International Journal of Medicinal Plants and Natural Products (IJMPNP)
Volume 1, Issue 3, 2015, PP 24-30
ISSN 2454-7999 (Online)
www.arcjournals.org

Preliminary Evaluation of the Antimicrobial Potency of an Ectohydric Moss Plant

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Abstract: *Approximately 15,000 to 25,000 bryophyte species are known in the world with moss species being 8000-9000 yet very little is known about the phytochemistry of bryophytes and information concerning research results is very scattered. This study therefore evaluated the phytochemicals and antimicrobial activities of an ectohydric moss plant using standard procedures. Ten principle bioactive compounds were investigated. In all, eight were present in three solvent extracts. These include tannins, steroids, triterpenoids, flavonoids, phenols, cardenolides, terpenoids and balsams.The antibacterial activities of the three different extracts from the moss plant were tested against four bacterial and a yeast isolate by agar well diffusion method. It was observed that chloroform and n-hexane extracts were active on some of the bacteria. In addition, extract of chloroform possessed the highest antibacterial activity against P. aeruginosa, E. coli and Candida albican with minimum inhibition concentration of 100 mg/ml, 100 mg/ml and 200 mg/ml respectively. The current study indicates that extracts of moss plant may be exploited for antimicrobial drugs in the future for the treatment of various ailment*

Keywords: *Ectohydric moss, Bryophyte, Phytochemicals, Antimicrobial activities*

1. INTRODUCTION

For thousands of years man has turned to nature for cures to the numerous diseases that afflict him. In fact with the advancement of medicinal chemistry, some of the earlier drugs were purified compounds from plants, examples include morphine and quinine. Over time, Chemistry has been found effective in utilizing some natural products to produce semi-synthetic compounds such as the case of aspirin and later the penicillins [1,2]. However, in recent time, pharmaceutical companies have moved away from investing in natural product research, yet natural products are still being developed into drugs for treatment of ailments. In fact natural products, natural product derivatives, and synthetic compounds containing a natural product pharmacophore have been reported to constitute 38% of the small molecule pharmaceuticals currently in use and 52% of all anticancer drugs currently available [3]. In the current pharmaceutical research environment, small companies invest in natural product leads and then licensing them to larger companies [2]. This is of vital importance for the treatment of diseases that are capable of developing resistance to current drugs and for those to which no current treatments exist [4]. For these reasons, new chemical entities with new mechanisms of action are needed of which nature has proven to be the ultimate source of such compounds with unique modes of action [5].

Bryophytes are the largest group of plants with about 8000-9000 species of mosses, 6000 species of liverworts and 100 species of hornworts, existing Worldwide [6]. Hundreds of medicinal bryophytes have been identified and classified in ethno botanical literature as potential antimicrobial agents [7]. However, only few of the plants have been thoroughly evaluated by pharmaceutical industries and there has only been a preliminary screening of bryophytes for biological activities [8]. Several hundreds of new compounds have been isolated from bryophytes and their structures elucidated [9]. Chinese, Europeans and North Americans have used bryophytes as medicine for hundreds of years. For example, Chinese have used bryophytes such as *Fissidens* sp. and *Polytrichum* sp. as diuretics and hair growth stimulation tonics. Hence, bryophytes have expressed interesting bioactivities [10]. They are known to posses various relationships with microorganisms and contain a set of various known and unknown secondary metabolites [11]. Previous investigations by various researchers have showed that bryophytes possess extremely high amounts of secondary metabolites such as terpenoids, phenolics (flavonoids and bibenzyl derivatives), glycosides, fatty acids, as well as some rare aromatic compounds [12,13].

This study aimed at extraction of bioactive compounds present in moss plant and hence evaluates the microbial activities of the compounds present.

2. EXPERIMENTAL

2.1 Plant Collection and Preparation

Moss plant sample was collected from wall of buildings around Gwagwalada, Abuja and was identified at the herbarium of the Biological Science Department, University of Abuja. The plant was washed under running water and air-dried for two weeks and then grounded into fine powder using an electric blender. The ground samples were stored in an air-tight, well labelled container from which the samples were removed for further chemical analysis.

2.2 Methanol Extract of Plant Material

The method of extraction adopted was the cold maceration method. About 60 g of the powdered plant material was packed into a conical flask and 250 ml of methanol was added. The flask was sealed with a stopper, shook vigorously and left to stand for 48 hours. The resulting extract was then filtered and concentrated by evaporation to dryness on a water bath. The extract was weighed and stored in a labelled sample bottle for further analysis.

Partitioning of the aqueous solution of the extract in separating funnel was carried out with equal volume of n-hexane, chloroform and n-butanol solvents. Each extract were evaporated over a water bath, stored in separate sterile bottles and refrigerated for further analysis.

