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Antibacterial Potential of *Bryophyllum pinnatum* Leaf Extracts on Bacteria Obtained from Infected Infant Respiratory Tract

Lawrence B. Etim[1], Godwin Attah Obande[2*], Chuku Aleruchi[2] and V. E. Bassey[1]

[1]Department of Biological Sciences, Faculty of Science, Cross River University of Technology, Calabar, Cross River State, Nigeria.
[2]Department of Microbiology, Faculty of Science, Federal University Lafia, Nasarawa State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors LBE and VEB designed the study, wrote the protocol and drafted the first manuscript. Author GAO managed the analyses of the study and literature searches. Authors CA and GAO read and reviewed the first and second drafts of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Annual incidence of respiratory infection and death among infants aged between one (1) to two (2) years is increasing, especially in remote areas of the world. Herbal medicines have been the basis of treatment and cure for various diseases and physiological disorders. Plants contain various bioactive components that have been explored in ethnomedicine. Nigeria has abundance of such plants.
Aim: To evaluate the *in vitro* antibacterial activity and potency of *Bryophyllum pinnatum* leaf extracts on pathogens isolated from infants with respiratory tract infection.
Place of Study: The study was conducted at the Microbiology laboratory of the Cross River University of Technology in Cross River, Nigeria.
Methodology: The *in vitro* antibacterial potency of methanol and aqueous extracts of *B. pinnatum* leaf against *Staphylococcus* sp. and *Streptococcus* sp. isolated from sputum of five infants was

**Corresponding author: E-mail: obandegodwins@gmail.com;*

tested. The susceptibility of the isolates to the extracts as well as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were assayed on Mueller-Hinton Agar by the disc diffusion method, using concentrations of 100, 80, 60, 40 and 20 mg/ml. Selected antibiotics and the respective solvents used, served as positive and negative controls respectively.

Results: *Staphylococcus* sp. (52%), *Streptococcus* sp. (36.6%), *Klebsiella* sp. (9.4%) and pus cells (2%) were found in the samples. Both the methanol and aqueous extracts of *B. pinnatum* leaf showed strong antibacterial activity against *Streptococcus* sp (16.7; 17.3 mm) and *Staphylococcus* sp. (13.0; 16.3 mm). Antibacterial activity of both the methanol and aqueous extracts was more pronounced against *Streptococcus* sp. than against *Staphylococcus sp.* Methanol extract was more inhibitory to both isolates than the aqueous extract. The MIC of the aqueous extract was observed to be at 80 mg/ml and 100 mg/ml, while that of the methanol extract was 100 mg/ml and 80 mg/ml for *Staphylococcus* sp. and *Streptococcus* sp. respectively. The MBC of both the methanol and aqueous extracts was at 100 mg/ml concentration. Isolates were resistant to over 60% of the tested commercial antibiotics. Leaf extracts of *B. pinnatum* showed more antibacterial activity than some of the broad spectrum antibiotics.

Conclusion: *B. pinnatum* leaves 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.

Keywords: Ethnomedicinal; sputum; phytoextracts; bioactive; healthcare.

1. INTRODUCTION

Plants have formed the essential part of African and global society since civilization in traditional healthcare delivery systems [1]. Recently, African traditional medicine has been brought into recognition and acceptance as alternative healthcare delivery practice. Herbal medicines have been the basis of treatment and cure for various diseases and physiological disorders in traditional methods among the people of sub-Saharan Africa [2].

Plant components that formed the basis of traditional medicine play an important role in conventional medicine and were instrumental to early pharmaceutical drug discovery and industry. Therefore, plant derived drugs are considered part of human evolution, existence and sustainable healthcare delivery system [3].

