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Evaluation Studies of the Phytochemical Constituents and Antimicrobial Activities of *Bryophyllum pinnatum* (Lam.) Oken (Crassulaceae)

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Abstract: Resistance of many microbes against antibiotics is distressingly increasing and the adverse effects linked with the usage of several antibiotics are also a major delinquent in treatment of infectious diseases. *Bryophyllum pinnatum* (Lam.) Oken is a perennial herb growing widely and used in folkloric medicine. The impulse of the study is to investigate the phytochemical compositions and antimicrobial activities of *Bryophyllum pinnatum*. Phytochemical study was carried out on the methanol extract of *Bryophyllum pinnatum* to determine its phytoconstituents using standard procedures. The antimicrobial activities of the stem, root and leaves extracts of *B. pinnatum* were also investigated and the Minimum Inhibitory Concentration (MIC) was determined. The presence of tannins, alkaloids, saponins, phenols, sterol, HCN, glycerol and flavonoids, were accessed qualitatively and quantitatively and it was revealed that all the phytochemicals assayed were all present in the leaves and root while HCN was absent in the stem but the leaves extract gave highest composition of tannin (1.74±0.021 mg/100g), alkaloid (2.08±0.021 mg/100g), saponin (0.44±0.021 mg/100g), glycerol (1.47±0.014 mg/100g) and flavonoid (1.64±0.021 mg/100g) and higher composition of HCN (2.44±0.290 ml/kg). The stem extract gave highest composition of saponin (0.81±0.014 mg/100g). While the root extract gave highest composition of sterol (0.49±0.014 mg/100g). The antimicrobial result reviewed that the inhibition of pathogens increased with increased concentration of the plant extracts. The leaf extract showed superiority in the inhibition of *S. aureus* and *E. coli* and *S. typhi* at 200 mg/ml concentration with values of 9.35±0.212, 7.55±0.071 and .40±0.001 respectively while the stem gave highest inhibition of *A. flavus* with value of 9.50±0.141 suggesting its superiority in the control of *A. flavus* than the leaves and root extract. However, the inhibition of *C. albicans* and *R. stolonifer* varied with plant extract and concentration signifying that plant extract had varied minimum inhibitory concentrations. There was a much higher antimicrobial activity displayed by the standard against all tested organisms compared to methanol extracts of root, leaves and stem at all used concentrations. These findings have demonstrated that *B. pinnatum* should not be exploited for treatment of various human diseases caused by pathogens.

Key words: Antimicrobial · *Bryophyllum pinnatum* · Phytoconstituents · Antibiotics

INTRODUCTION

Infectious diseases are caused by pathogenic microorganisms such as *Escherichia coli, Klebsiella spp., Salmonella spp., Staphylococcus aureus, Pseudomonas spp., Shigella spp.* Many organisms live in and on our bodies. Some of them are normally harmless or even helpful, while under certain conditions, some organisms may cause diseases. Some infectious diseases can be passed from person to person. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world [1-3]. With the continuous use of antibiotics, microorganisms have become resistant. In addition to this problem, antibiotics are sometimes associated with side effects on host which include hypersensitivity, depletion of beneficial gut and mucosal microorganism, immune suppression and allergic reactions [4]. This has created enormous clinical problems in the treatment of infectious diseases [5]. Therefore, there is a need to develop alternative antimicrobial drugs for the

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treatment of infectious diseases, one approach is to screen plants with medicinal values for its possible antimicrobial properties and also to isolate compounds with antimicrobial properties.

Bryophyllum pinnatum (Lam.) Oken is a perennial herb growing widely and used in folkloric medicine in tropical Africa, tropical America, India, China and Australia. It is a member of the family Crassulaceae. Vernacular names for *Bryophyllum pinnatum* include 'Cathedral Bells', 'Air Plant', 'Life Plant', 'Miracle Leaves', 'Goethe Plant' and the 'Katakataka'. *B. pinnatum* is commonly used traditionally as a medicine in different regions of India mainly to treat urinary stones, as well as in other parts of world. The traditional practitioners in various parts of world use this plant in numerous conditions like hypertension, skin disorders, asthma, cold, insect stings and abscesses. *Bryophyllum pinnatum* has been recorded in Trinidad and Tobago as being used as a traditional treatment for hypertension [6]. The juice made of *Bryophylum* is useful for cure of kidney stones, although there is no evidence-based clinical indication for these uses in modern medicine and, indeed, such usage could prove dangerous and even fatal in some cases. Bufadienolide compounds isolated from *Bryophyllum pinnatum* include bryophillin A which showed strong anti-tumor promoting activity *in vitro* and bersaldegenin-3-acetate and bryophillin C which were less active [7]. Bryophillin C also showed insecticidal properties [8]. The root of this plant is believed to protect the liver and proved useful for the treatment of hepatitis. It is proved diuretic therefore, it cures difficult urination [9].