2.3 Phytochemical screening

Qualitative chemical tests were carried out on the three partitioned extracts. Tannins, steroids and triterpenoids (Salkowski Test), glycosides (Boron Trager's test), saponins, cardenolides, terpenoids, flavonoids, balsams and presence of phenols were all tested for using standard procedures [14].

2.4 Antimicrobial Test

The test organisms used were pure isolate of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and yeast* of candida albican. Pure cultures of the test isolates were obtained from the Microbiology laboratory, University of Abuja Teaching Hospital. The bacteria isolates were first subcultured in a nutrient agar and incubated at 37 ^{o}C for 24 hours.

Antimicrobial activity of the plant extracts were carried out using agar diffusion method. Six different dilutions of each of the extracts were made by measuring 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg from the different extracts and 1 ml of the corresponding solvent was added to the measured extract in a test tube to obtain 100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml, 500 mg/ml, 600 mg/ml concentrations of each of the extract. The bacteria and yeast colonies on the nutrient agar were streaked to cover the entire surface of a new nutrient containing six wells and 0.1 ml each of the resulting mixture was transferred into each of the 6 well and incubated again for 24 hrs at 37^{o}C. Zone diameter of inhibition of growths were measured and recorded to the nearest millimeter. Standard antibiotic drugs (septrin, gentamycin and tetracycline) were used as positive control to provide and evaluate the degree of inhibition of the extracts due to its broad spectrum.

2.5 Minimum Inhibitory Concentration (Mic)

Minimal inhibitory concentration (MIC) was carried out on the sample extracts. The MIC was considered the lowest concentration of the sample that prevented visible growth. Minimum bactericidal concentrations (MBCs) were determined by subculturing, 10 ml from each negative tube and from the positive growth control. MBCs were defined as the lowest concentration yielding negative subcultures or only one colony. All samples were examined in duplicate in three separate experiments [15].

3. RESULT AND DISCUSSION

The results of the phytochemical screening and antimicrobial activities of moss plant sample are as presented below:

Table1. *Phytochemicals from moss plant extract*

Phytochemicals	n-Hexane	Chloroform	n-Buthanol
Tannins	+	+	+
Steroids	+	-	-
Triterpenoids	+	-	-
Glycoside	-	-	-

Saponins	-	-	-
Phenols	+	+	-
Cardenolides	+	+	-
Terpenoids	-	+	+
Flavonoids	+	+	+
Balsams	+	-	-

Keys: **+** = *present*, **-** = *absent*

Table2. *Zone of inhibition (mm) of the antimicrobial activities of the n-hexane, chloroform, and n-butanol extract of moss plant against test isolates*

Isolates	Concentration (mg/ml)	n-Hexane	Chloroform	n-Butanol
P. aeruginosa	100	NI	10	NI
	200	NI	13	NI
	300	NI	14	NI
	400	NI	14	NI
	500	NI	16	NI
	600	NI	17	NI
E.coli	100	13	18	NI
	200	16	20	NI
	300	17	27	NI
	400	17	29	NI
	500	23	31	NI
	600	25	32	NI
K. pneumonia	100	NI	NI	NI
	200	NI	NI	NI
	300	NI	NI	NI
	400	NI	NI	NI
	500	NI	NI	NI
	600	NI	NI	NI
S. aureus	100	NI	NI	NI
	200	NI	NI	NI
	300	NI	NI	NI
	400	NI	NI	NI
	500	NI	NI	NI
	600	NI	NI	NI
C. albican	100	NI	NI	NI
	200	NI	4	NI
	300	NI	5	NI
	400	NI	5	NI
	500	NI	8	NI
	600	NI	8	NI

Keys*: E.coli- Escherichia coli, S. aureus - Stapylococcus aureus, K. pneumonia – Klebsiella pneumonia, P. aeruginosa - Pseudomonas aeruginosa, C. albican - Candida albican, NI = No inhibition*

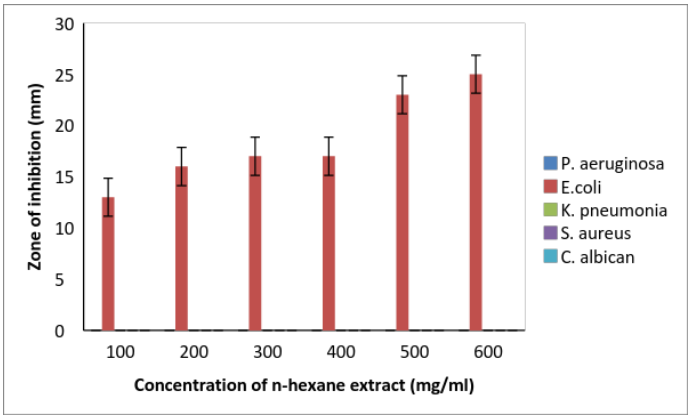


Figure1. *Zone of inhibition (mm) of the antimicrobial activities of the n-hexane extract of moss plant against test isolates*

