<https://doi.org/10.36293/sfj.2019.0046>

ANTIMICROBIAL SPECTRUM OF *BRYOPHYLLUM PINNATUM* EXTRACT (LEAF) ON SOME CLINICAL BETA-LACTAM RESISTANT TEST ORGANISMS

ZAKARI ADEIZA DAVID^{1*}, BELLO KIZITO ENEYE¹, YUSUF LAMIDI¹, ADEJOH PATIENCE OMEBIJE¹, MUSA OIZA AISHA¹, OLAITAN CATHERINE OHUNENE¹, OSAZUWA CHRISTOPHER OLOJEMARIN², OLUMIDE OLUYELE²

¹Microbiology Department, Faculty of Natural Sciences, Kogi State University, Anyigba.
P.M.B 1008, Anyigba, Kogi State, Nigeria.

²Department of Microbiology, Faculty of Life Sciences, Adekunle Ajasin University, Akungba
PMB 001, Akoko, Ondo State

Abstract

The trend of emerging drug resistant clinical pathogens are on the rise, therefore it is imperative that newer sources of potent antibiotics be explored. Plants offer a vast array of opportunities. This study examined the antimicrobial activity of both the ethanolic and aqueous extracts of *Bryophyllum pinnatum*. The clean dry leaves were macerated and various concentrate of the extract was prepared and screened for their antimicrobial activities against selected Beta lactam drug resistant organisms; Methicillin Resistant *Escherichia coli*, Methicillin Resistant *Staphylococcus aureus*, Oxacillin Resistant *Pseudomonas aeruginosa* and Cefoxitin Resistant *Candida albicans*, via the disc diffusion method, Ciprofloxacin was used as control antibiotic. The Minimum Inhibitory Concentration and Minimum Lethal Concentration of the extracts was determined via the broth dilution method at concentrations ranging from 1.0g/ml, 0.5 g/ml, 0.25 g/ml, 0.125 g/ml for 100%, 50%, 25% and 12.5% vol/vol respectively. The ethanolic extract was the most reactive while the aqueous extract showed lesser antimicrobial activity. Methicillin Resistant *Escherichia coli* have the highest susceptibility to both ethanolic and aqueous extract with a zone of inhibition of 22 mm and 11 mm respectively at $p \leq 0.05$. There was no statistical difference in the concentration and the zone of inhibition as the concentration decreases. *Bryophyllum pinnatum* possesses pharmacological potentials worthy of exploration to curb the menace of microbial drug resistance.

Keywords: *B.pinnatum*, *E.coli*, *S.aureus*, *P.aeruginosa*, *C.albicans*, Minimum Inhibitory Concentration) and Minimum Lethal Concentration.

Corresponding Author Email: David.z@ksu.edu.ng

Introduction

Over the last decade, new emerging and re-emerging antibiotic resistant bacteria strain have

become a day to day problem for clinicians and researchers (Alam *et al.*, 2016), the need for a more potent, cost effective and efficient solution to this emerging hitch in medicine requires

strange and new approaches. The latter stirs the need to consider plants and its varying body parts as potential antimicrobial agent to tame the highly emerging bacteria drug resistance (Nascimento *et al.*, 2014).

A substance is said to be antimicrobial if it possesses the inherent ability to impede the multiplication and proliferation of a micro-organism and plant represent over 70% of all known sources of potent antibiotic bioactive agents (Kingston, 2014).

Bryophyllum, commonly known as Sprouting leaf is usually found growing in various climatic belts and regions across the tropical rainforest, India China and Northern Australia (Oliver, 2014; Dehbuti *et al.*, 2012). It belongs to the Family **CRASSULACEAE**. The medicinal importance of the plant is enormous ranging from its application in severe disease treatments and therapy and as an important source of novel bioactive substances that are now used in modern oncological treatments, hence its name; Life plant (Gill, 2012). *Bryophyllum pinnatum* is usually called several names among the over 100 languages within Nigeria, but basically it is known as “oduaopua” in Igbo land (Igoli *et al.*, 2015), where it grows freely as a perennial herb or planted as ornamentals in individuals households or gardens.

