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ANTIMCROBIAL ACTIVITY OF EXTRACT OF *BRYOPHYLLUM PINNATUM* AND *CITRUS AURANTIFOLIA*ON SOME SELECTED PATHOGENS.

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ABSTRACT

The inhibitory properties of Bryophyllum pinnatum and lime juice on some selected microorganisms (Staphylococcus aureus,Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae, Aspergillus niger and Candida albicans) were investigated. The various concentrations of the extracts were prepared by double dilution method while the ability of the various extracts to inhibit the growth of the pathogenic organisms was determined using the agar well technique. The highest in-vitro antimicrobial activity of methanolic extract of Bryophyllum pinnatum was (13.0±2.83^{a} mm at 200 mg/ml) against Klebsiella pneumoniae while the least antimicrobial activity was (10.5±2.12^{b} and 10.0±0.00^{e} mm at 200 mg/ml) against Streptococcus pyogenes and Candida albicans respectively. The lime juice extracts had the highest in-vitro antimicrobial activity was (11.0±0.00^{b} mm at 200 mg/ml) against Staphylococcus aureus while the least antimicrobial activity was (7.0± 0.0^{b} mm at 200 mg/ml) against Streptococcus pyogenes. The methanol and aqueous soluble plant extracts were active against all the test isolates but methanol extract of Bryophyllum pinnatum demonstrated greater inhibitory activity (MIC) on Staphylococcus aureus, Escherichia coli, Streptococcus species and Aspergillus niger at the range of 12.5 mg/ml. The lime juice extracts demonstrated greater inhibitory activity (MIC) on Escherichia coli at 12.5 mg/ml. The results in the findings showed that the methanolic and aqueous extracts of Bryophyllum pinnatum as well as lime juice extracts have broad-spectrum antimicrobial activity and can serve as natural therapeutic agent against some enteric pathogens.

Keywords: *Bryophyllum pinnatum,* inhibitory properties, microorganisms, methanol, aqueous.

INTRODUCTION

The plant kingdom consists of a variety of plants which are of immerse importance to humans and of valuable use in the treatment of various illnesses (Igbinosa *et al.*, 2009; Akinyeye *et al.*, 2014).The medicinal value of these plants lies on their chemical and phytochemical substances they contain. Different parts (bark, root, twig, fruit and leaves) of different plants have been studied and found to be sources of antimicrobial agents. Many of these indigenous medicinal plants are used as spices and food plants that are sometimes added to foods for pregnant and nursing mothers for medicinal purposes (Akinyeye *et al.*, 2014). Medicinal plants contain biologically active chemical substances such as saponins, tannins, essential oil flavonoids, alkaloids and other chemical which have curative properties. These complex chemical substances of different composition are found as secondary plant

metabolite in one or more of these plants (Kayode and Kayode, 2011).

Bryophyllum pinnatum belongs to the crassulaceae family and is commonly found in tropical Africa, India, China, America and Australia (Obaid *et al*., 2019). *Bryophyllum pinnatum* is used in ethnomedicine. The leaves and leaf juice have been used traditionally as anti-inflammatory, antipyretic, antimicrobial, anti-oxidant, antitumour, antidiabetic, anti-ulcer, antiseptic, hypocholosterolemic, and cough suppressant (Ali *et al*., 2013; Ghasi *etal*., 2011; Devbhuti *et al.,* 2012). The leaves or the whole plant are used as analgesic and generally for the treatment of ear ache, cough, asthma, diarrhoea, dysentery, jaundice, abscesses, ulcers, insect bites, heart troubles, epilepsy, arthritis, dysmenorrheal, whitlow and other ailments. (Ghasi *etal*., 2011). This wide range of traditional uses justifies its being called "life plant", "resurrection plant", "goodluck", love plant, cathedral bells or miracle leaf and is distinctive for the profusion of miniature plantlets that form on the margins of its Phylloclades, a trait it has in common with some other members of its genus (Ghasi *et al*., 2011). The plant is a good source of ascorbic acids, riboflavin, thiamine and niacin.

