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ANTIBACTERIAL ACTIVITY OF *BRYOPHYLLUM PINNATUM* LEAVES EXTRACT AGAINST *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI*

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ABSTRACT

Pathogenic microorganisms cause several infections of great public concern. These pathogens from many studies were found to be resistant to many antibiotics. Thus, there is the need for new antimicrobial agents of plant origin to overcome the problem of resistance. The aim of this study was to screen the antibacterial activity of aqueous leave extract of *Bryophyllum pinnatun*. The aqueous leave extract of the *B. pinnatun* prepared by percolation method was investigated for phytochemical constituents and activity against *Staphylococcus aureus* and *Escherichia coli* using agar well diffusion method. The phytochemicals detected in the extract include saponins, tannins, alkaloids and flavonoids. The extract was found to be active on both the organisms with zone of inhibition ranging from 8-19mm for *S. aureus* and 7-16mm for *E. coli*. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were found to be higher at 50mg/ml and 100mg/ml, respectively. This study shows that leaf extract of *B. pinnatum* have antibacterial properties, and effective on both Gram positive and Gram-negative bacteria. Therefore, this result supports the traditional use of the *B. pinnatum* leaves in infection treatment.

Keywords: *Bryophyllum pinnatum, Sensitivity test, Styaphlococous aureus, Escherichica coli*

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1. INTRODUCTION

Antibiotics have been widely used in the treatment of several microbial infections [1]. They have significant impact in alleviating the rate of morbidity and mortality due to human microbial infections especially in underdeveloped countries where there is inadequate public health infrastructure [2]. However, the antibiotics have been misused and overused in the general populace leading to an increasing rate of microbial resistance [3].

As a result of rapid development of resistance to the available potent and cost–effective antibiotics by the clinical isolates, the search for alternative antimicrobial agents is indispensable. Several medicinal plants have been acknowledged as potential sources of natural antimicrobial compounds [4]. The World Health Organization (WHO) shows that medicinal plants are the best sources of various drugs [5]. A vast number of medicinal plants have been investigated for numerous secondary metabolites like tannins, alkaloids, phenolic compounds, and flavonoids. These metabolites have been confirmed to be responsible for plants' *in vitro* antimicrobial properties [4].

Bryophyllum pinnatum has been descried as an environmental weed belonging to the family Crassulceae. This plant is a succulent glabrous herb that is approximately 0.3 -1.2 m high. The *B. pinnatum* grows predominantly in the tropics, being a native to Madagascar and Southern Africa [6]. Some common names of this plant include life plant, air plant, love plant or miracle leaf [7-8].

The *B. pinnatum* is used in ethno-medicine generally for the treatment of ear-aches, cough, diarrhea, dysentery, abscesses, ulcer, insect bites, heart conditions, epilepsy, arthritis, dysmenorrhea, whitlow [9], treat urinary stones, hypertension, cold, asthma and other ailments [8]. This is also applied on ulcers, burns and on the body of young children when they are ill. The leaves of this plant contain bryophyllum, potassium, malate, ascorbic acid and citric acid. The plant is also rich in both macro and micro elements, vitamins, calcium, phosphorus, ascorbic acid, insulin [10] and other compounds like saponin, flavonoids. Anthrafuinones, xanthones, bryophyllum A and B [11]. Anti-inflammatory hypoglycaemic, antidiabetic and anti-cancer properties have been reported [9].

2. RESULTS AND DISCUSSION

2.1 Physical Properties and Phytochemical Screening of the Extract

The physical properties which include colour, texture, and consistency as well as percentage yield of the extract are shown in Table 1. The *Bryophyllum pinnatum* leaves extract was brown in colour, smooth textured, intermediate in consistency and with a percentage yield of 7.34%. Phytochemical screening of the aqueous leaves extracts of the *B. pinnatum* showed the presence of saponins, tannins, flavonoids and alkaloids, while steroids was found to be below the detectable limit.

The percentage yield of 7.34% was high, which may be due to high polarity and solubility of the water used as the extraction solvent [12]. This is in line with the work of Aibinu et al.,(2007) [13] that showed high yield of aqueous extract second to the methanolic extract. Similar to this finding, Mudi and Ibrahim, (2008) [14] confirmed the presence of tannins, flavonoids, and saponins in the aqueous extracts of *B. pinnatum*. In contrary, polyphenols, steroids and terpenoid were the key phytochemicals identified from the leaves of *B. pinnatum* in another study [15].