In Africa, India and South America it is used for the treatment of insect bites, wounds, ulcers, abscesses and burns, inflammations, swellings and discolorations [10-15] rheumatic afflictions, erysipelas and boils [16-18] conjunctivitis, earache and sore throats [19] and in the induction of labour and the removal of ovarian cysts [20].

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. It is well-known that plant produces these phytochemicals to prevent them but recent researches demonstrate that they can also protect humans against diseases. Recently, it is clearly known that they have roles in the protection of human's health, when their dietary intake is significant [21]. Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds. Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals. These compounds are known as secondary plant metabolites and have biological properties. In view of the above pharmacological activity we therefore investigated its phytochemical compositions and antimicrobial activities.

MATERIALS AND METHODS

Collection of Sample: The plant sample *Bryophyllum pinnatum* was collected from an abandoned farm land in Nibo, Awka South L.G.A of Anambra State in the month of March 2017. The plant specimen was authenticated by Mr. A.O. Ozioko (Taxonomist) of International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka and the plant material with voucher no ICEED/17/0245 was deposited in their herbarium.

Preparation and Extraction of Plant Material: The fresh plant parts were cut into bits with knife and then dried under shade for 21 days. The samples were ground in a mortar with a pestle and then in a blender (Omega, USA) into powdered form. The powdered samples were stored in an air tight container for analysis. The powdered sample of the leaves, stem and root where macerated in 100% methanol for 48 hours. The filtrate was concentrated in vacuo. The extract was stored in an air tight container at 4°C until required.

Qualitative Phytochemical Analysis: Qualitative tests were carried out to determine the presence or absence of different phytochemicals including alkaloids, saponins, tannins, hydrogen cyanide, sterols, phenols, anthraquinone and flavonoids using standard methods [21, 22].

Isolation and Characterization of Bacteria and Fungi

Preparation of Microorganism for the Experiment: The pure culture of the microorganisms was obtained from the Pathology Department of National Root Crop Research Institute, Umudike, Abia State. The stock cultures of bacteria were sub-cultured in nutrient agar (NA) slants while yeast and mold on Sabouraud and dextrose agar (SDA) slants and stored at 4°C

The test organisms were checked for their susceptibility to the herbal extract by carrying out antimicrobial screening using the extracts and by determining the Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC). The surface of the nutrient agar plate was flooded with

2ml of 18 hours broth culture standardized according to National Committee for Clinic Laboratory Standard (NCCLS, 2002) by gradually adding normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1.0x10 1.5 x 108 CFU/ml. The surface was allowed to dry and sterile No.4 cork borer was used to bore six holes of about 2.5cm equal size on the surface and 0.1ml of the extract at different concentrations of 6.25%w/v, 12.5%w/v, 25%w/v, 50%w/v and 100%w/v was dropped into each hole and the plate was kept for about 1hour at room temperature and incubated at 37°C for 18hours. The diameter of zones of inhibition was measured after incubation to the nearest millimeter (mm). The experiment was repeated three times and the mean diameter was taken. The effects of the extract on bacteria and fungi pathogens were compared with those of the standard antibiotic amoxicillin and fungabacter for bacteria and fungi respectively as standard control.

Statistical Analysis: Analysis of Variance (ANOVA) using SPSS version 21 was employed in analyzing the data collected from the study. The Duncan's Multiple Range Test (DMRT) was used to test the difference among the treatments means with more than two levels. All statistical analysis was carried out at 5% level of significance. The data were expressed as mean ±standard deviation of triplicate determinations.

RESULTS

Qualitative and Quantitative phytochemical composition of the extracts of the leaves, stem and root of *Bryophyllum pinnatum are presented in* Tables 1 and 2 respectively.