To the best of our knowledge, this study reports the first antibiotic activity of the leaf extract of *Bryophyllum pinnatum* on Beta Lactamase producing bacteria that are of clinical significance. There is dearth information on the antimicrobial activity of *Bryophyllum pinnatum* extracts on Methicillin and Oxacillin resistant bacteria within the study population (Igoli *et al.*, 2015).

Materials and Method of Study

All the glassware required for this experiment was properly washed with common brand detergent and rinsed with clean sterile distilled water after which they were sterilized at 160°C for 2 hours in hot air oven while adhering strictly to established standard guidelines for dry air sterilization in an oven.

The working bench surfaces were disinfected before carrying out any experiment to avoid contamination and to ensure aseptic working conditions.

2.1 Collection and identification of plant

The plant and leaves were collected from plant garden in Kogi State University, Anyigba and was identified in the Botanical lab of Plant science department of Kogi State University as *Bryophyllum pinnatum* using established standard keys as described by Chinaza and Chinweizu (2019).

Preparation of *Bryophyllum pinnatum* extracts

Collected *Bryophyllum pinnatum* leaves were washed shredded and pounded using mortar and pestle. Cold extraction method was implemented. Two sets of 400g of the crushed leaves were measured. Each set of the crushed leaves was soaked in 800ml of ethanol and 800ml distilled water in two 1000ml capacity flasks respectively. The flasks were then left standing on a bench with occasional shaking for 72 hours. The extracts were filtered with muslin filter and evaporated to dryness by gentle heating at 70°C on a hot plate. The dried concentrates (ethanol and aqueous extracts) were weighed and stored in a desiccator for antimicrobial assay on the isolates.

Preparation of medium

Nutrient agar, nutrient broth, and peptone water were prepared and sterilized according to the manufacturer's specifications.

Preparation of plant extract concentration

Different concentration (v/v) of the extract of *Bryophyllum Pinnatum* was prepared. For antimicrobial screening; A 1.0 gram (g) of extract were dissolved each in 1 milliliter (ml), 2 ml, 4 ml and 8 ml of distilled water giving concentrations of 1.0 gram-per-milliliter (g/ml), 0.5 g/ml and 0.25 g/ml, 0.125 g/ml at 100%, 50%, 25% and 12.5%, respectively. The Minimum Inhibitory Concentration (MIC) was determined from the 1.0 g/ml, 0.5 g/ml, 0.25 g/ml and 0.125 g/ml concentrations, as a 0.2ml of extract was dispensed into three different broth tubes containing the various test organisms.

Antimicrobial evaluation

Antimicrobial Evaluation on each of the different concentration of the *Bryophyllum pinnatum* was done using Kirby-Bauer diffusion susceptibility test method (also called the *disc diffusion test*). Discs (3mm) were obtained by perforating filter paper. The discs were sterilized using oven and impregnated with known concentrations of plant extracts then allowed to dry. Nutrient agar and peptone water was prepared following manufacturer's instruction, autoclaved at 15psi for 15minutes, allowed to cool then poured in the petri-dishes and were left to set under aseptic environment, a pure cultures of Methicillin resistant *Escherichia coli*, Oxacillin resisitant *Pseudomonas aeruginosa*, Methicillin Resistant *Staphylococcus aureus*, and cefoxitin resistant *Candida albicans* inoculums adjusted to 0.5 McFarland's standard was well seeded on the surface of the agar plate with a sterile swab stick via the spread plate inoculating method. Impregnated discs and antibiotic disc (Ciprofloxacin which served as control) were placed on plates using sterile forceps. The resulting culture was incubated at 37°C for 24hrs for the bacteria test organisms and 72hrs for the fungal test organism. The zone of inhibition was measured in millimeters and was recorded appropriately.