Citrus aurantifolia (Lime) belongs to the Citrus family. It is sour in taste and serves as a rich source of vitamin C and often used to accent the flavours of foods and beverages. Limes contain nutrients such as carbohydrates, sugar, soluble and insoluble fiber, sodium, vitamins, minerals, fatty acids and amino acids (Bina *et al*., 2010). Limes possess unique flavonoid compounds that have antimicrobial, antioxidant and anti-cancer properties (Tomotake, 2006). Limes are also effective in the treatment of stomach ache, colds, fevers, sore throats, sinusitis, bronchitis and asthma (Khan*et al*., 2012). The present study was designed to evaluate the antimicrobial activity and potency of*B.pinnatum* and *Citrusaurantifolia* leaf extracts respectively on pathogens.

MATERIALS AND METHODS

Collection of samples

Fresh plant of *Bryophyllum pinnatum* was collected from the horticulture department of the National Root Crop Research Institute (NRCRI), Umudike and was identified by Mrs Flora Mukah from the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. The lime fruit was purchased from Ndioru market at Umudike, Abia State, Nigeria and was washed with distilled water.

Preparation and extraction of the leaf extracts

The leaves of *Bryophyllum pinnatum* were washed severally to remove dirt, dried in shade at room temperature and ground to powder using electric blender of model W-BL5085M. Fifty (50) grams of the leaf powder was macerated in 500 ml of methanol and 500 ml distilled water (aqueous extract) for 24 hours, and centrifuged at 120 rpm to enable proper diffusion of the active ingredients. After that, the contents were filtered using Whatman's filter paper No 1. The filtrates were evaporated to dryness separately in a water bath and stored for analysis (Karabi and Sankar, 2015).

Preparation and extraction of *Citrus aurantifolia* juice extract

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Citrus aurantifolia fruits free from defects and decay were washed with distilled water to remove dirt and further sterilized with methanol to remove any form of contaminant. The fruits were aseptically cut with a sterile knife and the juice extracted. The extracted juice was stored in a sterile container at a temperature of 4°C to 8°C (Ugwu *et al.*, 2018).

Preparation of concentration of the extracts

One gram (1g) each of the methanol and aqueous crude extract of the plant was added to 5 ml of aqueous and methanol respectively to give a concentration of 200 mg/ml. Other concentrations of 150, 100, 50, 25 and 12.5mg/ml were prepared by double dilution method as described by Iqbal and Arina (2001). Also, the lime juice was diluted with distilled water to give concentrations of 200, 150, 100, 50, 25, and 12.5 mg/ml respectively.

Test microorganisms

Bacterial and fungal cultures: *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae, Candida albicans* and *Aspergillus niger* were obtained from Microbiology Laboratory stocks in Michael Okpara University of agriculture, Umudike, Abia State, Nigeria. The test organisms were resuscitated and the bacteria identities confirmed using cultural, morphological and biochemical tests as described by Cheesbrough, (2006). The test fungi were identified and characterized based on their macroscopic and microscopic characteristics by comparing them to taxonomic guides (Barnett and Hunter, 1972; Cheesbrough, 2006). They were maintained at 4°C on agar slants.

Standardization of bacterial inoculum:

Suspension of microorganisms was made in nutrient broth and incubated at 37°C for 24 hours. Turbidity produced was adjusted using sterile normal saline to 0.5 MacFarland standards (10^8CFU/ml).