Similarly, the results of the antimicrobial assays are presented in Table 3, 4, 5, 6 and 7 below:

Table 1: Qualitative phytochemical composition of the leaves, stem and root of *B. pinnatum*

Extracts	Tannin	Alkaloid	Saponin	Phenol	Sterol	HCN	Glycerol	Flavonoid
Leaves	++	++	+	+	+	+	+	++
Stem	++	+	+	+	+	-	+	+
Root	+	+	+	+	+	+	+	+

++: deeply present, +: present, -: absent

Table 2: Quantitative phytochemicals of the leaves, stem and root of *B. pinnatum*

Extracts	Tannin (mg/100g)	Alkaloid (mg/100g)	Saponin (mg/100g)	Phenol (mg/100g)	Sterol (mg/100g)	HCN (ml/kg)	Glycerol (mg/100g)	Flavonoid (mg/100g)
Leaves	1.74±0.021^{c}	2.08±0.021^{c}	0.75±0.014^{b}	0.44±0.021^{c}	0.27±0.014^{a}	2.44±0.290	1.47±0.014^{c}	1.64±0.021^{c}
Stem	1.49±0.014^{b}	1.85±0.028^{b}	0.81±0.014^{c}	0.25±0.021^{a}	0.34±0.000^{b}	-	1.24±0.014^{b}	0.73±0.014^{a}
Root	0.85±0.007^{a}	1.47±0.028^{a}	0.65±0.007^{a}	0.37±0.021^{b}	0.49±0.014^{c}	0.49±0.050	0.78±0.028^{a}	0.84±0.028^{b}
p-value	0.000	0.000	0.002	0.007	0.001	0.001	0.000	0.000

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different (p>0.05)

Table 3: Effect of methanol extracts of root, leaves and stem of *Bryophyllum pinnatum* on pathogens at 50mg/ml concentration

	Zone of Inhibition (mm)					
Extracts	*S. aureus*	*E. coli*	*S. typhi*	*C. albicans*	*A. flavus*	*R. stolonifer*
Standard	8.43±0.035^{d}	7.63±0.035^{d}	8.41±0.156^{c}	11.52±0.028^{c}	12.52±0.028^{d}	11.78±0.028^{d}
Root	1.63±0.035^{a}	2.43±0.04^{ba}	2.20±0.283^{a}	3.53±0.117^{b}	3.53±0.177^{c}	1.92±0.028^{a}
Leaves	3.82±0.028^{c}	2.67±0.021^{c}	3.18±0.028^{b}	3.22±0.039^{a}	2.47±0.021^{a}	2.40±0.141^{b}
Stem	2.35±0.014^{b}	1.77±0.021^{a}	1.83±0.035^{a}	3.08±0.106^{a}	3.27±0.021^{b}	2.79±0.014^{c}
p-value	0.000	0.000	0.000	0.000	0.000	0.000

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different (p>0.05)

Note: amoxicillin and fungabacter as standards.

Table 4: Effect of methanol extracts of root, leaves and stem of *Bryophyllum pinnatum* on pathogens at 100mg/ml concentration

	Zone of Inhibition (mm)					
Extracts	*S. aureus*	*E. coli*	*S. typhiityphi*	*C. albicans*	*A. flavus*	*R. stolonifer*
standard	12.58±0.106^{c}	11.5±0.141^{d}	12.28±0.106^{d}	13.77±0.099^{d}	15.33±0.177^{d}	16.15±0.212^{c}
Root	4.55±0.354^{a}	4.61±0.014^{c}	5.22±0.028^{b}	6.09±0.127^{c}	6.21±0.078^{c}	5.87±0.042^{a}
Leaves	5.83±0.099^{b}	4.33±0.035^{b}	6.47±0.021^{c}	5.77±0.021^{b}	5.41±0.014^{a}	5.83±0.035^{a}
Stem	5.08±0.113^{a}	3.43±0.035^{a}	4.63±0.035^{a}	5.41±0.014^{a}	5.77±0.021^{b}	6.38±0.106^{b}
p-value	0.000	0.000	0.000	0.000	0.000	0.000

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different (p>0.05)

Note: amoxicillin and fungabacter as standards.

Table 5: Effect of methanol extracts of root, leaves and stem of *Bryophyllum pinnatum* on pathogens at 150mg/ml concentration

	Zone of Inhibition (mm)					
Extracts	*S. aureus*	*E. coli*	*S. typhi*	*C. albicans*	*A. flavus*	*R. stolonifer*
Standard	15.41±0.014^{d}	16.13±0.177^{d}	14.55±4.172^{b}	18.51±0.127^{c}	17.50±0.141^{d}	18.55±0.212^{d}
Root	5.82±0.028^{a}	5.22±0.021^{b}	6.00±0.000^{a}	7.68±0.247^{b}	6.77±0.042^{a}	7.55±0.071^{c}
Leaves	7.00±0.000^{c}	6.28±0.035^{c}	8.30±0.002^{a}	7.23±0.035^{b}	7.70±0.152^{c}	6.88±0.035^{b}
Stem	6.52±0.182^{b}	4.73±0.042^{a}	5.82±0.028^{a}	6.33±0.177^{a}	7.23±0.035^{b}	6.33±0.106^{a}
p-value	0.000	0.000	0.039	0.000	0.000	0.000

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different (p>0.05)

Note: amoxicillin and fungabacter as standards.

