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Inhibitory Activities of *Bryophyllum pinnatum* leaf extract on Selected Strains of Microorganisms

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Bryophyllum pinnatum Lam. (Crassulaceae) called '*Oda-opue*' in Igbo, '*Eru-odundun*in Yoruba and '*Abomoda'* in Hausa languages are widely used as food and as medicines in traditional medical practice. They are found widely in tropical Africa, America, India and China. This study investigates the inhibitory activities of hydro-ethanolic leaf extract of *B. Pinnatum* against some strains of microorganisms (*Staphylococcus aureus, Klebsiella pneumoniae, Psuedomonas aeruginosa, Escherichia coli, Basillus subtilis, Aspergillus niger* and *Penicillum marneffei*) using standard inoculate method. Results showed that hydro-ethanolic leaf extract of *B. pinnatum* exhibited a considerable antimicrobial activity at the lowest concentration (15.625 mg/ml). The results in the findings showed that the leaf of *B. pinnatum* has high inhibitory activities against pathogenic organisms and could be employed to formulate new plant-based drug to improve human health.

Keywords: Bryophyllum pinnatum; pathogenic organisms; inhibitory activities; human health.

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1. INTRODUCTION

In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant [1, 2]. The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality [2]. Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs [3, 4].

Plants have been known from the inception of time to be an important source of medicine, with such plants commonly referred to as "medicinal plants". The plant kingdom being diverse, consists of a variety of plants which are of value in the treatment of various infections and diseases. While many of these plants have been discovered overtime, quite a lot are yet to be discovered [5]. Medicinal plants as a group are believed to consist of approximately 800 species, and thus account for about 50% of all higher flowering plants [6]. These are further divided into 386 families and 200 genera respectively. Traditional or herbal medicine is said to be an age long practice which has developed overtime, with man being able to distinguish plants which are useful as medicine and those which are not [7].

Bryophyllum pinnatum (Crussulaceae) is one of the most promising plants which could help to ameliorate diseases and consequently improve health.

It is a perennial herb that grows in the wild and used as a traditional medicine as well as ornamental plant in tropical Africa, China, Australia, tropical America and India. The Genus belongs to the family of Crassulaceae and it is cultivated as ornamental house plant on rocks or in the garden [8]. It is commonly called air plant, canterbury bell, cathedral bell, life plant and resurrection plant [9]. The major three Nigeria tribes refer to it as *Eru-odundun* (Yoruba), *Odaaopue* or *Alupu* (Igbo) and *Abomoda* (Hausa). It is found in many parts of the world largely because it is easy to cultivate. This may also be the reason for its extensive use in herbal medicine.

The leaves are fleshy dark green that are distinctly scalloped and trimmed in red. The plant flowers in November-March and fruits in April. The leaves and bark is bitter tonic, astringent to the bowels, analgesic, carminative, useful in diarrhea, ulcer and vomiting [10], and well known for its haemostatic and wound healing properties [11]. The plant has considerable attention for its medicinal properties and finds application in folk medicine, as well as in the contemporary medicine. The plant is a good source of vitamins [12], and contains bioactive substances such as alkaloids, flavonoids, saponins, triterpenes, steroids, glycosides, and tannins [13]. The plant has anti- leishmanial activity [14] as well as hepato-protective and nephro-protective effect [15]; anobesic effect [16]. Other reported activities of the leaf extract include Anti-hypertensive [17] and anti-allergic activity [18].

In spite of numerous reports on *B. pinnatum*, there is a dearth of literature report on its inhibitory role in strains of clinically important organisms. Hence, it is of great significance and necessity that research explores its effectiveness against these organisms.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Fresh green leaves of *B. pinnatum* were collected from International Center for Ethnomedicine and Drug Development (InterCEDD). Identification and authentication of the plant was carried out by Mr. Ozioko of InterCEDD with voucher specimen number (UHAE.2018/068) and a specimen was deposited at the herbarium. The plant material was shredded with a knife and air-dried under shade.

2.2 Preparation of Plant Extract

The dried plant (leaf) was pulverized using a laboratory blender and the fine powders obtained stored in an air-tight polythene bag at room temperature until further use. The powdered sample was weighed and used for the extraction with solvent combination (via maceration) of 70% ethanol for 72 hr. The extract yielded was used for the study.