The MICs were determined using broth dilution technique. About 3ml of the nutrient broth and peptone water were introduced into test tubes. Approximately 0.2ml of the extract at different concentrations was introduced into each of the test tubes. The test organisms (20ul) were inoculated into the tubes and then incubated at 37°C for 24 hours and 72hrs after which the Minimum Inhibitory Concentration was observed as the least concentration of extract which resulted in no visible growth and was thus recorded. The Minimum Lethal Concentration (MLC) was determined by sub culturing the MIC assay hat did not indicate any growth after incubation for 24 hours and 72 hours. The latter

was carried out by sub-culturing inoculums from MIC positive cultures into sensitivity test agar dish, incubated for 24 hours and 72 hours. The highest dilution of the concentration that yielded no single bacterial colony was taken as the Minimum bactericidal Concentration as described by Akinyemi *et al.*, (2005). The MLCs were obtained when broth tubes containing no visible growth were sub cultured unto a solid media without any antimicrobial agent and no colonies were formed.

Results and Discussion

This study shows that the antimicrobial activities of the ethanolic extracts of *Bryophyllum pinnatum* leaves against the test organisms were the most reactive at all concentrations. The zones of inhibition of the extracts also showed that Methicillin Resistant *Escherichia coli* was the most susceptible organism to both ethanolic and aqueous extract with the highest zone of inhibition (22mm for ethanolic extract and 13mm for aqueous extract respectively). Cefoxitin Resistant *Candida albican* was the least susceptible in both ethanolic and aqueous extract of the tested plant leave (12mm for ethanolic extract and 11mm for aqueous extract respectively) as shown in Table 1 and Table 2.

At concentration of 25% (0.25g/ml), Methicillin Resistant *Escherichia coli* and Oxacillin Resistant *Pseudomonas aeruginosa* did not show any sign of growth (clear) thus was taken as the MIC, while Methicillin *Staphylococcus aureus* and Cefoxitin *Candida albicans* 50% (0.5g/ml) was taken as the MIC as shown in Table 3. Methicillin Resistant *Escherichia coli* and Oxacillin Resistant *Pseudomonas aeruginosa* had MLC at 25% (0.25g/ml), Methicillin *Staphylococcus aureus* and Cefoxitin *Candida albicans* had MLC at 50% (0.5g/ml) as shown in Table 3.

Table 1: Antimicrobial activity of ethanolic extract of *Bryophyllum pinnatum*

Test Organisms	Zone of inhibition (Concentration in %)				Antibiotic Control (10µg/ml)
	100	50	25	12.5	
<i>Methicilin Resistant Escherichia coli</i>	22mm	13mm	9mm	6mm	20mm
<i>Methicilin Resistant Staphylococcus aureus</i>	14 mm	12mm	5mm	Nil	18mm
<i>Oxacillin Resistant Pseudomonas aeruginosa</i>	19mm	17mm	10mm	8mm	31mm
<i>Cefoxitin Resistant Candida albicans</i>	12mm	7mm	Nil	Nil	15mm

Table 2: Antimicrobial activity of aqueous extract of *Bryophyllum pinnatum*

Test Organisms	Zone of inhibition (Concentration in %)				Antibiotic Control (10µg/ml)
	100	50	25	12.5	
<i>Methicilin Resistant Escherichia coli</i>	13mm	8mm	Nil	Nil	11mm
<i>Methicilin Resistant Staphylococcus aureus</i>	11mm	7mm	9mm	Nil	16mm
<i>Oxacillin Resistant Pseudomonas aeruginosa</i>	11mm	9mm	5mm	Nil	10mm
<i>Cefoxitin Resistant Candida albicans</i>	8mm	5mm	Nil	Nil	12mm

Table 3: Minimum inhibitory concentration of ethanolic leaf extracts and aqueous extracts of *Bryophyllum pinnatum*

