ife, Nigeria

Kehinde O. OLASOJI[1]*, Amos M. MAKINDE[1], Bolajoko A. AKINPELU[2], Oluwatoyin A. IGBENEGHU[3], Musibau O. ISA[1]

[1]*Obafemi Awolowo University, Faculty of Science, Department of Botany, Ile-Ife, Osun State, Nigeria; Kennykayy10@gmail.com (*corresponding author), makmorak@gmail.com; alamuola25@gmail.com*
[2]*Obafemi Awolowo University, Faculty of Science, Department of Biochemistry and Molecular Biology, Ile-Ife, Osun State, Nigeria; baakinpelu@gmail.com*
[3]*Obafemi Awolowo University, Faculty of Pharmacy, Department of Pharmaceutics, Ile-Ife, Osun State, Nigeria; igbeneghuoluwatoyin@gmail.com*

Abstract

The present study aimed to evaluate antimicrobial activity of ethanol, methanol, schnapp (40% alcohol), oil palm wine and *Raffia* palm wine extracts of moss species *Archidium ohioense*, *Pelekium gratum* and *Hyophila involuta* against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Candida pseudotropicalis*. The antimicrobial activities of the alcoholic extracts were tested against selected microorganisms using agar well diffusion method. Minimum inhibitory concentrations (MIC) of the extracts were determined using standard methods. The antimicrobial test of the extracts on selected organisms revealed that the methanolic and ethanolic extracts of the mosses studied were inactive against all the bacteria and fungi screened, while the schnapp, Oil palm wine and *Raffia* palm wine extracts showed significant activity against the selected organisms. The minimum inhibitory concentration (MIC) value of the extracts on the test organisms ranged from 1.25 to 40 mg/ml. The study concluded that the extracts of the mosses studied contain pharmacologically active constituents which may be responsible for their antimicrobial properties.

Keywords: antimicrobial; *Archidium ohioense*; *Hyophila involute*; *Pelekium gratum*; phytochemicals

Introduction

Bryophytes are the second largest group of land plants after flowering plants, which consist of about 15,000 to 25,000 species worldwide and it is divided into three separate divisions, the Marchantiophyta (liverwort), Anthocerotophyta (hornwort) and Bryophyta (mosses) (Gradstein *et al*., 2001; Asakawa *et al*., 2013). Bryophytes are known to possess fungi as endobionts, as well as to develop mycorrhiza. However, relationships of bryophytes and fungi remain under-investigated. Bryophytes have been reported to have antifeeding effect and are known to possess various relationships with microorganisms; for example protozoa, fungi, bacteria, algae (Sabovljevic *et al*., 2001) contain a set of various known and unknown secondary metabolites (Xie and Lou, 2009).

The phytochemical study of bryophytes for pharmaceutical "lead" compounds has been overlooked because of their minuscule nature which makes it difficult to identify and to collect large quantity of pure samples for producing drugs. However, recent studies of some *in vitro* cultures of bryophytes have shown that they synthesize active substances which are antibiotic (Ilhan *et al*., 2006; Isa *et al*., 2014).

Bryophytes produce a broad range of antibiotics and the degree of antibiotic activity within a given species may depend on the age of the gametophyte (Subramoniam and Subhisha, 2005).

Antimicrobial resistance is one of the biggest challenges facing global public health at the beginning of the third millennium. According to World Trade Organization, about 440,000 new cases of multi drug resistant tuberculosis are reported every year, causing at least 150,000 deaths (WHO, 1994). Extract of many bryophytes have been shown to possess varying levels of antimicrobial potential and many chemicals were isolated from them which inhibited the growth of phytopathogenic microorganisms

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(Deora and Narendra, 2007; Deora and Vishwakarma, 2012).

One danger in using bryophytes is that the same compounds that may have antibiotic properties may also be toxic or allergenic, or may 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 (Akande, 1992). According to Nweze *et al*. (2004) the presence of these bioactive components gives them resistance against bacterial, fungal and pathogens. Such bioactive components are said to be responsible for the antimicrobial properties of plant extracts *in vitro*.

The present study was carried out to investigate the antimicrobial activity of *Archidium ohioense*, *Pelekium gratum* and *Hyophila involuta* on some selected microorganisms. The investigation was done with a view to exploring their potentials in manufacturing oral antibacterial drugs that can be used to treat infections caused by the tested organisms.

