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In Vitro Antibacterial Activity of Two Mosses: *Calymperes Erosum* C. Mull and *Bryum coronatum* Schwaegr from South-Western Nigeria

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Abstract

Bryophytes are poikilohydric in nature and the oldest known land plant. Their striking resistance to microbial attack suggests their inherent production of antibacterial compounds. The antibacterial activity of acetone, ethanol, methanol and hexane extracts of *Calymperes erosum* C. Mull and *Bryum coronatum* Schwaegr were investigated against twenty clinically important bacteria pathogens. Agar dilution method was used to assess the effectiveness of the extracts on the test organisms. The minimum inhibitory concentrations of the extracts of *C. erosum* were between <0.625 and >5.0 mg/ml. *Klebsiella pneumoniae* ATCC 10031, *Enterococcus feacalis* ATCC 29212, *Bacillus pumilis* ATCC 14884 and *Enterobacter cloaca* ATCC 13047 in decreasing order are most sensitive to the extracts while *Proteus vulgaris* KZN, *Staphylococcus aureus* OK2 and *Shigella sonnei* ATCC 29930 were resistant to the extracts. Ethanolic extract was the most effective among the extracts followed by acetone extract. *B. coronatum* had relatively lower activity. While the mosses screened proved to be promising sources of antimicrobial and biologically active compounds, their toxicity and action mechanism still needed to be investigated.

Keyword: *Calymperes erosum*, *Bryum coronatum*, Mosses, Antibacterial, Pathogen, bryophytes

1. Introduction

Bryophytes represent the second largest group of land green plants after angiosperms and are taxonomically placed between algae and pteridophytes (Asakawa, 2007). The bryophytes group consist of three subgroups: Bryophyta (mosses), Marchantiophyta (liverworts) and Anthocerotophyta (hornworts) (Campbell and Reece, 2002). It is estimated that there are between about 15000 and 25000 bryophyte species known in the world (Goffinet and Shaw, 2009).

Bryophytes have long been considered to be insignificant in the economy of man except for those used in packing, plugging and decoration. However, Saxena and Harrinder (2004) reported that the ecological role of bryophytes in any ecosystem is significant. In China where herbal medicine is extensively and widely accepted, bryophytes have been used as "crude drugs". Many medicinal bryophytes have been recognized (Madson and Pates, 1952; Wu, 1977; Ding, 1982; Ando, 1983). In particular, the occurrence of antibiotic substances in bryophytes has been documented by scientists (Dulger, 2005; Bodade *et al*, 2008; Russell, 2010; Elibol *et al*, 2011; Savaroglu *et al*, 2011). Although bryophytes normally grow in humid habitats, they are relatively free from microbial attacks and this scarcity of disease indicates that bryophytes are able to elaborate constitutive or inducible small – molecule antimicrobials. In fact, bryophytes have been proven to be a rich source of antibiotics and attempts to find potent, non-toxic broad spectrum antibiotics from the sources have been widely undertaken (Xie and Lou, 2009). However, reports of ethnobotanical researches into this plant group are minimal (Zhu *et al*, 2006). The reasons for this are the difficulty that researchers have with their identification, the limited amount of the same species available for analyses due to their inconspicuous position in the ecosystem and the difficulty with which analysis can be conducted since it relies on sophisticated methods (Savaroglu, 2011). Though, few studies have successfully investigated the chemistry of bryophytes (Asakawa, 2001; Jockovic *et al*, 2008), it is generally known that bryophytes possess extremely high amounts of terpenoids, phenolics (flavonoids and bibenzyl derivatives), glycosides, fatty acids as well as some rare aromatic compounds (Jockovic *et al*, 2008; Sabovljevic *et al*, 2008).

Recently, the public demand for herbal medicine and the rise of antibiotic-resistant bacteria have motivated scientists to look for new natural sources with potential pharmaceutical capabilities (Cowan, 1999). Zhu *et al* (2006) suggested that bryophytes are one of the most significant and promising sources of antibiotics and biologically active compounds in nature.

This study analysed the antimicrobial activities of different extracts of *Calymperes erosum* C. Mull and *Bryum coronatum* Schwaegr against some bacterial species.

2. Materials and methods

2.1 Plant Materials

Plant materials were collected in March 2012 in growing areas in Ekiti State University, Ado-Ekiti. The species was authenticated at the Herbarium section of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria. Voucher specimens were prepared and deposited in the Herbarium of the University for reference. Plant samples were air dried for

four days and pulverized. Powdered plant material (40 g each) was separately extracted in acetone, ethanol and methanol for 48 h with periodic manual shaking after which the extracts were filtered through Whatman No. 1 filter paper. The extracts were evaporated to dryness under reduced pressure at 40 ℃ using a rotary evaporator (Laborota 4000-efficient, Heldolph, Germany). Individual crude extracts was diluted using 5% dimethylsulphoxide to give 50 mg/mL stock solution (Taylor *et al*., 1995). This was then diluted to the required concentrations for the bioassay.