Key; NG=NO growth, G=growth

Test Organisms	Ethanol leaf extract (%)				Aqueous leaf extract (%)			
	100	50	25	12.5	100	50	25	12.5
<i>Escherichia coli</i>	NG	NG	NG	G	NG	G	G	G
<i>Staphylococcus aureus</i>	NG	NG	G	G	NG	G	G	G
<i>Pseudomonas aeruginosa</i>	NG	NG	NG	G	NG	G	G	G
<i>Candida albicans</i>	NG	NG	G	G	NG	G	G	G

Table 4: Minimum lethal concentration of ethanolic *bryophyllum pinnatum*

Test Organisms	Ethanol leaf extract (%)		
	50	25	12.5
<i>Methicilin Resistant Escherichia coli</i>	NG	NG	G
<i>Methicilin Resistant Staphylococcus aureus</i>	NG	G	G
<i>Oxacillin Resistant Pseudomonas aeruginosa</i>	NG	NG	G
<i>Cefoxitin Resistant Candida albicans</i>	NG	G	G

Key; NG=NO growth, G=growth

Methicilin resistant Escherichia coli and *Oxicallin Resistant Pseudomonas aeruginosa* had MLC at 25% (0.25g/ml), while *Staphylococcus aureus* and *Candida albicans* had MLC at 50% (0.5g/ml).

The ethanol extract of the leaves was the most potent showing antimicrobial activity on one or all of the test organisms at low to higher concentrations (0.125 - 1.0g/ml), the probable reason for this is unclear but it could be due to established knowledge that ethanol is an efficient extraction solvent either due to its polarity or concentration. Aquil and Ahmad (2013) reported that phytochemicals have a high dissolution index in solvent with high polarity. The latter can be attributed to the reason for the high efficacy of the ethanolic extract of *Bryophyllum pinnatum*. This study is in agreement with the findings of Aquil and Ahmad (2013) where they reported that the ethanolic extract of *Bryophyllum pinnatum* has a broad spectrum activity against bacteria and fungi. The aqueous extract of *Bryophyllum pinnatum* showed moderate antimicrobial activity. The latter is in agreement with the independent reports of Ofokansi (2015) and Akinpelu (2000).

The extracts were effective on Gram positive, Gram negative bacteria and a fungus. Methicillin Resistant *Escherichia coli* showed significant susceptibility at all concentrations of the ethanol extract with inhibition values ranging from 6-22mm. In contrast to the commonly reported resistance of Gram negative bacteria to antimicrobial agents, the extracts showed better activity against them than the Gram positive bacterium used. The latter is in agreement with Akinpelu (2000). The MIC of the ethanolic extract for Methicillin Resistant *Escherichia coli* and Oxacillin Resistant *Pseudomonas aeruginosa* is taken at 25% (0.25g/ml) while for Methicillin Resistant *Staphylococcus aureus* and

Cefoxitin Resistant *Candida albicans* 50% (0.5g/ml) was taken as the MIC, the low concentration Minimal inhibition is a strong indication that the extract is a high reference candidate in the quest for new effective antibiotic discovery from plants, this is also in strong agreement with the reports of CDC (2014), where they reported that plants offer vast opportunities for novel bioactive compound discovery against drug resistant microorganisms. Methicillin Resistant *Escherichia coli* and Cefoxitin Resistant *Candida albicans* have the highest and lowest inhibition respectively. The ethanol extract showed lethal effects on Methicillin Resistant *Escherichia coli* and Oxacillin Resistant *Pseudomonas aeruginosa* at concentration of 25% (0.25g/ml) while Methicillin Resistant *Staphylococcus aureus* and Cefoxitin Resistant *Candida albicans* at concentration of 50% (0.5g/ml).

Conclusions

The heavy burden of antimicrobial drug resistance on researchers and clinicians has increased the need for the discovery of novel bioactive agents from natural sources to which plant stands as king. Our study shows that extracts of *Bryophyllum pinnatum* have moderate to high antibiotic activity on the tested organisms and its MIC and MLC values are acceptable. Therefore more findings should be made on the bioactive constituents of *Bryophyllum pinnatum* as it demonstrates dynamic pharmacological potentials.

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