Nigeria as a sub-Saharan nation has a rich and diverse indigenous flora that is widely distributed throughout the country and is used as herbal medicine to cure various diseases. One of such medicinal plants is *Bryophyllum pinnatum*. *B. pinnatum* is a succulent perennial shrub that grows erect, wild in bushes and are propagated (seeds, leaves and bulbils) in gardens around the country [4-5]. *B*. *pinnatum* in Nigeria especially in Efik ethnomedicinal culture, is used for the treatment of earache, burns, diarrhea, chest and respiratory related diseases such as fever, cough and blood in sputum among infants. This suggests that *B. pinnatum* contains bioactive substances that are active against pathogens responsible for these infections. Leaves and other parts of *B. pinnatum* has been reported to contain alkaloids, saponins, tannins, flavonoids anthraquinones, xanthones and bryophyllin A and B [6-7]. According to [8-9], the green callus of the plant contain malic acid, quinones and tocopherol. The presence of phenolic compounds in *B. pinnatum* as confirmed by [4] suggests that the plant has antibacterial potential against bacterial pathogens. Other works have also shown that this plant possesses analgesic, anticonvulsion, antiinflammatory, antiarthritic and antispasmolytic properties [10].

The world over, reports showed that the annual incidence of respiratory infection and death among infants aged between one (1) to two (2) years) is increasing, especially in remote areas of the world. Infants not receiving proper medical care from birth due to lack of good medical facilities or poor hygienic practice, become very susceptible to infections such as cough, diarrhea and discharge of sputum. In Efik ethnomedicinal practice, infants aged between 1 to 2 years with these ailments are treated with extracts obtained from the succulent parts (leaves and buds) of *B. pinnatum*.

The present study was designed to evaluate the antibacterial activity and potency of *B. pinnatum* leaf extracts on pathogens isolated from infants with respiratory tract infection and to determine its *in vitro* minimum inhibition concentration (MIC)

and minimum bactericidal concentration (MBC) against the isolates in comparison with some selected broad spectrum antibiotics.

2. MATERIALS AND METHODS

2.1 Sputum Sample Collection

Early morning sputum samples were collected from five patients aged between 2 – 5 years in the outpatient unit of the General Hospital Calabar, Cross River State, Nigeria. The sputum samples were collected into sterile screw cap bottles containing physiological saline solution (0.85% w/v). Informed consent of parents were sought before collection of samples from the children. Before collection, each patient's mouth was rinsed with clean water. The children were aided to produce sputum by professional health workers, while ensuring that saliva did not mix with it. The sputum samples were immediately transferred within 2 hours to the laboratory for microbiological assay in an ice-packed container.

2.2 Collection of Plant and Selected Antibiotics

Correctly identified and confirmed *Bryophyllum pinnatum* plant leaves were collected at homestead a flower garden within Calabar metropolis. Ten broad spectrum antibiotics were purchased from a reputable pharmacy in Calabar, Cross River State, Nigeria. The antibiotics used in this study were obtained from a standard pharmacy in Calabar, Cross River state, and comprised of amoxacillin (Amx), ofloxacin (Ofl), streptomycin (Str), chloramplenicol (Chl), ceftriazone (Cef), gentamicin (Gen), perfloxacin (Pef), cotrimoxazole (Cot), ciprofloxacin (Cip) and erythromycin (Ery).

2.3 Preparation of *Bryophyllum pinnatum* Extracts

Two sets of 100g of *B. pinnatum* leaves were washed, shredded and blended with electric blender (Model 244: Super Interjet Japan). Each set of the crushed leaves was soaked in 300ml of methanol (95%) and 300ml deionized distilled water in two 1000ml capacity flasks respectively. The flasks were then left standing on a bench with occasional shaking for 72 hours. The aqueous extraction and methanol extraction were done using a Soxhlet extractor. At the end of the extraction process, the pure extract collected were filtered with No. 1 Whatman filter paper and evaporated to dryness by gentle heating at 40^{o}C on a hot plate. The dried concentrates (methanol and aqueous extracts) were weighed and stored in a desiccator for antimicrobial assay on the bacterial isolates.

2.4 Isolation and Identification of Bacteria from Sputum Samples

The isolation methods followed the process described by [11-12]. In these methods, the samples were cultured on blood agar and EMB agar using the streak plate method. Culture plates were then incubated at 37℃ for 24 hours, after which discrete colonies were further purified by sub-culturing on appropriate media and incubated at 37℃ for another 24 hours before characterization. Cultures were Gram stained and characterized based on their cultural, morphological and biochemical characteristics.