Antimicrobial properties of lime juice and plant extracts against test organisms

The ability of the various extracts to inhibit the growth of the bacterial and fungal pathogenic organisms was determined using the agar well diffusion technique. Standardized 50µl inoculum of each selected bacteria was aseptically inoculated onto Mueller-Hilton agar plates and the fungi on SDA plates using the spread plate method. A sterile cork borer of 5 mm diameter was used to make wells on the plates and 0.1ml of each varying concentrations of the plant extracts, lime juice and antibiotic control (Gentamycin/Ketoconazole), i.e. 200, 150, 100, 50, 25 and 12.5 mg/ml was dispensed into the wells. The plates were allowed to stand at room temperature for 30 minutes to allow for easy diffusion of the extract into the agar before being incubated at 37°C for 24 hours and room temperature (25^oC) for 72 hours for fungi. After incubation, the diameter of the zones of inhibition around each well was measured and recorded in millimeter representing the antimicrobial activities of the extracts (Ahmad and Beg, 2001).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of both leaf extracts and lime juice

were determined for each of the test bacterial and fungal pathogen at varying concentrations of 200, 150, 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml. One milliliter (1 ml) of nutrient broth was added and then one milliliter (1ml) of the standardized bacterial suspension was added. A tube containing nutrient broth only was seeded with the test organism to serve as control. It was incubated at 37^{o}C for 24 hours and then examined for growth by observing turbidity (Kaya *et al.,* 2012). For fungal cultures, prepared sabouraud dextrose broth was used. The tubes were incubated at 25^{o}C for 72 hours and then examined for growth by observing for turbidity (Kaya *et al.,* 2012). The lowest extract concentration that showed growth was read as the MIC.

Determination of Minimum bactericidal/ fungicidal Concentration (MBC AND MFC)

The minimum bactericidal concentration/ Fungicidal Concentration (MBC/MFC) of all the extracts on the bacteria and fungi were carried out according to Ajaiyeoba *et al*. (2003). One (1ml) of each of the MIC tube cultures which showed no growth was pipetted onto nutrient and sabouraud dextrose agar plates and incubated at 37^{0}C and 25^{o}C for 24 hours and 72 hours respectively. After incubation, the lowest concentration at which there was no single colony of bacteria on nutrient agar and fungi on sabouraud dextrose agar plates was taken as MBC and MFC.

Statistical Analysis

One-way analysis of variance (one-way ANOVA) by using the statistical package for social sciences (SPSS Inc, Chicago, USA) program version 17.0 was used to analyze all data collected. The significant difference between the variables at $p < 0.05$ was determined by Duncan test.Triplicate determinations were carried out and standard errors were calculated for all results.

RESULTS AND DISCUSSION

The antimicrobial activity of the methanol and aqueous extracts of *Bryophyllum pinnatum* (Table 1) showed that *Bryophyllum pinnatum*exhibited varying degrees of antimicrobial activities against the test isolates, with the methanolic extract of *Bryophyllum pinnatum* showing more activity than the aqueous extracts.

Table 1: Antimicrobial activity of the methanol and aqueous extracts of *Bryophyllum pinnatum* against test organisms

Test Organisms	Concentration (mg/ml)/Zone of Inhibition (mm)									
	Methanol					Aqueous				
	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
S. aureus	12.5±0.71^d	10.0 ±1.41^a	8.0±0.10^d	6.0±0.0^b	0.0±0.0^b	6.0± 0.0^c	0.0± 0.0^c	0.0± 0.0^b	0.0± 0.0^e	0.0± 0.0^a
S. pyogenes	10.5±2.12^b	7.5±0.71^b	3.0±1.24^b	0.0±0.0^c	0.0±0.0^b	7.0± 1.41^a	3.0± 4.24^a	0.0 ± 0.0^e	0.0± 0.0^c	0.0± 0.0^d
E. coli	11.0±1.41^c	8.5±0.71^b	6.5±0.71^c	0.0±0.0^c	0.0± 0.0^c	8.0± 0.0^c	3.0± 4.24^a	0.0± 0.0^c	0.0± 0.0^c	0.0± 0.0^b
K. pneumoniae	13.0± 2.83^a	10.0± 1.41^a	7.5± 2.21^a	7.5±2.21^a	0.0± 0.0^d	8.0± 0.0^c	0.0± 0.0^c	0.0± 0.0^a	0.0± 0.0^b	0.0± 0.0^c
Candida albicans	10.0± 0.0^e	8.5± 0.0^c	6.0± 0.0^e	0.0± 0.0^c	0.0± 0.0^c	8.5± 0.71^b	6.5± 0.71^b	0.0± 0.0^c	0.0± 0.0^a	0.0± 0.0^b
Aspergillus niger	10.5± 0.71^d	9.5± 0.71^b	7.0±1.41^b	0.0± 0.0^c	0.0± 0.0^e	9.0± 1.41^a	8.0± 0.0^c	6.0± 0.0^d	0.0± 0.0^c	0.0± 0.0^d

Values and means of triplicate analysis ± standard deviation. Means with different superscripts in the same column are significantly different($P \leq 0.05$).