Table 1. Physical Properties and Phytochemical Screening of the Extract

Physical Properties				Phytochemical Screening				
Cobur	percentage	yield	consistency	Tannins		Saponins		Alkaloids
texture				Flavonoids	St			
Brown			7.34%	+++		+++		+
Int.	smooth			+	-			

Key: + = presence of compound

+++ = presence of compound in high amount

- = the compound is below detectable limit

Int. =Intermediate

St=Steroids

2.2 Sensitivity of the test organisms to the extract

Table 2 shows the extent of sensitivity of the test organisms (zone of inhibition) at various concentrations of 100, 50, 25, 12.5 and 6.25 mg/ml. The extract shows remarkable degree of antibacterial activity against the tested organisms particularly at the higher concentrations of 100 and 50mg/ml. The zones of inhibition observed with *S.aureus* was almost the same with that of *E. coli*. This correlates well with the work of Ofokansi, (2005) [16] that showed strong activity of the *B. pinnatum* extract against some Gram-positive organisms, and the work of Aibinu et al., (2007) [13] that showed the strong activity of the *B. pinnatum* extract against Gram-negative organism.

Table 2. Sensitivity of test organisms to leaves extract of *B. pinnatum*

Test Organisms	Concentrations (mg/ml)					Cren
	40mg/ml					
	100	50	25	12.6	6.25	
	Zone of inhibition (mm)					
Escherichia coli	16	11	9	8	7	25
Staphylococcus aureus	19	15	10	8	8	32

2.3 Minimum Inhibitory Concentration (MIC) of the Extract

The results of MIC test of the *B. pinnatum* aqueous leaves extract showed that 50 mg/ml was the lowest concentration of the extract that inhibited the growth (absence of turbidity) of the test organisms following an overnight incubation (Table 3). This implies that the MIC of the extract on the *S. aureus* and *E. coli* is 50 mg/ml. The antibacterial effect of the aqueous extract against these organisms may be due to the ability of water to extract some of the active compounds of the *B. pinnatum* plants like phenolic compounds, saponins, bryophyllum and other secondary metabolites which are reported to be antimicrobial [17,18]. The result of minimum inhibitory concentration shows that, the aqueous extract was not the most effective by having MIC at higher concentration of 50mg/ml

Table 3. Minimum Inhibitory Concentration of the extract

Concentration (mg/ml)	Change in Broth Turbidity
100	No
50	No
25	Yes
Control (Gentamycin 40mg/ml)	No

3.4 Minimum Bactericidal Concentration (MBC) of the extract

The result of MBC test of *B. pinnatum* leaves aqueous extract shows that total cell death from the MIC test occurred in the 100 mg/ml concentration (Table 4). Therefore, MBC of the extract on the test organisms was taken as 100 mg/ml. This is contradictory to the work of Akacha (2016) [19] that showed the most effective of the extract of *B. pinnatum* with MIC at 5 mg/ml and MBC at 10 mg/ml. This contradiction may be due to variation of the solvents used since in the work of Akacha (2016) [19], ethylacetate and hexane were the extraction solvents.

Table 4. Minimum Bactericidal Concentration of the Extract

Concentration (mg/ml)	Appearance of Growth
100	Negative
50	Positive

3. MATERIALS AND METHODS

3.1 Collection and authentication of samples

The leaves of *Bryophyllum pinnatum* were collected from Kailaro Garden, opposite Government Science Secondary School (GSSS) Doma, Gombe, Gombe State. The samples were identified by a botanist at the Herbarium of Biological Science Department, Gombe State University and obtained a voucher number (100).

3.2 Preparation and extraction of samples

The leaves of *B. pinnatum* were shade dried at room temperature for two weeks and ground into powder using laboratory motor and pestle. One hundred and fifty gram (150g) of the

leaves powder was added into 1500 ml of distilled water (1:10w/v) and extracted using percolation method [20]. The mixture was shaken intermittently using rotary shaken for three days at room temperature. Finally, the resulting mixture was filtered using Muslim cloth and whatman No1 filter paper. The filtrate was evaporated using rotary evaporator at 40˚C and then stored at 4˚C until further analysis.

3.3 Phytochemical analysis

Phytochemical analysis was conducted on the leaves crude extracts to detect the phytochemical constituents as reported previously [21-22].

