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Antimicrobial Activity and Phytochemical Constituents of Methanolic Extract of *Bryophyllum pinnatum* Stem Bark

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ABSTRACT

The study screened the antimicrobial activity and phytochemical constituents of stem bark extract of Bryophyllum pinnatum. The antimicrobial activity was carried out using the method of Kirby Bauer, and the phytochemical screening was carried out using the method of Habourne (1973). The phytochemical screening revealed that saponin, flavonoid, alkoloid and Tannin were present while phenol was absent. The antimicrobial activity result showed that Staphylococcus aureus zones of inhibition for 125 mg, 250 mg, 500 mg, 100 mg and standard drugs 20 mg Ampiclox were 10.0 mm, 19.0 mm, 23.0 mm, 23.0 mm and 26.0 mm respectively. Streptococcus zones of inhibition at 250 mg, 500 mg, 1000 mg and 20 mg of Ampiclox standard were 12.0 mm, 26.0 mm, 27.0 mm and 27.0 mm respectively. Candida albicans zones of inhibition at 125 mg, 240 mg, 500 mg, 1000 mg and 20 mg Standard Ampiclox were 24.0 mm, 22.0 mm, 24.0 mm, 24.0 mm, 24.0 mm and 25.0 mm respectively. Escherichia coli and Streptococcus spp. *was not inhibited by 125 mg but was inhibited at other concentrations. The Minimum inhibitory concentration for Staphylococcus aureus, Streptococcurs* spp., *Escherichia coli and candida albicans observed at 125 mg, 250 mg, 500 mg, 1000 mg and Ampiclox 20 mg showed that S. aureus has MIC at 1000 mg, E. coli at 20 mg Ampiclox, Staphylococcus* spp. *at 1000 mg and C. albicans at 20 mcg Ampiclox.*

Keywords: Antimicrobial; Phtyochemistry; *B. pinnatum*; Methanol

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INTRODUCTION

Some strains of clinically important pathogens have increased in antimicrobial resistance, thus leading to the emergence of new bacterial strains that are multi-resistant. Antimicrobial resistance was become a global concern. The clinical efficacy of many existing antimicrobials is being threatened by the emergence of multi drug-resistant pathogens [1].

Researchers are increasingly turning their attention to traditional medicine, looking for a new leads to develop better drug against bacterial infections. *Bryophyllum pinnatum* bark is used traditionally in treatment of diarrhea (Kiritikar, 1975), pains and inflammations, upper respiratory infection, stomach ulcers, hypertension and various bacterial, viral and fungal infections (Silva et al., 1995). This study was designed to undertake phytochemical screening and in-vitro antimicrobial activities of *Bryophylum pinnatum* stem bark against clinically important pathogen

MATERIALS AND METHODS

Plant Materials

Stem bark of *Bryophyllum pinnatum* were collected from Okwuosa's compound in Ihiala Local Government area of Anambra State and identified by a botanist from department of Biology, Federal Polytechnic Nekede Owerri, Imo State.

Processing of the Sample

The stem barks collected were carefully checked to pick out impurities and the twigs were thereafter rinsed with tap water. The stem was separated into the stem bark and the woody portions and then air dried at room temperature (25°C) to constant weight over a period of 3-4 weeks. It was then powdered in an electronic blender and stored in a sterile air tight glass bottle protected from light and heat until required for analysis [2,3].

Extraction of *Bryophyllum pinnatum*

The extraction was carried out according to the method as described by Harboune, 1973. Soxhlet extraction method was employed using methanol as solvent. After extraction, the extraction was concentrated in a water bath and in an air tight container.

Media Preparation

The media used for the antimicrobial analysis were prepared according to the manufacturer's instruction. The prepared media was sterilized in autoclave at 121°C for 15 minutes and 15 psi.

Disc Preparation

A clean filter paper (Whatman No.1) was perforated using a perforator and the discs obtained were collected in a clean Petri dish. The Petri dish containing the disc was properly wrapped with a foil and placed in a hot-air oven for sterilization.

Antibacterial Bioassay

The antibacterial activity test of the plant was carried out using disc diffusion method (Kirby Bauer method). The sterile discs were impregnated with the methanolic extract of *Bryophyllum pinnatum* and were allowed to dry in an oven. Mueller Hinton agar plates were inoculated with the test organisms; *Staphylococcus aureus, Escherichia coli, Streptococcus* spp. *and Candida albican*s. The organisms were aseptically spread all over the plate using spread plate method. The sterile impregnated disc at different concentration (125 mg, 250 mg, 500 mg and 1000 mg) were placed on the plate 2 cm apart from each other, using sterile forceps, the discs were pressed down in the agar. The plates were properly labeled according to the organisms and were incubated for 24 hours and the zone of inhibition was observed (Table 1) [4].