Key:

- indicates no growth ; + indicates growth

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The antimicrobial activity of the lime juice (*Citrus auranifolia*) soluble extracts (Table 2) shows thatthe highest *in-vitro* antimicrobial activity (11.0±0.00^{b}mm) was exhibited by the lime juice extracts at the concentration of 200 mg/ml against *Staphylococcus aureus*, while the least antimicrobial activity (8.0±0.00^{b}mm) exhibited at the concentration of 200 mg/ml was against *Aspergillus niger* and *Candida albicans*.

Table 2: Antimicrobial activity of the lime juice (*Citrus auranifolia*) extract against test organisms

Test Organisms	Concentration (mg/ml)/Zone of Inhibition (mm)				
	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5 mg/ml
Staphylococcus aureus	11.0 ± 0.0^{b}	7.5± 0.71^{a}	3.0±4.24^{a}	0.0± 0.0^{d}	0.0± 0.0^{d}
Streptococcus pyogenes	7.0± 0.0^{b}	0.0± 0.0^{c}	0.0± 0.0^{b}	0.0± 0.0^{b}	0.0± 0.0^{b}
Escherichia coli	9.5± 0.71^{a}	7.5± 0.0^{c}	0.0± 0.0^{b}	0.0± 0.0^{b}	0.0± 0.0^{e}
Klebsiella pneumoniae	10.5± 0.71^{a}	8.0± 1.41^{b}	3.0±4.24^{a}	0.0± 0.0^{c}	0.0± 0.0^{b}
Candida albicans	8.0± 0.0^{b}	0.0± 0.0^{c}	0.0± 0.0^{b}	0.0± 0.0^{e}	0.0± 0.0^{d}
Aspergillus niger	8.0± 0.0^{b}	0.0± 0.0^{c}	0.0± 0.0^{b}	0.0± 0.0^{c}	0.0± 0.0^{e}

Values and means of triplicate analysis ± standard deviation. Means with different superscripts in the same column are significantly different($P \leq 0.05$).

Key:

- indicates no growth

+ indicates growth

The zone of inhibition produced by the standard antimicrobial agent (Gentamicin and Nystanin) against the test microorganisms showed higher antimicrobial activities than the extracts of *Bryophyllum pinnatum* and *Citrus auranifolia*.

Table 3: Zone of inhibition (mm) of standard antimicrobial agent (Gentamicin and Nystanin) against the test organism's positive control

Test Organisms	Concentration (mg/ml)/Zone of Inhibition (mm)				
	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5 mg/ml
Staphylococcus aureus	16.5 ± 2.12^{a}	14.5± 2.12^{a}	11.0±1.41^{b}	8.0± 1.0^{d}	6.0± 0.0^{b}
Streptococcus pyogenes	16.0 ± 1.21^{a}	13.5 ± 0.71^{e}	9.5± 0.51^{d}	7.0±1.31^{b}	0.0± 0.0^{b}
Escherichia coli	21.0 ± 1.48^{b}	17.0± 1.41^{c}	14.0± 1.52^{a}	9.0± 1.41^{a}	6.0± 00^{b}
Klebsiella pneumoniae	17.5± 0.71^{d}	15.0± 1.71^{b}	11.5± 0.71^{c}	9.0± 1.41^{a}	6.5± 0.71^{a}
Candida albicans	15.0± 1.41^{c}	12.0± 1.41^{d}	11.0± 0.70^{c}	7.0±1.31^{b}	0.0± 0.0^{b}
Aspergillus niger	13.0± 1.41^{c}	12.0± 1.41^{d}	9.5± 0.51^{d}	7.5±1.12^{c}	0.0± 0.0^{b}

Values and means of triplicate analysis ± standard deviation. Means with different superscripts in the same column are significantly different($P \leq 0.05$).