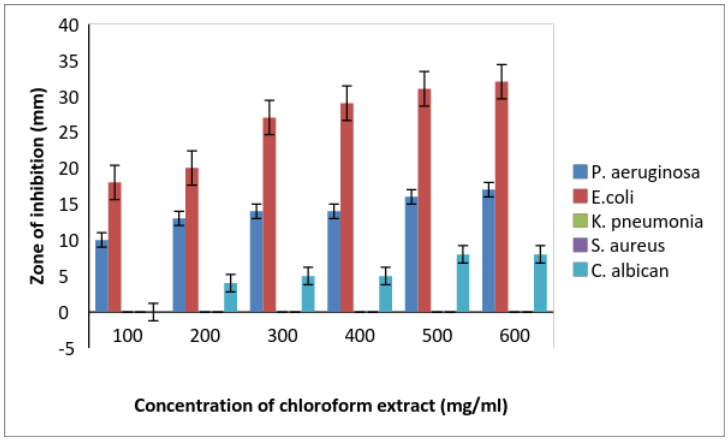


Figure 2. *Zone of inhibition (mm) of the antimicrobial activities of the chloroform extract of moss plant against test isolates*

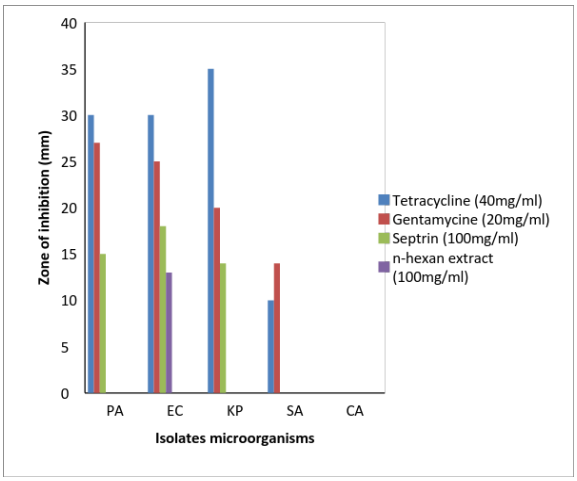
Table3. *Zone of inhibition (mm) of the antimicrobial activities of the control drugs against test isolates*

Isolates	Tetracycline (40 mg/ml)	Gentamycine (20 mg/ml)	Septrin (100 mg/ml)
P. aeruginosa	30	27	15
E.coli	30	25	18
K. pneumonia	35	20	14
S. aureus	10	14	NI
C. albican	NI	NI	NI

Table4. *Minimum Inhibitory Concentration (MIC) (mg/ml)*

Isolates	Extracts	MIC (mg/ml)					
		600	500	400	300	200	100
P. aeruginosa	n-Hexane						
	Chloroform						X
E.coli	n-Hexane						X
	Chloroform						X
K. pneumonia	n-Hexane						
	Chloroform						
S. aureus	n-Hexane						
	Chloroform						
C. albican	n-Hexane						
	Chloroform					X	

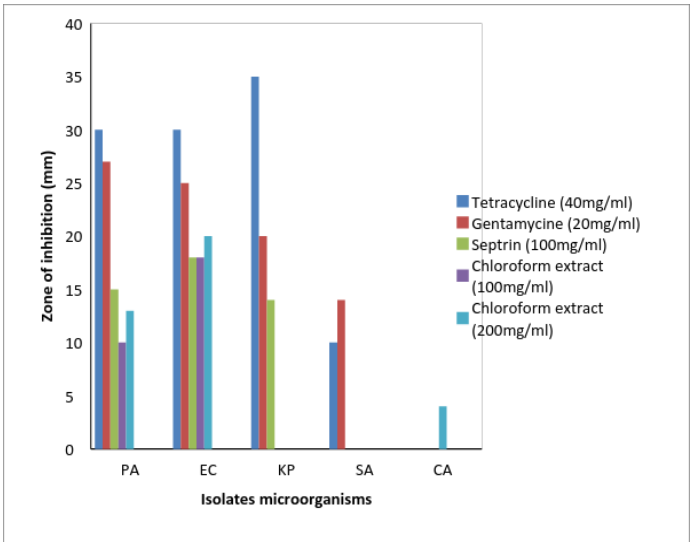
Key: *X= MIC*



Keys:

PA - *Pseudomonas aeruginosa*, EC- *Escherichia coli*, KP – *Klebsiella pneumonia* SA- *Stapylococcus aureus*, CA - *Candida albican*

Figure3. *Comparing the sensitivity of the MIC for n-hexane extracts with the control drugs against test isolates*