3.3.1 Detection of Alkaloids

About 2-4 drops of Dragendroff's reagent was added to 5 ml of the extract and observed for presence of alkaloids following a colour change.

3.3.2 Detection of saponins

A 0.5g of extract was shaken with 20 ml of water. Foam was produced and persisted for ten minutes which confirmed the presence of saponins.

3.3.3 Detection of tannins

About2-3 drops of ferric chloride were mixed with 2ml of the extract and observed for tannin detection.

3.3.4 Detection of Flavonoids

One millitre (1 mL) of concentrated NaOH was added to 2 ml of the extract and mixed, followed by the addition of a few drops of diluted hydrochloric acid.

3.3.5 Detection of Steroids

Chloroform (2-3 drops) was added to 2 ml of the extract and mixed followed by the addition of some drops of concentrated H_2SO4.

3.4 Sources and confirmation of test organisms

Staphylococcus aureus and *Escherichia coli* used as test organisms were obtained from stock culture collection of Microbiology laboratory, department of Microbiology, Gombe State University, Nigeria. The *E. coli* was confirmed based on Gram staining and biochemical tests such as Indole test, citrate utilization test and catalase test. Similarly, the *S. aureus* was confirmed based on Gram staining and biochemical tests including catalase and coagulase

tests and first been sub-cultured on mannitol salt Agar (MSA) [15].

3.5 Standardization of test organisms' inocula

The *E. coli* and *S. aureus* were sub-cultured on MacConkey and MSA agar plates, respectively, at 37˚C for 24 hours. Subsequently, a loopful discrete colonies of each organism was emulsified in 3-4 ml of sterile normal saline contained in test tube and matched to 0.5 McFarland turbidity standard [23].

3.6 Preparation of extract concentrations

A stock concentration of 100 mg/ml of the extract was prepared by dissolving 1g of the extract in 10 ml of DMSO. Four different concentrations of 50, 25, 12.5, and 6.25 mg/ml of the extract was prepared from the stock solution by using two-fold serial dilution with the same DMSO as reported by [24].

3.7 Sensitivity testing

The antibacterial activity of the *B. pinnatum* leaves' extract was studied by using agar well diffusion method as reported by Garba et al 2021[17]. Mueller-Hinton Agar plates were prepared according to manufacturer's instruction and inoculated with the standardized inocula of the test organisms by using sterile swab stick. A sterile cock borer of 6mm diameter was used to make wells on the medium. An aliquot of 100 µl of the various extract's concentrations was dropped into their respective appropriately labelled wells. Gentamycin (40 mg/ml) and DMSO were used as positive and negative controls, respectively. The inoculated plates were kept aside for 1 hour to allow the extracts to diffuse into the agar and then incubated at 37 ˚C for 24 hours. Antibacterial activity of the *B. pinnatum* leaves extract was determined by measuring the diameter of zones of inhibition (mm) produced after incubation.

3.8 Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration of the extract was determined by using broth dilution method. Nutrient broth (10 ml) was prepared in test tubes according manufacturers instruction. The extract was serially diluted such that the broth tubes contained 50mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.2mg/ml concentrations. The standardized inocula of the test organisms were inoculated into their respective extract concentrations and incubated at 37^0C for 24 hours. Two control tubes, one tube containing extract without inoculum and the other tube

containing the growth medium and inoculum were used alongside. Following the incubation, the broth cultures were observed for turbidity or growth. The lowest concentration of extracts in the broth which showed no turbidity was recorded as the minimum inhabitation concentration (MIC) [19].

3.9 Minimum bactericidal concentration (MBC) determination

This was carried out to determine the concentration of extract that could stop the growth of the test organisms. Mueller Hinton agar plates were prepared according to manufacturer's recommendation and used to subculture broth cultures that showed no evidence of growth from the MIC determination tubes. The plates were incubated at 37 ˚C for 24 hours after which the plates were observed for any colony growth. The plate with lowest concentration of extract that show absence of any growth was considered as MBC [27].

3.10 Statistical analysis

The results were analysed using one-way analysis of variants (ANOVA) test.

4. CONCLUSION

The study shows that the leaves of *B. pinnatum* possessed antibacterial properties as it inhibited the growth of both the *S. aureus* and *E. coli* serving as representative organisms for Gram-positive and Gram-negative bacteria, respectively. Therefore, this result supports the traditional medicine use of the *B. pinnatum* leaves.

5. ACKNOWLEDGEMENTS

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