2.5 Assay of Antibacterial Activity of *B. pinnatum* Extracts

2.5.1 Determination of antibacterial activity

The Kirby-Bauer disc diffusion method as described by [13] and [14] was used. An 18 hours old broth culture of the test organisms (*Staphylococcus sp.* and *Streptococcus* sp) were respectively diluted with sterile physiological saline (0.85% w/v sodium chloride) to 0.5 McFarland standard (approximately 1.5×10^{8} cfu/ml). A 3.0ml portion of each inoculant was placed onto the surface of pre-dried Mueller-Hinton (Biomark Laboratory, India) agar plate and spread out evenly with a sterile cotton swab to ensure contact with agar. The plates were allowed to stand on the bench at room temperature for some time to enable absorption of the inoculum by the agar. Thereafter, sterilized 6mm No. 1 Whatmann filter paper disc were impregnated with approximately 1.5 mg/100 ml of the methanol and aqueous extracts of *B. pinnatum* suspended in DMSO. The impregnated discs where then placed separately, equidistant from each other on the surface of the seeded agar. The plates were allowed to remain for 15 minutes at room temperature for the extracts to diffuse across the surface of the seeded agar before incubation at 37^{o}C for at least 18 hours. The plates were examined for zone of inhibition. The diameter of each triplicate zone was measured to the nearest millimeter using a Vernier caliper and the mean zone of inhibition calculated.

2.5.2 Assay of antibacterial activity of selected antibiotics

The susceptibility of *Staphylococcus* sp. and *Streptococcus* sp. to commercial antibiotics was also tested, using the disc diffusion method. The selected antibiotics used were Amoxacillin (250 mg), Ofloxacin (250 mg), Streptomycin (500 mg), Chloramphenicol (250 mg), Ceftriazone (250 mg), Gentamycin (280 mg), Pefloxacin (500 mg), Cotrimoxazole (480 mg), Ciprofloxacin (500 mg) and Erythromycin (250 mg). The antibiotic solutions were each prepared in 100 ml of solvent. The antibiotics served as positive controls while the respective solvents served as negative control.

2.5.3 Determination of minimum inhibition concentration (MIC) of *B. pinnatum* extracts

The MIC was done using both extracts by the disc diffusion method [14-17]. To determine the MIC values, paper discs were made from filter paper soaked with different concentrations of each extract prepared at 20, 40, 60, 80 and 100 mg/ml respectively. The discs containing each extract concentration were separately placed equidistant from each other on Mueller Hilton agar plates already seeded with the 0.5 McFarland standardized test organisms (approximately 1.5 $\times 10^8$ cfu/ml), and incubated overnight, after which the zones of inhibition were read. The lowest concentration of mucus formulation which exhibited the largest inhibition zone was interpreted as the minimum inhibitory concentration of the formulation.

2.5.4 Determination of minimum bactericidal concentration (MBC) of *B. pinnatum* extracts

The MBC was assayed at extract concentrations of 20, 40, 60, 80 and 100 mg/ml respectively. Equal aliquots of each *B. pinnatum* extract was mixed with equal aliquots of the test organisms at 0.5 McFarland standard and cultured on Mueller Hilton agar for at least 18 hours at 37℃. The number of colonies formed was counted and the mean of each duplicate concentration was taken. The lowest concentration capable of reducing bacterial growth on the medium was considered the minimum bactericidal concentration.

2.6 Statistical Analysis

Data collected were analyzed using SPSS version 20 (IBM Corp., Armonk, New York). Analysis of variance (ANOVA), simple means, percentages and standard deviation were computed as appropriate.

3. RESULTS AND DISCUSSION

The bacterial types isolated from the sputum samples and their percentage frequency obtained were as shown in Table 1. *Staphylococcus* sp. had the highest mean percentage frequency of 52% followed by *Streptococcus* sp. with 36.6% and *Klebsiella* sp. having 9.4%. Pus cells occurred in 2% of the samples. This finding agrees with the report by [18] in which *Staphylococcus aureus* was most predominant. In another study however, *Streptococcus pneumoniae* and *Klebsiella pneumoniae* were higher in number than *Staphylococcus aureus* in the respiratory tract of patients [12]. The presence of these bacterial organisms is a clear indication that they are tenacious and resistant to commonly used antibiotics in the treatment of infant respiratory infections. Selective pressure exerted by antibiotic usage may also have allowed for selection of these bacteria which have been widely reported to display resistance to some antibiotics [19].