2.2 Test Organisms

Twenty bacterial strains used in this study were obtained from the Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria. Eight Gram positive were used, the isolates include: *Bacillus cereus* ATCC 10702, *Bacillus pumilis* ATCC 14884, *Bacillus subtilis* KZN, *Enterococcus feacalis* ATCC 29212, *Micrococcus luteus*, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* OK1 and *Staphylococcus aureus* OK2. The Gram negative organisms used include: *Acinetobacter calcoaceticus* CSIR, *Escherichia coli* ATCC 25932, *Escherichia coli* ATCC 8739, *Enterobacter cloaca* ATCC 13047, *Klebsiella pneumoniae* ATCC 10031, *Klebsiella pneumoniae* KZN *Pseudomonas aeruginosa* ATCC 19582, *Proteus vulgaris* KZN, *Proteus vulgaris* ATCC 6830, *Proteus vulgaris* CSIR 0030, *Serratia mercesscens* ATCC 9986 and *Shigella sonnei* ATCC 29930. Each strain was maintained on Nutrient Agar (Oxoid) plates. The grown cultures were used for preparation of bacterial suspensions in sterile distilled water with densities adjusted to 0.5 McFarland Standard.

2.3 Antibacterial Activity Assay

Antibacterial activity was determined by the methods of Afolayan and Meyer (1997) using Mueller-Hinton agar (Oxoid). Briefly, different concentrations of the extracts were prepared in 5% Dimethyl Sulfoxide (DMSO) while diluent (5% DMSO) was used as control. Molten medium containing the extracts at final concentrations of 0.625, 1.25, 2.50 and 5.00 mg/ml were poured into Petri dishes, swirled gently until the agar began to set, and left over night for solvent to evaporate completely. Agar plates containing 1% of the extracting solvents were used as controls. The test organisms were streaked in radial pattern on the agar plates, incubated under aerobic conditions at 37 ^{o}C and examined after 24 h. Each treatment was performed in triplicate and complete suppression of growth at a specific concentration of an extract was required for it to be declared active (Sindambiwe *et al*., 1999; Mathekga *et al*., 2000). The lowest concentration that inhibits the growth of the organisms was recorded and considered as the minimum inhibitory concentration (MIC) value.

Table 1. Minimum inhibitory concentrations (MICs) of extracts of *C. erosum*

Gram Reaction	Isolates	Extractants			
		Acetone	Ethanol	Methanol	Hexane
Positive	*B. cereus* ATCC 10702	2.50	<0.625	1.25	>5.0
	B. pumilis ATCC 14884	2.50	<0.625	2.50	<0.625
	B. subtilis KZN	0.625	>5.0	5.00	>5.0

	Enter. feacalis ATCC 29212	2.50	<0.625	1.25	0.625
	M. luteus	2.50	5.00	5.00	2.50
	St. aureus ATCC 6538	2.50	<0.625	>5.0	2.50
	St. aureus OK1	>5.0	1.25	5.00	5.00
	St. aureus OK2	2.50	5.00	>5.0	5.00
Negative	*A. calcoaceticus* CSIR	0.625	1.25	<0.625	2.50
	E. coli ATCC 25932	2.50	<0.625	>5.0	2.50
	E. coli ATCC 8739	1.25	<0.625	>5.0	2.50
	Ent. cloaca ATCC 13047	1.25	<0.625	2.50	5.00
	K. pneumonia ATCC 10031	1.25	<0.625	0.625	<0.625
	K. pneumonia KZN	2.50	1.25	>5.0	<0.625
	Ps. aeruginosa ATCC 19582	1.25	<0.625	2.50	<0.625
	P. vulgaris KZN	>5.0	5.00	5.00	2.50
	P. vulgaris ATCC 6830	<0.625	<0.625	5.00	>5.0
	P. vulgaris CSIR 0030	0.625	2.50	>5.0	2.50
	Se. mercescens ATCC 9986	2.50	1.25	>5.0	5.00
	Sh. sonnei ATCC 29930	5.00	5.00	>5.0	2.50

