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Antimicrobial Activity of Three Mosses, Calymperes Erosum Müll. Hal., Racopilum Africanum Mitt. , Cyclodictyon Mitt. From Southwest Nigeria

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Abstract: *Although antimicrobial activity of some European, U.S.A. and Asian mosses have been reported, testing of the sub-Saharan Africa species are comparatively new. Antimicrobial activity of three south western Nigerian mosses,Calymperes erosum, Racopilium africanum and Cyclodictyon sp. on pathogenic microorganisms was evaluated by standard microbial assay. Different solvent extracts of the mosses were obtained and the pH was adjusted to 7.0. Antimicrobial effects of these extracts were determined by agar diffusion method on test pathogenic microorganisms. The result was then compared with the synthetic standard antibiotics ampicillin (20 µg/ml), gentamycin (10 µg/ml) and nystatin (100 mg/ml). The moss extracts were found to be active against all of the test microorganisms (p<0.05). The extracts that displayed antibacterial activity were always effective against the same organisms and consistent in magnitude of inhibition. Ethanolic, methanolic and acetone extracts were found to be more effective on Staphylococcus aureus and Escherichia coli. Among the fungi Microsporum gypseum and Aspergillus niger were most sensitive to the ethanolic extracts of the three mosses.*
Keywords - *Calymperes erosum, Racopilum africanum, Cyclodictyon spp. Antimicrobial, Nigeria*

I. Introduction

Inspired of their wide distribution all over the world bryophytes (mosses, liverworts and hornworts) remain an untapped pharmaco-industrial resource [1] [2] [3]. To harness the potentials of mosses for human health and industry, their biological and chemical properties must be studied and documented. Although the occurrence of antibiotic substances in bryophyte have been documented by some bryologists and microbiologist [4] [5] [6] [7] [8]. The search for potent antimicrobial agents has been intensified in recent years, due to an increasing global concern about the rapid increase in the rate of infections by antibiotic resistant microorganisms [9]. Indeed pharmaceuticals corporations now randomly screen plants for new active compounds to promote new technologies and to intensify research in combating drug resistant microbes [10] [11] [12] [13].

Although efforts involving numerous surveys on antimicrobial bryophytes have been made in the United States, Asia and Europe testing antimicrobial activity of sub-Saharan African bryophytes is comparatively new. The biologically active compounds found in bryophytes are of pharmaceutical value [1] [2] [14] [15] [16]. In some ethnic groups the antimicrobial effect of bryophytes has been known from time immemorial to treat burns and open wounds [17]. Chinese traditional medicine names 40 kinds of bryophytes that were used to treat illnesses related to heart and circulatory system [18]. The extract of Rhodobryum giganteum a moss was clinically tested which produced an increased aorta blood transit by 30 % and protective effects against myocardial hypoxia/reoxygenation in mice [19] [20].

In the present work, three selected common mosses were evaluated for their antimicrobial activity against the selected bacterial and fungal pathogenic strains. This was done with a view to exploring their potentials as candidates for drug manufacture.

II. Materials And Methods

2.1 Plants material and sites

Calymperes erosum and Racopilum africanum were collected from a Apoje plantation in trunk pockets of Elaeis guineensis (Lat 7.018198 N; Long 4.110811E; altitude 87 m) The material of submerged Cyclodictyon sp. was collected from the wall of a 60 year old ancient water well (Lat 6.952579 N; Long 3.947676 E; altitude

63 m) in Oru-Ijebu town. The mosses were identified at the Herbarium of the Department of Botany and Microbiology University of Ibadan, Nigeria. Voucher Specimen Oyesiku 613; 154; 23 were deposited at the PE Herbarium IB-CAS Chinese Academy of Science.

2.2 Plant extraction procedure

The plant material was carefully picked from attached litter and decaying materials and washed under running tap water and air dried. Dried powder (500 g) of each moss material was exhaustively macerated for 48 h with 500 ml of 70% Acetone, 95% Ethanol and 100% Methanol. Each filtrate was centrifuged at 3,000 rpm for 10 min., sterile filtrate obtained from the supernatant using Millipore 0.20 µm (Minisart Non-pyrogenic) and concentrated over water bath. The pH of the extract was adjusted to 7.0 [8].

2.3 Microorganisms

Standard strains of pathogenic bacteria used were Bacillus subtilis Gram (+) FIIRO, Staphylococcus aureus Gram (+) FIIRO, Streptococcus pyogenes Gram (+) FIIRO, Escherichia coli Gram (-) FIIRO, Klebsiella pneumonia Gram (-) FIIRO. Standard strains of pathogenic fungi: Aspergillus niger FIIRO, Candida albicans FIIRO , Microsporum gypseum FIIRO, Saccharomyces cerevisiae FIIRO and Trichoderma spp. FIIRO were used in the study. Cultures were collected from the Department of Biotechnology, Federal Institute of Industrial Research Oshodi (FIIRO), Nigeria.