Key:

- indicates no growth

+ indicates growth

Table 4 and Table 5 shows the minimum inhibitory concentration (MIC) of *Bryophyllum pinnatum* and *Citrus auranifolia* extracts against test bacteria. Methanol and aqueous soluble plant extracts of *Bryophyllum pinnatum* were active against all the test isolates but the methanol extracts demonstrated greater inhibitory activity on *Staphylococcus aureus,*

Escherichia coli, Streptococcus pyogenes at the range of 12.5 mg/ml each. The *Citrus auranifolia* extract were active against all the test isolates but demonstrated greater inhibitory activity on *Escherichia coli* at the range of 12.5 mg/ml.

Table 4: Minimum inhibitory concentration of *Bryophyllum pinnatum* plant extract against test bacteria

Tests organisms/Extracts								
Concentration (mg/ml)	***Staphylococcus aureus***		***Streptococcus pyogenes***		***Escherichia coli***		***Klebsiella pneumoniae***	
	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous
200	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	+
25	-	+	-	+	-	+	+	+
12.5	+	+	+	+	+	+	+	+
6.25	+	+	+	+	+	+	+	+
3.125	+	+	+	+	+	+	+	+
MIC Value (mg/ml)	**12.5**	**25.0**	**12.5**	**25.0**	**12.5**	**25.0**	**25.0**	**50.0**
Control	**6.2**		**3.1**		**3.1**		**6.2**	

Key: – indicates no growth + indicates growth, MIC = Minimum Inhibitory Concentration, Control = Gentamicin

Table 5: Minimum inhibitory concentration of Lime juice (*Citrus auranifolia*)against test bacteria

Tests organisms				
Concentration (mg/ml)	***Staphylococcus aureus***	***Streptococcus pyogenes***	***Escherichia coli***	***Klebsiella pneumoniae***
200	-	-	-	-
100	-	-	-	-
50	+	-	-	+
25	+	+	-	+
12.5	+	+	+	+
6.25	+	+	+	+
3.125	+	+	+	+
MIC Value (mg/ml)	**50.0**	**25.0**	**12.5**	**50.0**
Control	**6.2**	**3.1**	**3.1**	**6.2**

Key:
– indicates no growth
+ indicates growth
MIC = Minimum Inhibitory Concentration
Control = Gentamicin

The minimum inhibitory concentration (MIC) of the *Bryophyllum pinnatum* and *Citrus auranifolia* extracts against test fungi are shown in Tables 6 and 7.Aqueous soluble extracts of *Bryophyllum pinnatum* showed greater inhibitory activity at the range of 12.5 mg/ml against *Aspergillus niger*. *Citrus auranifolia* extract was active against all the test fungi at the

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range of 25 mg/ml.The antibiotics Nystatin (control) showed lesser inhibitory activities on *Candida albicans* and *Aspergillus niger* at the range of 6.25 mg/ml.

Table 6: Minimum inhibitory concentration of *Bryophyllum pinnatum* Plant extract against test Fungi

Tests organisms/Extracts				
Concentration (mg/ml)	***Candida albicans***		***Aspergillus niger***	
	Methanol	Aqueous	Methanol	Aqueous
200	-	-	-	-
100	-	-	-	-
50	-	+	-	-
25	+	+	-	+
12.5	+	+	+	+
6.25	+	+	+	+
3.125	+	+	+	+
MIC Value (mg/ml)	**25.0**	**50.0**	**12.5**	**25.0**
Control	**6.2**		**6.2**	

Key: – indicates no growth + indicates growth, MIC = Minimum Inhibitory Concentration, Control = Nystatin

Table 7: Minimum inhibitory concentration of Lime Juice extract against test Fungi

