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Phytochemistry, Antimicrobial and Anti-Hyperglycemic Activity of *Bryophyllum pinnatum* Extracts in an Animal Model

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Abstract

This research was done to ascertain phytochemistry, antimicrobial and anti-hyperglycemic activity of *Bryophyllum pinnatum* extracts in an animal model. It also involves the discovering and identifying plants with antimicrobial properties without severe effects for the treatment of diabetes mellitus has become an important goal of research in biomedical science. The qualitative and quantitative phytochemical analyses of *Bryophyllum pinnatum* were evaluated. Qualitative analysis showed that the leaves of *Bryophyllum pinnatum* were rich in flavonoids, saponins, tannins, alkaloids and cardiac glycosides. Tannin gave the lowest percentage yield of 0.36% for both the aqueous and methanol extract while cardiac glycoside gave the highest percentage yield of 65% for the aqueous extract. The methanol and aqueous leaf extracts were screened for their antimicrobial activities against Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and a fungus (*Candida albicans*). Agar well diffusion method was used to determine the minimum inhibitory concentration at 100 mg/ml, 200 mg/ml and 400 mg/ml. All organisms except *Candida albicans* were susceptible to the extracts. The gram negative organisms were not inhibited by the aqueous extracts of *B. pinnatum*. The antidiabetic potential of both extracts of the plant was evaluated in the alloxan monohydrate induced-diabetic albino rats. Graded doses of the aqueous and methanol extracts were administered to experimental diabetic rats for 10 days. Significant reduction in fasting blood sugar was obtained in the diabetic animals. There was significant differences in the mean values of the parameters evaluated at $P < 0.05$ for the methanol-extract treated rats.

Keywords: *Phytochemistry; Anti-Hyperglycemic; B. pinnatum; Antimicrobial; Anti-Diabetic*

Introduction

Diabetes mellitus is a disorder characterized by hyperglycemia. According to World Health Organization [1], there were approximately 160,000 diabetics worldwide, the number of diabetics has doubled in the last few years and is expected to double in the year 2025. Due to its high prevalence and potential deleterious effect on a patient's physical and psychological state, diabetes is a major medical concern [2]. Over the years, several animal models have been developed for studying diabetes mellitus and testing anti-diabetic agents [3]. The oral anti-hyperglycemic agents currently used in clinical practice have characteristic profiles of serious side effects [4]. This leads to increasing demand for herbal products with anti-diabetic activity and less side effects [5]. *Bryophyllum pinnatum*, commonly known as life plant belongs to the plant family Crassulaceae. It is a perennial herb growing widely and used in folkloric medicine in tropical Africa, tropical

America, India, Australia and China. In Southwest Nigeria, the plant is called *'abamoda'* while in the Northern Nigeria, it is called '*shuka halinka'* or '*karan masallachi'*. It is known as '*odaa opuo'* by the Igbos'. In Mexico and Nicaragua, the plant is used to promote menstruation and assist in childbirth. In Nigeria and other West African countries, fleshy leaves are frequently used as herbal remedy for an array of human disorders, including hypertension, diabetes mellitus, bruises, wounds, boils, abscesses, insect bites, arthritis, rheumatism, joint pains, headaches and body pains. Ofokansi [6] reported that the plant is effective in the treatment of typhoid fever and other bacterial infections, particularly those caused by *S. aureus, E. coli, B. subtilis, P. aeruginosa*, *K. aerogenes, K. pneumoniae*, and *S. typhi*. In traditional medicine, the leaves of this plant have been used for antimicrobial [7], antifungal, antiulcer, anti-inflammatory, analgesic, antihypertensive [8], potent with anti- allergic activity. Fungitoxic and phytotoxic activity of the plant have been studied on plants [9]. Therefore, this research was carried out to evaluate the phytochemical constituents, antimicrobial and anti-diabetic activities of *B. pinnatum* in alloxan-induced diabetes.

Materials and Methods

Sample collection

Fresh leaves *of Bryophyllum pinnatum* plant were collected from Umuofor Okija, Anambra State, Nigeria. They were authenticated at the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State.

Preparation of extracts

The leaves were washed with distilled water and dried at room temperature for 21 days. They were pulverized using an electric blender (Sonik Electrical Appliance Company Ltd.). Exactly 100g of the powdered leaves were subjected to soxhlet extraction with 250 ml methanol and distilled water as the extraction solvents. Each extract (methanol and aqueous) was gently evaporated to dryness in a water bath at 40°C [10]. Each extract was transferred into clean and dried airtight vials until ready for use.