2.4 Antimicrobial assay

Antimicrobial activities of the sample extracts were determined by agar well diffusion method [21] [22]. Petri dishes containing sterilized Nutrient and Czapek's Dox agar (3% agar) were used as media for sample cultures. Solidified medium was seeded with 50 µl of broth (1.5% agar) pure culture strains and poured in Petri dishes and mixed gently to spread and allowed to diffuse. Wells of 6 mm diameter were cut with sterile cork borer to a depth of 12.5 mm. Each well was filled with 50 µl test extract of each sample.

All extraction solvents and sterile agar plates were used as negative controls. Standard antibiotics, Gentamycin (10 μgml^{-1}), Ampicilin (20 μgml^{-1}) for bacteria and Nystatin (100 $mgml^{-1}$) for fungi were used as positive control. Bacteria cultures were incubated at 37 ±0.5 ^{o}C for 24 h and antifungal cultures at 28 ^{o}C for 48 h. Inhibition zone diameters were expressed in millimeters.

2.5 Statistical analysis

Extracts were tested in triplicate and One-way ANOVA (SPSS version 16.0) was used for the analysis of the data. P values of <0.05 were considered statistically significant.

III. Results

Antimicrobial activities of three mosses extracted with three different solvents and tested on ten pathogenic microbes were shown in Figs 1, 2 and 3. Ethanolic extracts of the tested mosses showed a broad spectrum of antimicrobial potential with strong zones of inhibition while methanolic and acetone extracts had weak zones of inhibition.

The average inhibition zone of tested bacteria ranged from 11 mm (Streptococcus pyogenes) to 20 mm (Escherichia coli). Escherichia coli was significantly ($p < 0.05$) inhibited compared to other tested bacteria. Similarly the average inhibition zone of tested fungi ranged from 8 mm (Trichoderma spp.) to 20 mm (Aspergillus niger). Aspergillus niger and Microsporum gypseum) were significantly ($p < 0.05$) higher than other tested fungi (Fig.1).

The average inhibition zone of tested extracts ranged from 11 mm (Cyclodictyon spp.) to 16 mm (Calymperes erosum). Calymperes erosum exhibited higher inhibition zone, but there were no significant differences ($p > 0.05$) among the three moss extracts. The standard drugs showed strong activity against all tested bacteria and fungi with range of 20 mm to 30 mm and average of 25 mm. Ampicillin and Nystatin showed a significant difference ($p < 0.05$) against Gentamycin and the plant extracts (Fig. 2).

The average inhibition zone of solvent extracts ranged from 10 mm (Methanol) to 19 mm (Ethanol). Ethanol extract inhibited tested bacteria and fungi significantly ($p < 0.05$) while methanol was least (Fig. 3).

IV. Discussion

This study reports the antimicrobial activity of selected indigenous mosses with different extracting solvents. Three extracts showed levels of activity against the test microorganisms, the ethanol extract was found to be more active than other two, methanol and acetone extracts. Ethanol extract of mosses and liverworts is reported to show antibiotic activity. Ethanol extracts of dry shoots of Sphagnum magellanicum as reported by [5] show high inhibitory effect on certain bacterial strains and Bryum argenteum as reported by [23] show significant antimicrobial activity in vivo than in vitro. This suggests that specific antibacterial compounds are isolated by ethanol and are effective against specific bacteria spp. [24].

Sensitivity of B. subtilis, S. aureus and Streptococcus pyogenes to C. erosum and R. africanum acetone extracts were relatively high as shown by stronger inhibition zone (18 mm) in this study. The acetone extract had greater inhibitory activity compared to that of methanol extracts which showed no visible inhibitory activity. The zones of inhibition showed by B. subtilis and S. aureus with acetone and methanol extracts corroborate [25] report that acetone extract had greater inhibition zone (12 mm) against B. mycoids, while methanol extract exhibited weaker inhibition zone (8 mm). Both solvent extracts were inactive against tested fungi, C. albicans, Saccharomyces cerevisiae and Trichoderma spp. These results necessitate further investigation into the choice of solvents. [4] report extract of fresh S. magellanicum in mixture of acetone and hexane show positive results against some bacterial strains and fungal strains.

Among the three mosses, C. erosum and R. africanum extracts were most active against bacteria and fungi while Cyclodictyon extract was the most effective antifungal. The antimicrobial activity reported in this paper might be due to the presence of flavonoids, steroids, terpenoids and other polyphenolic compounds [2] [6] [25] which were not screened in this work.