Tests organisms		
Concentration (mg/ml)	***Candida albicans***	***Aspergillus niger***
200	-	-
100	-	-
50	-	-
25	+	+
12.5	+	+
6.25	+	+
3.125	+	+
MIC Value (mg/ml)	**25.0**	**25.0**
Control	**6.2**	**6.2**

Key: – indicates no growth + indicates growth, MIC = Minimum Inhibitory Concentration, Control = Nystatin

The minimum bactericidal concentration (Table 8) revealed that the methanol extracts of *Bryophyllum pinnatum* showed greater activity against *Staphylococcus aureus, Streptococcus pyogenes* and *Klebsiella pneumoniae* at 100 mg/ml, when compared to the aqueous plant extract. Table 9 revealed that the soluble extracts of lime juice were active against all the test bacteria, but showed greater activity against *Staphylococcus aureus*, *Streptococcus pyogenes* and *klebsiella pneumoniae* at 100 mg/ml each.

Table 8: Minimum bactericidal concentration of the *Bryophyllum pinnatum* extract against test bacteria

Tests organisms/Extracts								
Concentration (mg/ml)	***Staphylococcus aureus***		***Streptococcus pyogenes***		***Escherichia coli***		***Klebsiella pneumoniae***	
	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous
200	-	-	-	-	-	-	-	-
100	-	+	-	+	+	+	-	+
50	+	+	+	+	+	+	+	+

25	+	+	+	+	+	+	+	+
12.5	+	+	+	+	+	+	+	+
6.25	+	+	+	+	+	+	+	+
3.125	+	+	+	+	+	+	+	+
MBC Value (mg/ml)	**100**	**200**	**100**	**200**	**150**	**200**	**100**	**200**
Control	**6.2**		**3.1**		**3.1**		**6.2**	

Key: – indicates no growth + indicates growth, MBC = Minimum Bactericidal Concentration, Control = Gentamicin

Table 9: Minimum bactericidal concentration of the Lime Juiceextract against test bacteria

Tests organisms				
Concentration (mg/ml)	***Staphylococcus aureus***	***Streptococcus pyogenes***	***Escherichia coli***	***Klebsiella pneumoniae***
200	-	-	-	-
100	+	+	+	+
50	+	+	+	+
25	+	+	+	+
12.5	+	+	+	+
6.25	+	+	+	+
3.125	+	+	+	+
MBC Value (mg/ml)	**150**	**150**	**200**	**150**
Control	**6.2**	**3.1**	**3.1**	**6.2**

Key: – indicates no growth + indicates growth, MBC = Minimum Bactericidal Concentration, Control = Gentamicin

The minimum fungicidal concentration as shown in Table 10 revealed that the methanol and aqueous soluble extracts of *Bryophyllum pinnatum* were active against all the test isolates with the aqueous extracts showing greater antifungal activity against *Candida albicans* at the range of 100 mg/ml. The minimum fungicidal concentration revealed that the soluble extracts of lime juice were active all the test isolates but showed greater antifungal activities against *Aspergillus niger* at the range of 50 mg/ml (Table 11).

Table 10: Minimum fungicidal concentration of the *Bryophyllum pinnatum* extract against test fungi

Tests organisms/Extracts				
Concentration (mg/ml)	***Candida albicans***		***Aspergillus niger***	
	Methanol	Aqueous	Methanol	Aqueous
200	-	-	-	-
100	-	+	-	-
50	+	+	+	-
25	+	+	+	-
12.5	+	+	+	+
6.25	+	+	+	+
3.125	+	+	+	+
MFC Value (mg/ml)	**100**	**200**	**100**	**25**
Control	**100**		**100**	

Key: – indicates no growth + indicates growth, MFC = Minimum Fungicidal Concentration, Control = Nystatin

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Table 11: Minimum fungicidal concentration of the Lime Juiceextract against test fungi

Tests organisms		
Concentration (mg/ml)	***Candida albicans***	***Aspergillus niger***
200	-	-
100	+	-
50	+	-
25	+	+
12.5	+	+
6.25	+	+
3.125	+	+
MBC Value (mg/ml)	**200**	**50**
Control	**100**	**100**