Materials and Methods

Collection and identification of plant materials

The mosses investigated were *Archidium ohioense Schimp* ex. C. Mull, *Pelekium gratum* (Palis) Jaeg. and *Hyophila involuta* (Hook) Jaeg.; samples were collected from their natural populations at the Obafemi Awolowo University Campus, Ile-Ife, Nigeria, within Latitudes 7° 31' and 7° 34' N and Longitudes 4° 30' and 4° 32' E. The plant samples were identified and authenticated at IFE Herbarium of Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Sources of microorganisms

The test organisms employed for screening antimicrobial activities of the plant extracts were Gram-positive organisms: *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 29213); Gram-negative organisms: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145) and yeasts *Candida albicans* (ATCC 24433) and *Candida pseudotropicalis* (NCYC 6). All organisms were obtained from the Department of Pharmaceutics, Faculty of Pharmacy of Obafemi Awolowo University, Ile - Ife, Nigeria. The bacteria and yeast were sub-cultured into fresh nutrient agar plates and sabouraud dextrose agar plates respectively for 24 hours before use for antimicrobial test.

Preliminary screening for antimicrobial activity using agar well diffusion method

Duplicate nutrient agar plates were seeded with the test bacteria: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis* organism at 45 °C, while *Candida albicans* and *C. pseudotropicalis* were prepared using Sabouraud dextrose agar as the culture medium. The plates containing the bacteria and fungi were incubated at 37 °C and 25 °C for 20 minutes respectively. Holes of 8 mm diameter, equidistant to one another, were bored into the plates using sterile cork borer. Three drops of each test sample were introduced into the holes and allowed to diffuse for an hour before incubation for 24 hours. After the incubation time, the presence of clear zones of inhibition around each extract was noted.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations were determined using the micro broth dilution method as recommended by the Clinical Laboratory Standard Institute (CLSI, 2012). The test was performed in a 96 well micro plate. A volume of 100 µl of Mueller Hinton Broth was first dispensed into each well of the plate, then 100 µl of sample solution were added into the first well to achieve a concentration of 40 mg/ml. Serial dilutions (40, 20, 10, 5, 2.5, 1.25 mg/ml) were carried out by removing 100 µl of reaction medium from the first well into the second well and so on till the tenth well. A volume of 100 µl was then withdrawn from the tenth well and discarded. The eleventh well had no test sample, to serve as the negative control, while the twelfth well had ciprofloxacin at 2 mg/ml, as the positive control for antibacterial study; Ketoconazole at 4 µg/ml was added as the positive control for the antifungal study. A volume of 5 µl of the suspension of each test organisms contain approximately 10^5 cfu/ml was then added to the respective wells. The last row was not inoculated with any organisms to serve as the un-inoculated control. The experiment was carried out in duplicate and the plates were incubated at 37 °C for 24 hours. After the incubation period, the wells were subcultured onto over dried duplicate Mueller Hinton Agar plates using a multi-inoculator before adding a drop of tetrazolium salt to each well and inspecting for colour change, this indicating the presence of viable cells in the wells. The minimum concentration inhibiting the growth of a test organism was recorded as the MIC of test sample against the organism. For the yeast, the medium used was Sabouraud dextrose medium and the incubation temperature was 25 °C.

Determination of minimum bactericidal/fungicidal concentration

A multi-inoculator was used to transfer samples of reaction mixture from each well onto fresh duplicate Mueller Hinton agar plates. The plates were incubated at 37 °C for the bacteria and 25 °C for the yeasts and growth was observed after 72 hours. The wells with the minimum concentration from which no colony was recoverable were taken as indicative of the minimum bactericidal concentration/ fungicidal concentration.

Results

Antimicrobial investigation

The antimicrobial activity of the alcoholic extracts (methanol, ethanol, schnapp, oil palm wine and *Raffia* palm wine) against *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans, C. pseudotropicalis* are shown in Tables 1-5. Both the methanolic and ethanolic extracts of the selected mosses showed no activity against *Echerichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Candida*

albicans and *C. pseudotropicalis*. All other extracts (schnapp 40% alcohol, oil palm wine and *Raffia* palm wine) showed varying degrees of activities against the bacteria and fungi screened.

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) / minimum fungicidal concentration (MFC) of the extracts were shown in Table 6. All the extracts were either bacteriostatic or bacteriocidal / fungicidal against the microorganisms. *Archidium ohioense*, *Pelekium gratum* and *Hyophila involuta* extracts exhibited different degrees of growth inhibition against the tested bacteria and fungi species. The values of MIC ranged between 1.25 - 40 mg/ml and that of MBC were 20 - 40 mg/ml, while MFC ranged from 1.25 - 40 mg/ml for all the extracts. Some extracts were not tested because they were not active against the bacteria and fungi during the preliminary screening.