Both the methanol and aqueous extracts of *B. pinnatum* showed strong antibacterial activity against *Streptococcus* sp. (16.7; 17.3 mm) and *Staphylococcus* sp. (13.0; 16.3 mm) respectively (Table 2). The mean inhibition zones observed show that antibacterial activity of both the methanol and aqueous extracts was more pronounced against *Streptococcus* sp than against *Staphylococcus* sp., suggesting a higher susceptibility of *Streptococcus* sp. to the extracts, even though the observed differences were not statistically significant ($p>0.05$). Although analysis of variance (ANOVA) revealed no statistically significant difference in the activities of the both extracts against the isolates ($p>0.05$), the results reveal that the methanol extracts were more inhibitory to both isolates than the aqueous extracts. This finding also agrees with the report of similar works in which methanol extracts were observed to have shown more antibacterial activity than other extracts of *B. pinnatum* [4,20-21]. Stronger antibacterial activity of methanol extracts have been attributed to the ability of the solvent to extract some of the active properties of these plants like phenolic compounds, saponin, bryophyllin and other secondary metabolites which are reported to be antimicrobial [5,22].

The minimum inhibitory concentration (MIC) of the methanol and aqueous extracts was read as the lowest extract concentration that showed the largest inhibition zone. Results of the MIC showed that growth of the isolates was most inhibited by both the aqueous and methanol extracts between concentrations of 80 mg/ml and 100 mg/ml (Table 3). The MIC of the aqueous extract was observed to be at 80 mg/ml and 100 mg/ml, while that of the methanol extract was 100 mg/ml and 80 mg/ml for *Staphylococcus* sp. and *Streptococcus* sp. respectively. The MIC of the aqueous extract was observed to be higher against *Streptococcus* sp. than *Staphylococcus* sp., while the reverse was the case for the methanol extract. The reason for this observation however, is not clear.

The minimum bactericidal concentration (MBC) of the extracts was also determined as the lowest concentration of the extracts that exhibited the largest inhibition zone against the test isolates (Table 4). The MBC of both the methanol and aqueous extracts was at 100 mg/ml concentration. 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. (Table 2), although the observed differences were not statistically significant ($p>0.05$). A higher antibacterial activity was again observed against the isolates with the methanol extract than the aqueous extract. The result also reveal a correlating increase in antibacterial activity with increase in extract concentration.

The susceptibility of *Staphylococcus* sp. and *Streptococcus* sp. was also tested against some selected broad spectrum antibiotics (Table 5). The resistance of the isolates to the selected antibiotics was significant and was in the order Cot>Amx>Cip>Ery>Ofl>Pef. The isolates demonstrated significant sensitivity in the order Str>Chl>Cef>Gen. The results reveal a high rate of multidrug resistance by the isolates, as they were resistant to over 60% of the tested antibiotics. In comparison to the leaf extracts of *B. pinnatum* however, susceptibility of the bacterial isolates to both methanol and aqueous extracts was averagely higher than to the selected antibiotics. This could imply that leaf extracts of *B. pinnatum* showed more antibacterial activity against *Staphylococcus* sp. and *Streptococcus* sp. than some broad spectrum antibiotics.