KEYS: *PA - Pseudomonas aeruginosa, EC- Escherichia coli, KP – Klebsiella pneumonia SA- Stapylococcus aureus, CA - Candida albican*

Figure4. *Comparing the sensitivity of the MIC for chloroform extracts with the control drugs against test isolates*

From table 1, the phytochemical analysis of the moss extracts showed the presence of tannins, steroids, triterpenoids, flavonoids, phenols, cardenolides and balsams in at least one of the three extract but showed the absence of Glycosides and saponins in all the moss extracts. The absence of glycoside and saponins in the sample could be due to the conditions in which this work was carried out, ecological and geographical factors of the plant, age of plants, method of extraction or extracting solvents. Similar studies by Savaroglu et al. [16] on mosses species investigated had alkaloids, anthraquinones, terpenoids and flavonoids. The presences of some of these phytochemicals show the potentials of moss plant for medicinal use [17]. For example, flavonoids in the extract could indicate that the extract could be used for asthma and venereal diseases and also indicates the presence of natural occurring phenolic compound which has beneficial effects in the human diet as antioxidants and are able to scavenge and neutralize free radicals. Since flavonoids are known to be synthesized by plants in response to microbial infection, it is not surprising that they have been found to be effective antimicrobial substances against microorganisms. Their activity is probably due to ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes [18,19].

Also, presence of tannins in the plant extract suggests the ability of this plant to play a major role as anti diarrhea and anti hemorrhagic agent [20]. Tannins have been found to form irreversible complexes with prolinerich protein resulting in the inhibition of protein synthesis [20]. Tannins are known to react with protein to provide the typical tannin effect which is important for the tannin of inflamed or ulcerated tissues [21]. Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders, diarrhea and dysentery. Terpenoids represent another class of secondary metabolites which benefit the producing organisms with improved pathogen resistance [22]. In this study, one or more of the antimicrobial activities observed in the extracts of the moss plant could be said to be active terpenoids. Another compound observed is steroids which are known to have relationship with sex hormones. Plants with this chemical are used as vegetables for expectant mothers or breast feeding mothers to ensure balance, since steroidal structure could serve as a potent starting material of this hormone.

The preliminary results of screening showed that according to the extraction solvents used, the mosses plant exhibited a distinct difference in antimicrobial activities (Table 2). In this study, out of the three extraction solvent used, only hexane and chloroform extract showed some sensitivities on the microorganism isolates used (Figure 1 and 2). n-butanol extract showed no sensitivity on any of the microorganism. According to the size of the inhibition zones of different crude extracts, the chloroform extract gave results that were more appreciable than the n-hexane extract. In other words, while the n-hexane crude extracts considerably inhibited growth of only *E. coli* at all the prepared

concentration, the chloroform crude extracts inhibited both *P. aeruginosa* and *E. coli* at all the prepared concentrations and *Candida albican* from 200mg/ml to 600 mg/ml. It can therefore be concluded that the chloroform extract, being a non polar solvent, inhibited growth of microorganisms more than the other extracts that are polar in nature. Similar results has been reported by Savaroglu et al. [16], where methanol extracts from mosses demonstrated a poor effect against the selected bacteria species in general while chloroform, acetone, and ethyl acetate extracts had a higher potency. Russell reported that none of the mosses investigated in a study showed any antibiotic activity when methanol extracts were used [23]. In addition, Bodade et al. stated that ethanol, acetone, and chloroform extracts of Bryophytes were found to be more effective than methanol extract [24].

Table 4 illustrates the MIC ranges of selected extracts against microorganism strains. The MIC as compared to that of the control drugs values for n-hexane and chloroform extracts against pathogens ranged from 100 and 200 mg/ml (figure 3 and 4). The results of the microdilution method showed that chloroform extract of *moss* possessed the highest antimicrobial potency, with a MIC of 100 mg/ml on P. aeruginosa and *E. coli* and 200 mg/ml on *Candida albican*. Also, n-hexane extract has MIC value of 100 mg/ml on E. coli.

4. CONCLUSION

Of the mosses investigated, the inhibition effect seen against two bacteria and a yeast isolate by chloroform and hexane extracts suggests that it may be used as a broad spectrum antibiotic in the future. The presence of some secondary metabolites (tannins, steroids, triterpenoids, flavonoids, phenols, cardenolides and balsams) in the extracts might be the reason for this observation. Chloroform extracts demonstrated more antibacterial activity against *P. aeruginosa*, *E. coli* and *Candida albican*. These results indicate that the extracts investigated should find a practical application in the prevention of and protection against both gram (+) bacterial infections in plants, animals and humans. It is therefore recommended that further research should be carried out on moss plant especially on the isolation and characterization of the bioactive chemical constituents from the active fractions and their mode of action on microbial cells in order to determine the major components present for complete and accurate medicinal application.

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