Conventionally, antibiotics are generally more active against Gram (+) than Gram (-) bacteria. [24]. Compared with Gram (+) bacteria, Gram (-) bacteria are more resistant against antibiotics because of their protective outer coat of lipopolysaccharide. However, antibacterial activity of the tested mosses was found to be more active against Gram (-) bacteria. The sensitivity of E. coli and K. pneumonia to the test extracts in the present study seems to show that some mosses bioactive compounds may have advantage over drug resistant antibiotics used against Gram (-) bacteria.

Our study has shown that the selected fungi were generally susceptible to the extracts of the mosses. Of all the fungi, A. niger and M. gypseum were most sensitive to ethanolic extract of Cyclodictyon. Ampicilin and Nystatin seems to be most effective compared to all the tested moss extracts.

V. Conclusion

This study has shown that some tropical mosses may be useful as sources of natural antimicrobial compounds against drug resistant antibiotic microbes. In the light of this more sub-Saharan African mosses and other bryophytes need to be screened for antimicrobial activity. Furthermore, their bioactive compounds should be detected and characterized.

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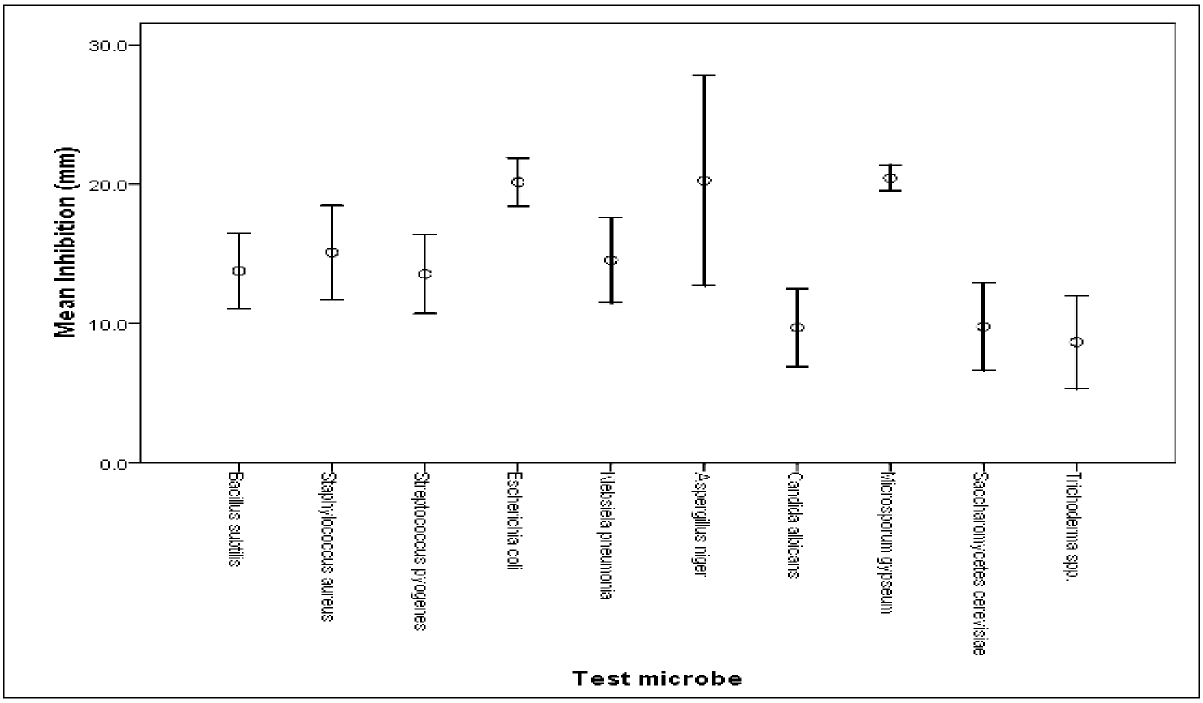

Fig. 1 Sensitivity of test microbes to test moss extracts. Error bars = 95% Cl, n=3