The following tests were done in this research work according to [11].

Determination of extractive value, preparation of stock solutions of the extracts, preparation of the test organisms.

Determination of phytochemical constituents of extracts

Other tests done as described by [11] and [12] include determination of saponins, emulsion test, frothing test, determination of tannins, determination of alkaloids, determination of flavonoids, saponin determination, determination of reducing sugar, determination of cardiac glycosides.

Determination of preliminary antimicrobial activity of extracts

The antimicrobial activity of the extracts was determined using agar well diffusion technique [13]. Nutrient agar plates were each seeded with 0.05 ml of an overnight culture of each bacterial isolate while the sabouraud dextrose agar (SDA) plates were similarly seeded with the fungal strain. The seeded plates were allowed to set and then dry. A sterile cork borer of 5 mm diameter was used to bore 5 uniform wells on the surface of the agar. Exactly 0.05 ml of each concentration of the aqueous and methanol extracts (400, 200, and 100 mg/ml) was placed in the wells respectively. Into the 4^{th} and 5^{th} wells were placed 0.05 ml chloramphenicol (25 mg/ml) and sterile distilled water to serve as positive and negative controls respectively for the bacterial strains, while 0.05 ml fluconazole (50 mg/ml) and sterile distilled water were used for the fungal strain. The plates were allowed on the bench for pre-diffusion for 40 minutes followed by an overnight incubation at 37°C for bacterial isolates, and 25°C for 48h for the fungal isolate. The degree of antimicrobial activity of each extract was measured as the inhibition zone diameter in millimeters. The sensitivity test was done in triplicates. The average of the three readings was taken to be the zone of inhibition of the bacterial and fungal isolates in question at the particular concentration.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was determined for only the extracts that showed inhibitory activity. The broth dilution technique was employed to determine the MIC of the potent extracts as described by [14].

Determination of minimum bactericidal concentration (MBC)

The broth culture tubes used in the minimum inhibitory concentration that showed no growth were gently shaken to homogenize the contents. This was fully described by [15].

Test animals

White star albino rats, 10-12 weeks old weighing 115-140g were procured from Nsukka, Enugu state. Animals were maintained under standard environmental conditions and had free access to feed and water *ad libitum.* The animals were acclimatized to laboratory conditions for one week prior to performing the experiment. The rats were divided into nine groups. Group 1 consists of diabetic rats (positive control) while Group 2 consists of normal rats (negative control). Group 3 consists of diabetic rats treated with 0.5 ml glibenclamide orally at 10 mg/kg body weight daily for 10 days. Groups 4, 5, and 6 consist of diabetic rats treated with 100 mg/ml, 200 mg/ml and 400 mg/ml *B. pinnatum* methanol extract daily for 10 days respectively. Groups 7, 8, and 9 consist of diabetic rats treated with 100 mg/ml, 200 mg/ml and 400 mg/ml *B. pinnatum* aqueous extract daily for 10 days at respectively.

Induction of experimental diabetes and treatment

Alloxan monohydrate was dissolved in normal saline and injected intraperitoneally after an overnight fast using 25 gauge syringe within 10 minutes of preparation (solution of 150mg/kg body weight). A rest period of 10 days was allowed during which the animals had free access to feed and water. In order to assess the effect of alloxan and to chemically establish the diabetic condition, the fasting blood sugar of the rats were determined using a Prestige Smart System glucose analyzer, by tail puncturing method. Thereafter, the animals were treated orally with 0.5ml of the extracts and glibenclamide with regards to their groupings for 10 days during which their FBS values were determined on the 4^{th}, 7^{th} and 10^{th} days of treatment. The level of blood glucose level considered normal in rats ranges from 50 - 135 mg/dl [15]. Rats whose blood glucose level exceeded 200 mg/dl were considered diabetic [16].

Statistical analysis

The data were analyzed using two-way analysis of variance (ANOVA). The level of significance was set at 0.05.