Table 1. Screening of antimicrobial activity of the methanolic extracts of *Archidium ohioense, Pelekium gratum, Hyophila involuta* against different microorganisms

Mosses/Organism	Activity on selected organisms					
	Esc*	Sta*	Bas*	Psa*	Caa^	Cap^
Archidium ohioense	-	-	-	-	-	-
Pelekium gratum	-	-	-	-	-	-
Hyophila involuta						
Ciprofloxacin 2 mg/ml	-	-	-	-	-	-
(+ve control)	+	+	+	+	NA	NA
Ketoconazole 4 µg/ml	NA	NA	NA	NA	+	+
(+ve control)						

Table 2. Screening of antimicrobial activity of the ethanolic extracts of *Archidium ohioense, Pelekium gratum, Hyophila involuta* against different microoganisms

Mosses/Organism	Activity on selected organisms					
	Esc*	Psa*	Bas*	Sta*	Caa^	Cap^
Archidium ohioense	-	-	-	-	-	-
Pelekium gratum	-	-	-	-	-	-
Hyophila involuta						
Ciprofloxacin 2 mg/ml	-	-	-	-	-	-
(+ve control)	+	+	+	+	NA	NA
Ketoconazole 4 µg/ml	NA	NA	NA	NA	+	+
(+ve control)						

Key:
Esc = *Escherichia coli*; Bas = *Bacillus subtilis*; Sta = *Staphylococcus aureus*; Psa = *Pseudomonas aeruginosa*; *Caa* = *Candida albicans*; Cap = *Candida pseudotropicalis*;
* = Bacteria, ^ = fungi, + = Presence of activity, - = Absence of activity, NA – Not Applicable

Table 3. Screening of antimicrobial activity of the schnapp extracts of *Archidium ohioense, Pelekium gratum, Hyophila involuta* against different microorganisms

Mosses/Organism	Activity on selected organisms					
	Esc*	Psa*	Bas*	Sta*	Caa^	Cap^
Archidium ohioense	-	-	+	+	-	-
Pelekium gratum	-	-	+	+	+	+
Hyophila involuta	-	-	+	+	+	+
Ciprofloxacin 2 mg/ml (+ve control)	+	+	+	+	NA	NA
Ketoconazole 4 µg/ml (+ve control)	NA	NA	NA	NA	+	+

Table 4. Screening of antimicrobial activity of the Oil palm wine extracts of *Archidium ohioense*, *Pelekium gratum*, *Hyophila involuta* against different microorganisms

Mosses/Organism	Activity on selected organisms					
	Esc*	Psa*	Bas*	Sta*	Caa^	Cap^
Archidium ohioense	+	+	+	+	-	-
Pelekium gratum	+	+	+	+	-	-
Hyophila involuta	+	+	+	+	-	-
Ciprofloxacin 2 mg/ml (+ve control)	+	+	+	+	NA	NA
Ketoconazole 4 µg/ml (+ve control)	NA	NA	NA	NA	+	+

Key:
Esc = *Escherichia coli*; Bas = *Bacillus subtilis*; Sta = *Staphylococcus aureus*; Psa = *Pseudomonas aeruginosa*; *Caa* = *Candida albicans*; Cap = *Candida pseudotropicalis*;
* = Bacteria, ^ = fungi, + = Presence of activity, - = Absence of activity, NA – Not Applicable

Table 5. Screening of antimicrobial activity of the *Raffia* palm wine extracts of *Archidium ohioense, Pelekium gratum, Hyophila involuta* against different microorganisms

Mosses/Organism	Activity on selected organisms					
	Esc*	Psa*	Bas*	Sta*	Caa^	Cap^
Archidium ohioense	+	+	+	+	-	-
Pelekium gratum	-	-	-	-	+	-
Hyophila involuta	-	-	-	-	+	-
Ciprofloxacin 2 mg/ml (+ve control)	+	+	+	+	NA	NA
Ketoconazole 4 µg/ml (+ve control)	NA	NA	NA	NA	+	+

Key:
Esc = *Escherichia coli*; Bas = *Bacillus subtilis*; Sta = *Staphylococcus aureus*; Psa = *Pseudomonas aeruginosa*; *Caa* = *Candida albicans*; Cap = *Candida pseudotropicalis*;
* = Bacteria, ^ = fungi, + = Presence of activity, - = Absence of activity, NA – Not Applicable

Table 6. The minimum inhibitory concentration (MIC) mg/ml, minimum bactericidal concentration (MBC) mg/ml and minimum fungicidal concentration (MFC) mg/ml of alcoholic extracts of *A. ohioense, P. gratum*, *H. involuta*