Key: – indicates no growth + indicates growth, MFC = Minimum Fungicidal Concentration, Control = Nystatin

The present study showed that the methanolic and aqueous extracts of *Bryophyllum pinnatum* exhibited variable degrees of antimicrobial activities against the test isolates investigated. The extent of sensitivity of the test organisms to the plant extracts was shown by the clear zones of inhibition produced by the extracts after the period of incubation. The highest *in-vitro* antimicrobial activity of (13.0 $\pm$2.83^{a} mm at 200 mg/ml) was exhibited by the methanolic extract of *Bryophyllum pinnatum* against *Klebsiella pneumoniae* while the least antimicrobial activity (10.5$\pm$2.12^{b} mm at 200mg/ml) was against *S. pyogenes*(Table 1). This study revealed that the methanol extract of *B. pinnatum* was most effective against the test organisms than the aqueous solvents. This is in-line with the assertion of Akinnibosun and Edionwe (2015), that methanol gives a higher antimicrobial effects than other extracting solvent such as aqueous and acetone. The highest *in-vitro* antimicrobial activity of (8.0$\pm$ 0.0^{c} mm at 200 mg/ml) was also exhibited by the aqueous extract of *Bryophyllum pinnatum* against *E. coli*and *K. pneumoniae* while the least antimicrobial activity (6.0$\pm$0.00^{c}mm at 200 mg/ml) was against *Staphylococcus aureus. Aspergillus niger*showed the highest susceptibility (10.5$\pm$ 0.71^{d} at 200 mg/ml) while *Candida albicans*showed the lowest susceptibility (10.0$\pm$ 0.0^{e} at 200 mg/ml) (Table 1).

The result also revealed that the highest *in-vitro* antimicrobial activity of (11.0$\pm$0.00^{b} mm at 200 mg/ml), was exhibited by the lime juice extracts against *Staphylococcus aureus*, while the least antimicrobial activity of (7.0$\pm$0.00^{b}mm at 200 mg/ml) was exhibited against *Streptococcus pyogenes. Aspergillus niger* and *Candida albicans* exhibited susceptibility of (8.0$^{a}\pm$ 0.0^{b} at 200 mg/ml) each(Table 2). The more significant inhibition was observed with a higher extract concentration which could be due to the stronger extraction capacity of methanol. This is in agreement with the observations of Ammara*et al*., (2009), who concluded that the stronger extraction capacity of methanol could have been responsible for the higher antimicrobial activity, although the observed differences were not statistically significant ($p \geq 0.05$). The stronger extraction capacity of methanol for *Bryophyllum pinnatum* could have been responsible for the higher antibacterial and antifungal activities as the biologically active components in the plant could have

been enhanced in the presence of methanol (Ammara*et al*., 2009).

The antimicrobial activity could be attributed to the presence of phenolic compounds which have been detected in this plant extract to include: saponin, tannins, alkaloids, bryophyllin and other secondary metabolites which are antimicrobial (Okwu and Josiah, 2006;Giteru *et al*., 2015). Aromatic phenolic compounds, flavonoids, saponin, tannin, steroids, curammin, bryophyllin and alkaloids have been confirmed to be strongly antagonistic to Gram positive and Gram negative human pathogens, especially *Staphylococcusaureus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis* (Adegoke *et al*., 2010). The plant *B. pinnatum* has also been reported to have very numerous medicinal applications ranging from its use in the treatment and management of inflammation, glycaemia, diabetes, cancer, headache, dysentery, smallpox, *convulsion*, arthritis and spasms (Theophil *et al*., 2006). Researchers have asserted that the phenolic compounds in the plant may be responsible for its therapeutic, antiseptic, antifungal or bactericides as well as antiviral and antitumor activities (Okwu and Josiah, 2006). The zone of inhibition produced by the standard antimicrobial agent (Gentamicin and Nystanin) against the test microorganisms showed higher antimicrobial activities than the extracts of *Bryophyllum pinnatum* and *Citrus auranifolia*.