Results

The percentage yield and macroscopic characteristics of the crude extract of dried leaves of *B. pinnatum* are presented in table 1. The yield was generally more for the aqueous extract than for the methanol extract. Qualitative phytochemical screening of the extracts of *B. pinnatum* demonstrated the presence of alkaloids, flavonoids, saponins, tannins, and cardiac glycosides while reducing sugar and steroids were absent as shown in table 2. Quantitative composition (in percentage) of the observed phytochemicals is presented in table 3 below. Tannin gave the least concentration while cardiac glycosides gave the highest concentration. The results of the antimicrobial activity of the extracts are presented in table 4. None of the extracts at any concentration showed any inhibitory effects on the fungal strain. All extracts showed varying degrees of inhibition on the tested bacterial isolates at concentrations of 400, 200, and 100 mg/ml. The methanol extract has a higher potency than the aqueous extract. Again, no activity was shown by the aqueous extract at any concentration against *P. aeruginosa* and *E. coli*. The antimicrobial activity of both extracts against *B. subtilis* was relatively low. With respect to the antimicrobial activity of both extracts on the test organisms, mean inhibitory zones ranging from 10-25mm was observed on *S. aureus* while 10 - 15 mm was observed on *P. aeruginosa*. *S. aureus* showed the highest zone of inhibition (25 mm) on both extracts while *B. subtilis* and *E. coli* showed the least zones of inhibition (7 mm). The methanol extract did not show any inhibition for any of the test organisms at 100 mg/ml. The controls (25 mg/ml of chloramphenicol and 50mg/ml of fluconazole for bacterial and fungal strains respectively) showed higher

zones of inhibition compared to the extracts except for *S. aureus.* Table 5 shows the minimum inhibitory concentration (MIC) of the leaf extracts. The MIC for the methanol extract of the bacterial organisms was shown to be at 200 mg/ml while the MIC for *S. aureus* and *B. subtilis* was shown at 100 mg/ml.

Extracting solvent	Percentage yield (%)	Color
Methanol	13.85%	Greenish-yellow
Distilled water	37.22%	Dark-brown

Table 1: *Extraction yield and macroscopic characteristics of the crude extract.*

Phytochemical Test	Observation	Methanol	Aqueous
Tannins			
Acid test	Reddish-brown ppt	-	+
Bromine H_2O	Greenish-red color	-	-
Saponins			
Frothing test	Thick persistent froth	+	+
Emulsion	Emulsion observed	-	+

Table 2: *Qualitative analysis of B. pinnatum leaf extracts.*

Phytochemical	Percentage yield
Alkaloids	1.01%
Tannins	0.36%
Flavonoids	2.8%
Saponins	4.6%
Cardiac glycosides (M)	50%
Cardiac glycosides (A)	65%

Table 3: *Quantitative composition (in %) of the observed phytochemicals.*

Key: M = Methanol Extract; A = Aqueous Extract.

Organisms	{Conc (mg/ml)}	IZD (mm)	
		[Methanol]	[Aqueous]
S. aureus	400	25	-
	200	15	25
	100	-	10
	25 (RSD*)	20	20
E. coli	400	10	-
	200	7	-
	100	-	-
	25 (RSD*)	25	28

B. subtilis	400	10	-
	200	7	8
	100	-	7
	25 (RSD*)	20	20
P. aeruginosa	400	15	-
	200	10	-
	100	-	-
	25 (RSD*)	25	15
C. albicans	400	-	-
	200	-	-
	100	-	-
	50 (RSD**)	10	10

Table 4: *Antimicrobial activity of the extracts of B. pinnatum.*

Key: - = No zone of inhibition; RSD = Reference standard drug- 25 mg/ml chloramphenicol; RSD** = Reference standard drug - 50 mg/ml fluconazole.*

Organisms	Extract	{Conc (mg/ml)}		
		400	200	100
S. aureus	M	-	-	+
	A	+	-	-
B. subtilis	M	-	-	+
	A	+	-	-
E. coli	M	-	-	+
P. aeruginosa	M	-	-	+

Table 5: *Minimum inhibitory concentration of B. pinnatum extracts.*

Key: - = Not Turbid; A = Aqueous Extract; + = Turbid; M = Methanol Extract.

Test organism	Log. conc. (intercept)	Antilog (mg/ml)
S. aureus	0.55	3.55
E. coli	0.20	1.58
B. subtilis	0.09	1.23
P. aeruginosa	0.30	2.00

Table 6: *MIC (log. conc. intercept) of methanol extract.*

Test organism	Log. conc. (intercept)	Antilog (mg/ml)
B. subtilis	2.08	120.23
S. aureus	2.34	158.49

Table 7: *MIC (log. conc. intercept) of aqueous extract.*

Test organism	Extract {Conc (mg/ml)}	MBC			
		400	200	100	
S. aureus	M	-	-	+	200
	A	+	-	-	100
B. subtilis	M	-	-	+	200
	A	+	-	-	100
E. coli	M	-	-	+	200
P. aeruginosa	M	-	-	+	200

Table 8: *Minimum bactericidal concentration of B. pinnatum extracts.*

Key: - = No Bacterial Growth; M = Methanol Extract; + = Bacterial Growth; A = Aqueous Extract.