Table 2. Minimum inhibitory concentrations (MICs) of extracts of *B. coronatum*

Gram Reaction	Isolates	Extractants			
		Acetone	Ethanol	Methanol	Hexane
Positive	*B. cereus* ATCC 10702	>5.00	>5.00	2.50	2.50
	B. pumilis ATCC 14884	1.25	>5.00	5.00	>5.00
	B. subtilis KZN	>5.00	5.00	>5.00	>5.00
	Enter. feacalis ATCC 29212	>5.00	>5.00	0.625	>5.00
	M. luteus	5.00	>5.00	>5.00	>5.00
	St. aureus ATCC 6538	>5.00	>5.00	>5.00	>5.00
	S. aureus OK1	>5.00	2.50	2.50	0.625
	S. aureus OK2	5.00	5.00	>5.00	>5.00
Negative	*A. calcoaceticus* CSIR	0.625	0.625	1.25	5.00
	E. coli ATCC 25932	>5.00	>5.00	>5.00	>5.00
	E. coli ATCC 8739	>5.00	0.625	2.50	>5.00
	Ent. cloaca ATCC 13047	>5.00	>5.00	>5.00	0.625
	K. pneumonia ATCC 10031	>5.00	5.00	>5.00	>5.00
	K. pneumonia KZN	>5.00	>5.00	>5.00	>5.00
	Ps. aeruginosa ATCC 19582	1.25	>5.00	5.00	0.625
	P. vulgaris KZN	0.625	0.625	>5.00	0.625
	P. vulgaris ATCC 6830	>5.00	>5.00	>5.00	>5.00
	P. vulgaris CSIR 0030	>5.00	>5.00	0.625	>5.00
	Se. mercesscens ATCC 9986	2.50	>5.00	0.625	>5.00
	Sh. sonnei ATCC 29930	2.50	>5.00	5.00	1.25

3. Results and Discussion

The emerging resistant bacteria pathogens proved to be serious threat to human health globally (Sanchez and Kouznetsov, 2010; Lopez-Pueyo *et al*., 2011). Treatment with orthodox medicine is ineffective in most cases due to different resistant mechanisms hence the need for cheap, effective and natural medicinal compounds (Basile *et al*., 1998; Saboljevic *et al*., 2006). Nearly all bryophytes are known to resist fungi and bacteria attack. They are also not consumed by molluscs, insects and mammals (Basile *et al*., 1998; Asakawa, 2001). Compared to vascular plants, the use of bryophytes as antibacterial agent is scanty despite their worldwide occurrence. The antibacterial properties of acetone, ethanol, methanol and hexane extracts of *C. erosum* and *B. coronatum* were screened against twenty medically important bacteria. The extracts showed varying degrees of inhibitory effects on the test organisms with the control (5% DMSO) has no inhibition on the organisms. Most of the active substances in medicinal plants are in aromatic or saturated organic nature. Some of the substances are reported to be easily extracted by polar and non-polar solvents (Cowan, 1999; Altuner *et al*., 2011). The MICs ranges were outside the test concentrations. They were between <0.625 and >5.0 mg/ml. *Klebsiella pneumoniae* ATCC 10031, *Enter. feacalis* ATCC 29212, *B. pumilis* ATCC 14884 and *Ent. cloaca* ATCC 13047 in decreasing are most sensitive to the extracts of *C. erosum* while *P. vulgaris* KZN, *S. aureus* OK2 and *S. sonnei* ATCC 29930 were resistant to the extracts. Mosses are a rich source of secondary metabolites with antimicrobial activity (Asakawa, 1981, Sabovljevic *et al*., 2006; Asakawa, 2007; Mellegard *et al*., 2009). Different antimicrobial agents have been isolated from the bryophytes and they are used for the treatment of infectious diseases (Dulger *et al*., 2005; Singt *et al*., 2006; Dulger *et al*., 2009; Bukvieki *et al*., 2012). Ethanolic extract was the most effective among the extracts followed by acetone extract. Ethanol and acetone invariably extract the active compound from the plant at a rate or amount higher than the other extractants. According to Nikolajeva *et al*. (2012), ethanolic extract has the best activity against pathogenic bacteria. They reported that extracts of seven out of 11 bryophytes were effective in curtailing the growth of *Staphylococcus aureus*. Khanam *et al*. (2011) reported ethanolic extracts of *Marchantia palmata* to have high antibacterial activity. However, Mukhopadhyay *et al*. (2013) reported low activity of ethanolic extracts of some bryophytes. Methanolic extract had the least effectiveness among the extracts. The extract of non-polar solvent (hexane) had low activity against the isolates though its activity is relatively better than methanol as shown in Table 1. The activity of the extracts of *B. coronatum* was very poor compared to the *C. erosum*. The activity of *B. coronatum* was pronounced against *A. calcoaceticus* CSIR, *P. vulgaris* KZN and *S. aureus* OK1. The action of the extracts may be due to their actions on the cell wall or any other parts of the test organisms as suggested by Olofin *et al.* (2013). Acetone extract was very active against *A. calcoaceticus* CSIR and *P. vulgaris* KZN while the hexane extract was active against *S. aureus* OK1 (Table 2). Relatively, Gram negative bacteria were more resistant / susceptible to the extracts of *C. erosum* while the extracts of *B. coronatum* of had low inhibitory activity on both Gram positive and negative bacteria. The activity of the mosses on the test organisms may be due to the presence of the secondary metabolites present in the mosses (Singh *et al*., 2007; Chaudhary and Kumar, 2011). The results of the preliminary screening the both organic and inorganic extracts of two bryophytes tested against the test organisms showed different biological activity considering the

extraction solvents. The active nature and the mechanism of action(s) of the active compounds in the two plants are open to further investigations.

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