The minimum inhibitory concentration (MIC) of the methanol and aqueous extracts was read as the lowest extract concentration that showed growth. The results showed that growth of the isolates was inhibited by both the aqueous and methanol extracts between the concentrations of 12.5 mg/ml and 50 mg/ml(Table 4 and Table 6). The MIC of the lime juice was observed to be between the concentrations of 12.5 mg/ml and 50 mg/ml for the bacterial isolates (Table 5) and 25mg/ml for the fungal isolates (Table 7).

The minimum bactericidal concentration revealed that the methanol soluble extracts of *Bryophyllum pinnatum* showed greater activity against *Staphylococcus aureus, Streptococcus pyogenes* and *Klebsiella pneumoniae* at 100 mg/ml, when compared to the aqueous plant extract (Table 8). The lime juice extracts were active against *Staphylococcus aureus, Streptococcus pyogenes,* and *Klebsiella pneumoniae* at 150 mg/ml (Table 9) while the minimum bactericidal concentration varied inhibition activity. This is in agreement with the study by Lawrence *et al*., (2016) who reported that the MBC of both the methanol and aqueous extracts of *Bryophyllum pinnatum* was at 100 mg/ml concentration and the bactericidal activity of both extracts was higher against *Streptococcus* sp. than *Staphylococcus* sp. This confirms the earlier observation that antibacterial activity of both the methanol and aqueous extracts were more pronounced against *Streptococcus* sp. than against *Staphylococcus* sp. Lawrence *et al*., (2016) also stated that a higher antibacterial activity was again observed against the isolates with the methanol extract than the aqueous extract which reveals a correlating increase in antibacterial activity with increase in extract concentration.

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In this present study, the lime juice and the methanol and aqueous soluble plant extracts of *Bryophyllum pinnatum* were active against all the test isolates but its methanol extract demonstrated greater inhibitory activity. The substances used in this study were more effective against the test organisms when compared to previous report by Akinnibosun and Edionwe (2015), who also worked on *Bryophyllum pinnatum* and *Citrus auranifolia*. This could be due to differences in the plant material, extraction method and the extracting solvent used.

The sensitivity of the test isolates to methanolic extracts of the plants is at variance with the findings of Obi and Onuoha (2000), who described ethanol as the best solvent for the extraction of bioactive substances from plants. The ability of the methanolic extract of *Bryophyllum pinnatum* to be more effective than that of the aqueous extract could be linked to the fact that, the active antimicrobial agent in the leaves are more soluble in methanol and as such, it is able to extract the antimicrobial constituents from the leaf (Okwu and Josiah, 2006).

Investigation of the antimicrobial activity of limejuice alone and in combination with other herbs has been investigated (Rodriguez *et al*., 2000;Onyeagba *et al*., 2004; Bina *et al*., 2010) and lime juice has been found to have high antimicrobial activity. The inhibitory effect of extracts of *B. pinnatum against* pathogenic strains makes the plant apotential drug development candidate fortreatment of ailments caused by thesepathogens.

CONCLUSION

The results of this finding indicates that the bacterial and fungal growth inhibition, evidenced by MIC and MBC values of both methanolic and aqueous extracts of *Bryophyllum pinnatum* as well as lime juice extracts have broad-spectrum antimicrobial activity when compared to the standard antibiotics used in this study, hence can serve as natural therapeutic agent against some enteric pathogens. Based on the findings of this research, methanol extract of *Bryophyllum pinnatum* demonstrated greater antimicrobial activity on *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes*, while the lime juice extracts demonstrated greater antimicrobial activity on *Streptococcus pyogenes*, and *Klebsiella pneumoniae*. Antimicrobial activity of these plants has been related to the presence of bioactive phytochemicals in parts of the plant. Hence, *B. pinnatum* leaves and lime juice could be useful in the treatment of infant respiratory infections and a potential source of antibacterial agents and raw material for the pharmaceutical industry if adequately explored.

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