Table 6: Effect of methanol extracts of root, leaves and stem of *Bryophyllum pinnatum* on pathogens at 200mg/ml concentration

	Zone of Inhibition (mm)					
Extracts	*S. aureus*	*E. coli*	*S. typhi*	*C. albicans*	*A. flavus*	*R. stolonifer*
Standard	18.83±0.106^{d}	19.45±0.071^{d}	20.37±0.184^{d}	21.77±0.042^{c}	22.38±0.106^{c}	21.65±0.071^{c}
Root	7.23±0.035^{a}	6.75±0.071^{b}	8.46±0.057^{b}	8.61±0.014^{b}	8.45±0.071^{a}	8.50±0.141^{a}
Leaves	9.35±0.212^{b}	7.55±0.071^{c}	9.40±0.001^{c}	8.30±0.141^{a}	9.30±0.424^{b}	9.35±0.212^{b}
Stem	8.43±0.247^{c}	6.27±0.042^{a}	7.00±0.000^{a}	8.78±0.035^{b}	9.50±0.141^{b}	8.75±0.071^{a}
p-value	0.000	0.000	0.000	0.000	0.000	0.000

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different (p>0.05)

Note: amoxicillin and fungabacter as standards.

Table 7: Minimum inhibitory concentration of the extracts of stem, root and leaves of *B. pinnatum*

	MIC (mg/ml)					
Extracts	*S. aureus*	*E. coli*	*S. typhi*	*C. albicans*	*A. flavus*	*R. stolonifer*
Control	3.5	3.5	3.5	3.5	3.5	3.5
Stem	12.5	25	12	12.5	6.55	12.5
Root	12.5	25	25	25	12.5	6.5
Leaves	6.5	12.5	12.5	6.3	6.5	12.5

DISCUSSION

Resistance of many microbes against antibiotics is distressingly increasing and the adverse effects linked with the usage of several antibiotics are also a major delinquent in treatment of infectious diseases. Therefore, search for new molecule with antimicrobial activity, fewer side effects has become imperative. Medicinal plants have been used in development of drugs for a long time and compounds with antimicrobial activity from plant origin are the possible alternative to the challenges faced by usage of synthetic antimicrobial molecules basically from nature. Most of the research in this area is going on development of newer antimicrobials with more effective activity either from the plant derived compounds or from their synthetic analogues. This study

examined the qualitative and quantitative phytochemical compositions of the extracts of the leaves, stem and root of *B. pinnatum*. Result of qualitative phytochemical composition of the extracts of the leaves, stem and root of *B. pinnatum* revealed that all the phytochemicals assayed were present in the leaves and root while HCN was absent in the stem (Table 1).

Result of the quantitative phytochemical composition of the extracts of the leaves, stem and root of *B. pinnatum* was shown in Table 2. The table revealed that the leaves extract gave highest composition of tannin (1.74±0.021 mg/100g), alkaloid (2.08±0.021 mg/100g), saponin (0.44±0.021 mg/100g), glycerol (1.47±0.014 mg/100g) and flavonoid (1.64±0.021 mg/100g) and higher composition of HCN (2.44±0.290 ml/kg).The stem extract gave highest composition of saponin (0.81±0.014 mg/100g)while the root extract gave highest composition of sterol (0.49±0.014 mg/100g).There was significant difference in all the phytochemicals assayed between the leaves, stem and root extracts of *B. pinnatum* ($p<0.05$) (Table 2).

Result of the activity of the root, leaves and stem extracts of *B. pinnatum* against pathogens at 50 mg/ml concentration was shown in Table 3. The table revealed that the root extract gave highest inhibition of *Candida albicans* (3.53±0.117 mm) and *Aspergillus flavus* (3.53±0.177 mm) than the leaves and stem extracts; the leaves extract gave highest inhibition of *Staphylococcus aureus* (3.82±0.028 mm), *Salmonella typhi* (3.18±0.028 mm) and *Escherichia coli* (2.67±0.021 mm) than root and stem extracts, while the stem extract gave highest inhibition of *Rhizopus stolonifer* (2.79±0.014 mm) than root and leaves extracts. In comparison between the control and the plant extracts, the control gave much higher inhibition of all the pathogens than the plant extracts used. There was significant difference in the inhibitory activity of root, leaves and stem extracts of *B. pinnatum* against all the pathogens investigated ($p<0.05$) (Table 3).