2.3 Antimicrobial Screening

2.3.1 Sources and maintenance of test organisms

Seven (7) bacterial isolates including *Staphylococcus aureus* (MSSA), *Klebsiella*

pneumonia (JH1), *Escherichia coli* (K-12), *Psuedomonas aeruginosa, Basillus subtilis, Aspergillus niger* and *Penicillium marneffei* obtained from the Department of Microbiology and Brewing Nnamdi Azikiwe University Awka were used in the study. All the bacterial species used were maintained on nutrients agar slopes and stored in the refrigerator at a temperature of 4°C from where they were sub-cultured unto fresh media prior to use.

2.3.2 Preparation and Standardization of Bacterial Inoculum

Standardization of bacterial inoculum was done by picking five colonies of each organism into Nutrient broth (Oxoid Thermo Fisher Microbiology, UK) and incubating at 37°C for 18-24hrs. Turbidity produced was adjusted to match 0.5 McFarland standard (10^8 CFU/ml) which was further adjusted to 10^5 CFU/ml.

2.3.3 Antimicrobial Bioassay

Suspension of microorganisms was made in nutrient broth and incubated for 24 hours. Turbity of test organism was adjusted using sterile normal saline to 0.5 MacFarland standards (10^8CFU/ml). From the stock of 500mg/ml extract prepared by dissolving 0.5 g in 1 ml of dimethyl sulphoxide (DMSO), serial dilutions were made to 250, 125, 62.5, 31.25 and 15.625 mg/ml. Each labeled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed. A sterile cork borer of 6mm diameter was used to make wells on the medium. 0.01ml of the various extract concentration were dropped into each appropriate labeled well. DMSO used for dissolving of the extract was tested neat for the organism. The inoculated plates were kept in the refrigerator for 1 hour to allow the extracts to diffuse into the agar. The Mueller Hinton Agar plates were incubated at 37°C for 24 hours. Antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation. 10µg of Ciprofloxacin (Optun disc, Nigeria) was used as control. Tests were carried out in triplicates [19, 20, 21].

2.4 Data Analysis

The results were expressed as Mean ± S.E.M. One way analysis of variance (ANOVA) was carried out on the results and significance was accepted at $p<0.05$.

3. RESULTS

The results of the inhibitory activities of the leaf extract of B. pinnatum on strains of selected microorganisms is presented in Tables 1a to 1g.

Table 1a presents the effect of *B. pinnatum* leaf extract of on *Escherichia coli.* The result showed that the extract exhibited a significant increase in antibacterial activities in a concentration dependent manner (15.625-500 mg/ml) with highest activity at a concentration of 15.625 mg/ml compared with the standard drug (ciprofloxacin).

Fig. 1. The *Bryophyllum pinnatum* plant

Table 1a. Effect of the leaf extract of *B. pinnatum* on *Escherichia coli*

Concentration (mg/ml)	Zone of Inhibition (mm)
15.625	12.50±0.04[b]
31.25	11.20±0.03[c]
62.50	9.50±0.04[d]
125.00	8.00±0.02[e]
250.00	3.50±0.01[f]
500.00	2.00±0.00[f]
Standard (ciprofloxacin)	19.50±0.03[a]

Values are means ± standard error of mean. Values with different alphabet superscript are significantly different at P< 0.05

Table 1b. Effect of the leaf extract of *B. pinnatum* on *Psedomonas aeruginosa*

Concentration (mg/ml)	Zone of Inhibition (mm)
15.625	15.00±0.02[b]
31.25	12.50±0.01[c]
62.50	10.05±0.04[d]
125.00	7.20±0.03[e]
250.00	5.00±0.02[f]
500.00	3.00±0.04[g]
Standard (ciprofloxacin)	20.00±0.04[a]

Values are means ± standard error of mean. Values with different alphabet superscript are significantly different at P< 0.05

Table 1c. Effect of the leaf extract of *B. pinnatum* on *Penicillum marnefei*

Concentration (mg/ml)	Zone of Inhibition (mm)
15.625	13.50±0.03[b]
31.25	12.25±0.04[c]
62.50	8.50±0.02[d]
125.00	7.25±0.03[d]
250.00	2.85±0.01[e]
500.00	2.02±0.00[e]
Standard (ciprofloxacin)	15.00±0.02[a]

Values are means ± standard error of mean. Values with different alphabet superscript are significantly different at P< 0.05

Table 1b presents the effect of the leaf extract of *B. pinnatum* on *Pseudomonas aeruginosa.* The result showed that the extract exhibited a significant increase in antibacterial activities in a concentration dependent manner (15.625-500 mg/ml) with highest activity at the lowest concentration (15.625 mg/ml) compared with the standard drug (ciprofloxacin).