Bacteria/plant extracts	*E. coli*		*P. aeruginosa*		*B. subtilis*		*S. aureus*		*C. albicans*		*C. pseudotropicalis*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
Pelekium gratum (schnapp)	NT	NT	NT	NT	1.25	>40	5.0	20	20	20	1.25	1.25
Archidium ohioense (schnapp)	NT	NT	NT	NT	2.50	>40	5.0	>40	NT	NT	NT	NT
Hyophila involuta (schnapp)	NT	NT	NT	NT	1.25	>40	2.50	20	20	20	5.0	5.0
Hyophila involuta (Oil palm wine)	10	20	20	20	40	>40	10	>40	NT	NT	NT	NT
Archidium ohioense (Oil palm wine)	20	20	20	20	20	>40	10	40	NT	NT	NT	NT
Pelekium gratum (Oil palm wine)	40	40	40	>40	>40	>40	40	>40	NT	NT	NT	NT
Archidium ohioense (*Raffia* palm wine)	20	>40	40	>40	40	>40	40	>40	NT	NT	NT	NT
Hyophila involuta (*Raffia* palm wine)	NT	NT	NT	NT	NT	NT	NT	NT	40	>40	NT	NT
Pelekium gratum (*Raffia* palm wine)	NT	NT	NT	NT	NT	NT	NT	NT	40	>40	NT	NT

Key:
NT = not tested; MIC = Minimum Inhibitory Concentration; MBC= Minimum Bactericidal Concentration; MFC = Minimum Fungicidal Concentration

Discussion

Microbiological tests carried out in the hereby study showed that different bryophytes possess different influence on the growth of microorganisms. Other investigator's report on the antimicrobial properties of bryophytes implies that these mosses could be used as an alternative for the treatment of infections that could be caused by these microorganisms (Isa *et al.*, 2014; Deora and Suhalka, 2016). The result of the extracts from the selected moss studied has varied antibacterial and antifungal activities against the test organisms, except the methanol and ethanol extracts that showed no activity. The possible reason for the data obtained might be varying solubility of various plant metabolites in different solvents (Aibinu *et al*., 2007). It also suggests that the extracts of this plant are broad spectrum in their activities and this agrees with the observation of previous workers that plants contain substances that are antimicrobial (Olukoya *et al*., 1986).

The implication of the broad spectrum action of some of these extracts is that they can be used in antiseptic and disinfectant formulation, as well as in chemotherapy if the active ingredients are isolated (Olukoya *et al*., 1993). The resistance shown by some of the tested organisms to some of the extracts e.g. methanol and ethanol extracts of *Archidium ohioense*, *Pelekium gratum*, *Hyophila involuta* on all the microorganisms screened, should not be seen as absence of antibiotic activity in those extracts, but it may be that the concentration used was too low to inhibit the growth of the organisms or are probably potent against other microorganisms (Isa *et al*., 2014).

In the current study, all the extracts of mosses showed more antibacterial activity than antifungal activity, and this concur with the work of Ertuk *et al.* (2015) who investigated the antifungal, antibacterial and antioxidant activity of 8 different acrocarpous mosses which were tested *in vitro* against 13 different microorganisms and 3 yeast strains; the authors reported that all crude extracts from mosses showed more antibacterial activity compared with antifungal activity. Also, from the study there is a clear indication that the solvent system plays a significant role in the solubility of the active principles in the plant and also influences antibacterial activities. This also indicates that different solvents have different polarities, dispersibility and penetrability and could selectively extract different phytochemicals (Zhang, 2015).

The antimicrobial test conducted hereby revealed that the oil palm wine extracts of these mosses had high activity against the bacteria screened. This implies that the use of palm wine as a solvent possibly releases some active ingredients which have high activity against some enteric organisms. This agrees with the work of Aibinu *et al*. (2007) who investigated the potency of *Citrus aurantifolia* (lime fruit) against pathogens using five different solvents as extractants (distilled water, schnapps, ethanol, Palm wine, fermented water from three days soaked ground maize). The researchers reported that apart from the lime oil, Palm wine extract had the highest activity against both gram-positive and gram-negative isolates and this was closely followed by the schnapps extract and ethanol extract and then aqueous extract and lime juice.

The present study also showed that *Candida albicans* and *Candida pseudotropicalis* were most sensitive to schnapp extracts of *Pelekium gratum* and *Hyophila involuta*. The results obtained are similar to some researchers' report that extracts from mosses displayed anti-fungal activities (Bodade *et al*., 2008). In the study, *Baccilus subtilis* and *Staphylococcus aureus* (Gram-positive bacteria) were found to be more sensitive to the extracts than *E. coli* and *Pseudomonas aeruginosa* (Gram-negative bacteria). The cell wall of Gram-positive bacteria is less chemically complex than that of the Gram-negative bacteria (Lamikanra, 2010). Excellent antimicrobial activities were observed for oil palm wine and schnapp extracts of the studied mosses which may be due to their low MIC and MBC/MFC values. According to El-Mahmood (2009) antimicrobial agents with low activity against a particular organism usually gives high minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) or minimum fungicidal concentrations (MFC) values, while a highly reactive agents gives low MIC and MBC/MFC values.

Conclusions

The investigated mosses can therefore be used to source for antibacterial drugs that can treat infections caused by the susceptible micro-organisms. In the presents study, the microbiological investigations carried out on several moss species have shown activity coherent with their use in medicine and also as a pointer to new source of novel drugs.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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