Table 1. Antibacterial Bioassay by using disc diffusion method (Kirby Bauer method)

Microorganisms	Zone of inhibition (mm)				
	125 mg	250 mg	500 mg	1000 mg	Standard (Ampiclox) 20 mcg
Staphylococcus aureus	10	19	23	23	26
Escherichia coli	-	21.7	22	24	27
Streptococcus spp.	-	12	26	27	27
Candida albican	24	22	24	24	25

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the plant extracts was determined according to the micro broth dilution technique. It was performed in test tubes for determining the MIC, and were labeled from 1000, 250, 125 and 62.5 mg/ml respectively. A stock sample of 1000 mg containing the stem bark extract was prepared. 4 ml of the nutrient broth was introduced into the first test tube and 2 ml to the rest of the test tubes.

2 ml of the extract was introduced into each of the test tube by serial dilution. 0.1 ml of the test organism were inoculated into each of the tubes and then incubated for 24 hours after which the minimum inhibitory concentration was observed and recorded (Table 2) [5,6].

Table 2. Minimum Inhibitory Concentration of the plant extracts by micro broth dilution technique (+ indicates presence of growth; - indicates absence of growth)

Microorganisms	Minimum inhibitory concentration				
	125 mg	250 mg	500 mg	1000 mg	Standard (Ampiclox) 20 mcg
Staphylococcus aureus	+	+	-	-	-
Escherichia coli	+	+	-	-	-
Streptococcus spp.	+	-	-	-	-
Candida albican	+	+	+	-	-

Determination of Minimum Bactericidal Concentration (MBC)

The MBCs were determined by first selecting tubes that showed no growth during MIC determination; a loopful from each tube was sub-cultured onto extract free agar plates, incubated for further 24 hours at 37°C (Table 3).

Table 3. Minimum Bactericidal Concentration (+ indicates presence of growth; - indicates absence of growth)

Microorganisms	Minimum bactericidal concentration				
	125 mg	250 mg	500 mg	1000 mg	Standard (Ampiclox) 20 mcg
Staphylococcus aureus			+	-	-
Escherichia coli			+	+	-
Streptococcus spp.		+	+	-	-
Candida albican				+	-

RESULTS

Phytochemical Constituents of the Extract

The results of the phytochemical screening of the extract reveals that saponins was moderately present, tannin was present in minute amount, alkaloid was moderately present, phenol was absent and flavonoids was moderately present (Table 4).

Table 4. Phytochemical Screening

Parameter	Observation
Saponin	++
Tannin	+
Alkaloid	++
Phenol	-
Flavonoid	++

DISCUSSION

The African traditional medicine is the oldest medicinal system and often culturally referred to as the crade of mankind. The result of the antimicrobial activity of *Bryophyllum pinnatum* stem bark extract revealed that *Staphylococcus aureus* had the highest inhibition (26.0 mm) by the standard drug (Apliclox 20 mcg) while the lowest zone of inhibition (10.0 mm) was observed at 125 mg. the zone of inhibition increased as the concentration of the extract increased, this shows that antimicrobial activity of the extract is concentration dependent. The zones of inhibition of *E. coli* at concentration of the extract; 125 mg, 250 mg, 500 mg, 1000 mg and for the standard drug were nil, 21.0 mm, 22.0 mm, 24.0 mm and 27.0 mm respectively. In other words, concentration 1000 mg has approximate inhibition as the standard drug. At 125 mg, *Streptococcus* spp. was not inhibited, but there was inhibition at other concentration in which 250 mg has 120 mm, 500 mg had 26.0 mm, 1000 mg and the standard drug has the same zone of inhibition (27.0 mm). This shows that the stem bark extract of *Bryophyllum pinnatum* could serve as a promising antimicrobial drug at a higher dosage. The zone of inhibition of *Candida albican* for 125 mg, 250 mg, 500 mg, 1000 mg and standard drug were 24.0 mm, 22.0 mm, and 25.0 mm respectively.

Moreover, the MIC Test, the concentration for the extract that was the lowest concentration for growth inhibition for *Staphylococcus aureus, E. coli, Staphylococcus* spp. *and C. albicans* was 1000 mg, 20 mcg of the standard drug, 100 mg and 20 mcg of the standard drug respectively.

CONCLUSION

The secondary metabolites detected in the extract include saponin, alkaloid and flavonoid which were moderately present, tannin which was present in minute amount and phenol which was totally absent.

Based on the study, the antimicrobial activity of the extract is concentration dependent which means that increase in the extract increased the level of inhibition. In other words, the extract inhibited antimicrobial effect on the organism.

This research may serve as a scientific basis and lend credence that stem bark of *Bryophyllum pinnatum* have antimicrobial potentials.

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