Discussion

The difference in the macroscopic characteristics of the aqueous and methanol extracts of *Bryophyllum pinnatum* leaves shows that certain components of the plant leaves showed varied solubility in the different solvents used for the extraction as shown in table 1. The aqueous extract gave a higher percentage yield (32.66%) than the methanol extract (13.85%). The qualitative phytochemical analysis of *B. pinnatum* leaf extracts showed the presence of alkaloids, tannins, saponins, flavonoids and cardiac glycosides (Table 2). Alkaloids, tannins, saponins, flavonoids and cardiac glycosides derived from plants have been shown to have antimicrobial and pharmacological activities. The antimicrobial activity of *B. pinnatum* observed in this study could be attributed to the presence of these compounds. However, the methanol extract showed a higher inhibitory activity against the test organisms. This correlates positively with the findings of [7]. The antimicrobial effect of methanol extract against these organisms may be due to the ability of the methanol to extract some of the active constituents of these plants like phenolic compounds, saponin, bryophyllin and other secondary metabolites which are reported to be antimicrobial. The aqueous extract showed inhibitory activity only against the Gram-positive organisms. This may be as a result of loss of some of the plant's active principle when drying, or the inability of the solvent to dissolve some of the active principles of the plant. El-Mahmood [17] explained that the low activity of aqueous extract of plants could be as a result of the modulatory effects of some phenolases and hydrolases released by plant material when they are ground in water. The result of this research is in agreement with the report of [18], that showed methanol, local gin and squeezed leaf extracts of *B. pinnatum* to have higher antimicrobial activity in that order than the aqueous extract. The quantitative analysis showed that *B. pinnatum* is rich in flavonoids (2.8%), cardiac glycoside (65% and 50% for aqueous and methanol extract respectively), tannin (0.36%), alkaloid (1.01%) and saponin (4.6%) as shown in table 3. The higher aqueous cardiac glycoside percentage yield could be attributed to its solubility in water. Although tannins showed the least percentage yield, its presence cannot be underestimated in the antimicrobial activity of the plant. The mechanism of action of tannin is based on their ability to bind protein thereby inhibiting cell protein synthesis [20]. From table 4, it was observed that the test organisms showed higher inhibition zones at higher concentrations of both extracts. This means that higher doses of the antimicrobial agent will be needed in the treatment of infections caused by these organisms provided they are not toxic to the tissues. This correlates positively with the findings of [19], that the activity of antimicrobial agent is concentration-dependent. The present work showed that both extracts of the plant were not active against the fungal strain tested. This agrees with the work of [7] who observed that *C. albicans* resisted the action of *B. pinnatum* extracts. The resistance of the extract is an indication that this plant does not possess a potential as a source of antifungal agent. The result of table 5 shows that all the bacterial isolates requires a higher concentration of the methanol extract (200 mg/ml) to inhibit the organisms than the aqueous extract that inhibited only the Gram-positive bacteria at 100 mg/ml concentration. Table 6 shows *S. aureus* to give the highest intercept while *B. subtilis* gave the least intercept showing that *S. aureus* showed the highest sensitivity with the methanol extract and *B. subtilis* the least. Table 7 also shows the intercept of *S. aureus* to be higher than that of *B. subtilis* showing also that *S. aureus* is more sensitive to the aqueous extract than *B. subtilis*. Table 8 also correlates with the previous findings of this study that

the test organisms require a higher concentration of 200 mg/ml methanol extract to kill the test bacteria while the gram-positive bacteria require only 100 mg/ml of aqueous extract to kill the organisms. Further studies on other extracting solvents may be conducted in search of antifungal activity. The implication of broad spectrum action of *B. pinnatum* methanol extract is that it can be useful in antiseptic and disinfectant formulation as well as in chemotherapy if the active principles can be isolated [21].

Conclusion and Recommendations

The results of this research have shown that the aqueous and methanol extracts of *B. pinnatum* have excellent antibacterial effect on the test bacteria namely *S. aureus, E. coli, P. aeruginosa,* and *B. subtilis* while only the methanol extract showed anti-hyperglycemic property. It was observed that the methanol leaf extracts was used in the treatment of alloxan-induced diabetes in rats hence could be valuable to humans.

Further efforts should be directed towards extracting the active phytochemicals in *B. pinnatum* leaf extracts, and elucidation of mechanism of anti-hyperglycaemic action of the methanol leaf extract.

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