Table 1c presents the effect of the leaf extract of *B. pinnatum* on *Penicillum marnefei.* The result showed that the extract exhibited a significant increase in antifungal activities in a concentration dependent manner (15.625-500 mg/ml) with highest activity at a concentration of 15.625 mg/ml.

Table 1d presents the effect of the leaf extract of *B. pinnatum* on *Bacillus subtilis.* The result showed that the extract exhibited a significant increase in antibacterial activities in a concentration dependent manner (15.625- 500 mg/ml) with highest activity at a concentration of 15.625 mg/ml compared with the standard drug (ciprofloxacin).

Table 1e presents the effect of the leaf extract of *B. pinnatum* on *Staphylococcus aureus.* The result showed that the extract exhibited a significant increase in antibacterial activities in a concentration dependent manner (15.625- 500 mg/ml) with highest activity at a concentration of 15.625 mg/ml compared with the standard drug (ciprofloxacin).

Table 1f presents the effect of the leaf extract of *B. pinnatum* on *Aspergillus niger.* The result showed that the extract exhibited a significant increase in antifungal activities in a concentration dependent manner (15.625- 500 mg/ml) with highest activity at a concentration of 15.625 mg/ml compared with the standard drug (ciprofloxacin).

Table 1g presents the effect of the leaf extract of *B. pinnatum* on *Klebsiella pneumoniae.* The result showed that the extract exhibited a significant increase in antibacterial activities in a concentration dependent manner (15.625- 500 mg/ml) with highest activity at a concentration of 15.625 mg/ml compared with the standard drug (ciprofloxacin).

4. DISCUSSION

The antimicrobial activity of *B. pinnatum* leaf extract is presented in tables 1a to 1g. The presence of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* among others in wounds is of public health concern as their presence can lead to serious complication of nosocomial infections if adequate attention is not given [22], especially in situation where the wounds are left opened. As depicted in tables 1a to 1g, there were significant inhibitions of the tested microorganisms by ethanol extract of the plant at minimum inhibitory concentration (15.625 mg/ml), and all the isolates were sensitive to ciprofloxacin. Extensive use of this antibiotic and other related one has led to reported cases of multi-drug resistant plasmids and transposons found in strains of bacterial genera associated with nosocomial infections [23]. However, multidrug resistant saga has continued to pose critical health challenging issues especially in remote villages with little or non-existence of functional health infrastructure to cater for increasing population of the citizens.

The minimum zone of inhibition displayed by the extract corresponds to the level of susceptibility of the test organism to the extract. All the tested organisms showed a concentration dependent activity with highest activity at minimum inhibitory concentration of 15.625 mg/ml. However, *Pseudomonas aeruginosa* is the most susceptible of the tested organisms (table 1b). *Pseudomonas aeruginosa* is a common gram-negative, rod shaped prototypical multidrug resistant (MDR) pathogenic bacterium that can cause disease in plants and animals, including humans [24]. It typically infects the airway, urinary tract, burns, and wounds, and also causes other blood infections [25]. Although found in the normal flora of the mouth, skin and intestines, it can cause destructive changes to human and animal lungs if aspirated, specifically to the alveoli resulting in bloody sputum [26].

Moderately susceptible of the test organisms include: *Escherichia coli*, *Penicillum marnefei* and *Aspergillus niger* (tables 1a, 1c and 1f). *Escherichia coli* are a gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm blooded organisms [27]. Most *E. coli* strains do not cause disease, but virulent strains can cause gastroenteritis, urinary tract infections, and neonatal meningitis [28]. It can also be characterized by severe abdominal cramps, diarrhea that typically turns bloody within 24 hours, and sometimes fever [28]. In rare cases, virulent strains are also responsible for bowel necrosis and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and gram-negative pneumonia [29].