Result of the activity of the root, leaves and stem extracts of *B. pinnatum* against pathogens at 100 mg/ml concentration was shown in Table 4. The table revealed that the root extract gave highest inhibition of *E. coli* (4.61±0.014 mm), *C. albicans* (6.09±0.127 mm) and *A. flavus* (6.21±0.078 mm); the leaves extract gave highest inhibition of *S. typhii* (6.47±0.021 mm), while the stem extract gave highest inhibition of *R. stolonifer* (6.38±0.106 mm).In comparison between the control and the plant extracts, the control gave much higher inhibition of all the pathogens than the plant extracts used. There was significant difference in the inhibitory activity of root, leaves and stem extracts of *B. pinnatum* against all the pathogens investigated ($p<0.05$) (Table 4).

Result of the activity of the root, leaves and stem extracts of *B. pinnatum* against pathogens at 150 mg/ml concentration was shown in Table 5. The table revealed that the root extract gave highest inhibition of *C. albicans* (7.68±0.247 mm) and *R. stolonifer* (7.55±0.071 mm) while the leaves extract gave highest inhibition of *S. aureus* (7.00±0.000 mm), *E. coli* (6.28±0.035 mm), *S. typhii* (8.30±0.002 mm) and *A. flavus* (7.70±0.152 mm). In comparison between the control and the plant extracts, the control gave much higher inhibition of all the pathogens than the plant extracts used. There was significant difference in the inhibitory activity of root, leaves and stem extracts of *B. pinnatum* against all the pathogens investigated ($p<0.05$) (Table 5).

Result of the activity of the root, leaves and stem extracts of *B. pinnatum* against pathogens at 200 mg/ml concentration was shown in Table 6. The table revealed that the leaves extract gave highest inhibition of *E. coli* (7.55±0.071 mm), *S. typhii* (9.40±0.001 mm) and *R. stolonifer* (9.35±0.212 mm) while the stem extract gave highest inhibition of *S. aureus* (8.43±0.247 mm), *C. albicans* (8.78±0.035 mm) and *A. flavus* (9.50±0.141 mm) which could be attributed to its higher composition of tannins, alkaloids, phenols, HCN, glycerol and flavonoids [23]. The stem gave highest inhibition of *A. flavus*, suggesting its superiority in the control of *A. flavus* than the leaves and root extract. However, the inhibition of *C. albicans* and *R. stolonifer* varied with plant extract and concentration. In comparison between the control and the plant extracts, the control gave much higher inhibition of all the pathogens than the plant extracts used. There was significant difference in the inhibitory activity of root, leaves and stem extracts of *B. pinnatum* against all the pathogens investigated ($p<0.05$) (Table 6).

The MIC of the root, leaves and stem extracts of *B. pinnatum* revealed that the stem extract required 12 mg/100g concentration to prevent the growth of *S. typhii*; the root extract required 6.5 mg/100g to prevent the growth of *R. stolonifer* while the leaves extract required 12.5 mg/100g, 6.5 mg/100g, 6.3 mg/100g and 6.5 mg/100g to prevent the growths of *S. aureus*, *C. albicans* and *A. flavus*, respectively. In comparison between the control and the plant extracts, the control only required 3.5 mg/100g to prevent the growth of all the organisms investigated (Table 7). The result on MIC revealed that the MIC values for the leaves extract are the lowest while that of the root extract are the highest. This confirms the study of Acuna [2] that plant extract have varied minimum inhibitory concentrations. However, some bacteria pathogens like the *S. typhii* and *E. coli* show special mechanism of resistance to the plant extracts. This may be

attributed to ability to develop resistant traits [13]. Meanwhile, in the comparison of inhibitory activity of the standard drug with the plant extracts, it was revealed that the standard drug gave highest inhibition of all the test organisms than the plant extracts which suggest the need for further improvement of plant extract for clinical purposes.

CONCLUSIONS AND RECOMMENDATIONS

These findings have demonstrated that *B. pinnatum* should *not be* exploited for treatment of various human diseases caused by pathogens.

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