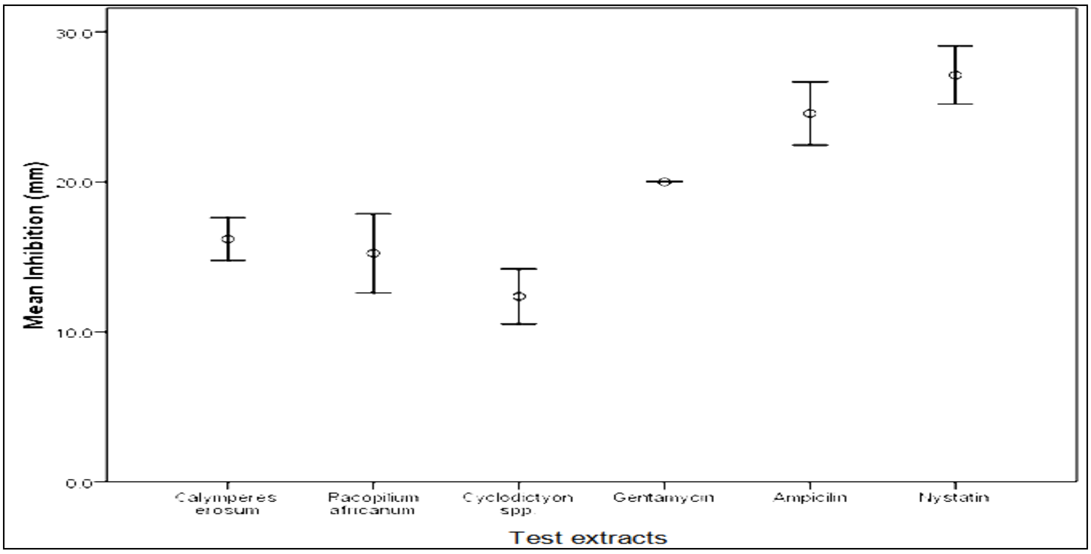

Fig. 2 Strength of test moss extracts in inhibiting the growth of test microbes. Error Bars: 95% Cl, n=3

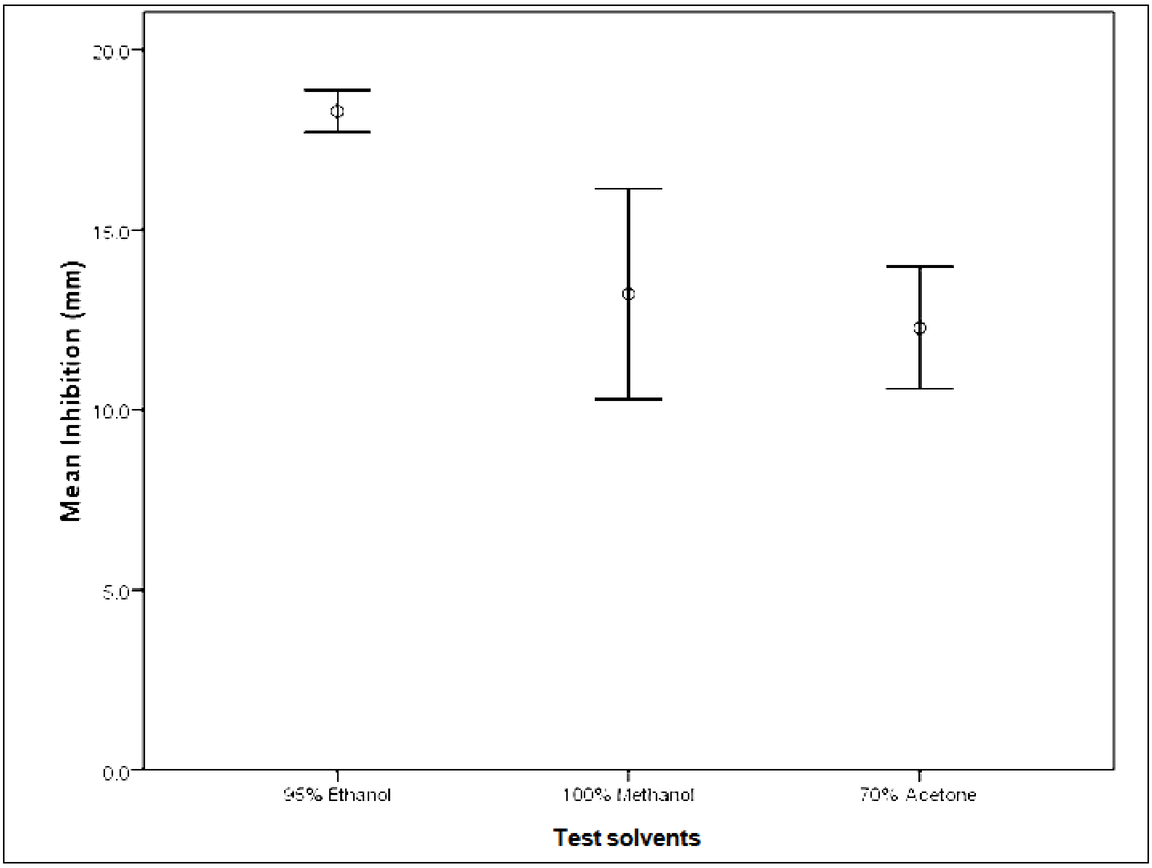

Fig. 3 Solvents extraction tendencies. Error bars = 95% Cl, n=3