Another susceptible organism is *Staphylococcus aureus* (*S. aureus*) (table 1e). *Staphylococcus aureus* is a gram-positive coccal bacterium that is a member of the *Firmicutes,* and it is frequently found in the nose, respiratory tract, and on the skin. While *S. aureus* usually acts as a commensal bacterium, asymptomatically colonizing about 30% of the human population, it can sometimes cause disease [30]. In particular, *S. aureus* is one of the most common causes of bacteremia and infective endocarditis. Additionally, it can cause various skin and soft tissue infections, particularly when skin mucosa barriers have been breached [30].

The antimicrobial potential of the plant extract was likely due to the presence of alkaloids and flavonoid in the plant extract [31]. The antimicrobial potentials of alkaloids and flavonoid are well documented [32, 33, 34, 35].

Table 1d. Effect of the leaf extract of *B. pinnatum* on *Bacillus subtilis*

Concentration (mg/ml)	Zone of Inhibition (mm)
15.625	14.85 ± 0.02^{b}
31.25	13.50 ± 0.04^{c}
62.50	12.14 ± 0.01^{c}
125.00	8.25 ± 0.00^{d}
250.00	4.00 ± 0.02^{e}
500.00	2.50 ± 0.01^{f}
Standard (ciprofloxacin)	20.00 ± 0.03^{a}

Values are means ± standard error of mean. Values with different alphabet superscript are significantly different at P< 0.05

Table 1e. Effect of the leaf extract of *B. pinnatum* on *Staphylococcus aureus*

Concentration (mg/ml)	Zone of Inhibition (mm)
15.625	9.00±0.04[b]
31.25	8.50±0.03[b]
62.50	7.25±0.03[c]
125.00	6.25±0.01[d]
250.00	6.25±0.02[d]
500.00	5.00±0.00[e]
Standard (ciprofloxacin)	11.00±0.04[a]

Values are means ± standard error of mean. Values with different alphabet superscript are significantly different at P< 0.05

Table 1f. Effect of the leaf extract of *B. pinnatum* on *Aspergillus niger*

Concentration (mg/ml)	Zone of Inhibition (mm)
15.625	12.00±0.02[b]
31.25	8.25±0.04[c]
62.50	8.00±0.04[c]
125.00	7.50±0.03[d]
250.00	2.50±0.02[e]
500.00	1.00±0.01[f]
Standard (ciprofloxacin)	13.00±0.03[a]

Values are means ± standard error of mean. Values with different alphabet superscript are significantly different at P< 0.05

Table 1g. Effect of the leaf extract of *B. pinnatum* on *Klebsiella pneumonia*

Concentration (mg/ml)	Zone of Inhibition (mm)
15.625	14.30±0.04[b]
31.25	14.00±0.04[b]
62.50	13.50±0.02[c]
125.00	10.00±0.05[d]
250.00	5.50±0.02[e]
500.00	2.00±0.03[f]
Standard (ciprofloxacin)	18.00±0.05[a]

Values are means ± standard error of mean. Values with different alphabet superscript are significantly different at P< 0.05

The sensitivity of the test isolates to ethanolic extract of the plant is consistent with the findings of Obi and Onuoha [36], who described ethanol as the best solvent for the extraction of bioactive substances from plants. The ability of the extract to be more effective at lower concentration could probably be that the active antimicrobial agents in the leaves are more soluble in lower concentration than higher concentrations. Okwu and Josiah [31] made similar assertion. However, the sensitivity of the *P. aeruginosa* to the ethanol extract of the plant is in contrast to the study carried out by Faleye and Ogundaini [37], who reported that the *Aspilia africana* extracts obtained using six different solvents, had no activity on *P. aeruginosa*. This may be because the solvents used in the extraction process could not extract the active antimicrobial agent against *P. aeruginosa*. Nonetheless, the observed antimicrobial potential of the extract partly rationalizes the ethno-medicinal uses of the plant [22].

5. CONCLUSION

The plant extract also showed noteworthy inhibitions of clinically significant microorganisms, including the ones implicated in lungs, urinary tract, skin, blood and wound infections; gastroenteritis; neonatal meningitis, abdominal cramps; diarrhea; bowel necrosis and gram-negative pneumonia. The antimicrobial potential of the plant extract is accredited to the presence of pharmaceutically important secondary metabolites in the plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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