The occurrence of multidrug resistance in human pathogens and high cost of medicare in poor countries necessitated the interest to conduct this study. Infants in poor nations are exposed to inadequate healthcare facilities that often results in fatal consequences. *Staphylococcus*, *Streptococcus* and *Klebsiella* species are human pathogens with ability to develop resistance to many antibiotics. The strong antibacterial activity of *B. pinnatum* observed in this study against *Staphylococcus* sp. and *Streptococcus* sp. isolated from the respiratory tract of infants, seems to hold a promise for the development of alternative antibacterial agents for the treatment and management of bacterial infections. The antibacterial activity expressed by this plant, has been related to the presence of bioactive compounds in parts of the plant. The plant has been reported to contain phenolic compounds, saponin, bryophyllin and other secondary metabolites which are antimicrobial [5,22]. The reported presence of phenolic compounds in *B. pinnatum* is noteworthy, considering that phenols and phenolic compounds are extensively used in disinfections and remain the standard with which other bactericides are compared [23]. 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 *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis* [24]. Furthermore, [25] reported the presence of two new novel flavonoids namely, 5I Methyl 4I, 5, 7 trihydroxyl flavone 1 and 4I, 3, 5, 7 tetrahydroxy 5-methyl 5I-propenamine anthocyanidines 2, in *B. pinnatum* leaves which successfully inhibited *P. aeruginosa, K. pneumoniae, E. coli, S. aureus, Candida albicans* and *Aspergillus niger.* 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, general debility, dysentery, smallpox, *convulsion*, arthritis and spasms [10,26]. 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 [5]. All the foregoing make *B. pinnatum* a plant that requires attention and adequate exploration considering its very rich phytochemical composition and potential to serve as a source of medically important agents.

Table 1. Percentage frequency of bacterial isolates from sputum samples

Specimen no.	No. of isolates	Isolates	Frequency (%)
1	3	*Staph, Strep, Kleb*	36, 46, 18
2	2	*Strep, Staph*	44, 56
3	2	*Strep, Staph, Kleb*	52, 40, 8
4	1	*Staph,* pus cell	90, 10
5	3	*Staph, Strep, Kleb*	38, 41, 21

Key: Staph = Staphylococcus sp., Strep = Streptococcus sp., Kleb = Klebsiella sp.

Table 2. Antibacterial activity of *Bryophyllum pinnatum* leaf extract against sputum isolates

Extract	Isolate	Replicate trials (mm)			Mean inhibition zone (mm)
		1	2	3	
Methanol	*Staphylococcus* sp.	16	18	15	16.3±1.5
	Streptococcus sp.	17	19	16	17.3±1.5
Aqueous	*Staphylococcus* sp.	14	12	13	13.0±1.0
	Streptococcus sp.	15	20	15	16.7±2.9

Mean inhibition zones are mean of replicates ± Standard deviation

Table 3. Minimum inhibitory concentration (MIC) of *B. pinnatum* extracts on bacterial isolates from sputum

Extract	Isolate	MIC (mg/ml)
Methanol	*Staphylococcus* sp.	100
	Streptococcus sp.	80
Aqueous	*Staphylococcus* sp.	80
	Streptococcus sp.	100

Table 4. Minimum bactericidal concentration (MBC) of *B. pinnatum* leaf extracts against viable count of isolates (Log_n cfu/ml)

Extracts concentration (mg/ml)	Aqueous extract		Methanol extract	
	I	II	I	II
100	3.3±0.1	3.0±0.01	3.6±035	2.8±0.02
80	4.0±0.02	3.6±0.3	3.8±0.4	3.1±0.1
60	4.3±0.2	4.0±0.2	4.1±0.2	3.5±0.1
40	4.4±0.1	4.2±0.08	4.7±0.1	3.9±0.3

Values are viable counts ± Standard deviation. I = Staphylococcus sp.; II = Streptococcus sp.

Table 5. Susceptibility of isolates to selected antibiotics

Antibiotic	Conc./100 ml	Mean zone of inhibition (mm)	
		Staphylococcus sp.	**Streptococcus *sp.***
Amx	250	8(R)	10(R)
Ofl	250	12(R)	12(R)
Str	500	12(S)	14(R)
Chl	250	10(R)	13(S)
Cef	250	16(S)	14(R)
Gen	280	18(S)	16(R)
Pef	500	27(R)	25(R)
Cot	480	7(R)	10(R)
Cip	500	11(R)	8(R)
Ery	250	11(R)	9(R)

Key: Amx = Amoxacillin, Ofl = Ofloxacin, Str = Streptomycin, Chl = Chloramphenicol, Cef = Ceftriazone, Gen = Gentamycin, Pef = Pefloxacin, Cot = Cotrimoxazole, Cip = Ciprofloxacin, Ery = Erythromycin. S = Sensitive, R = Resistant; Conc. = Concentration

4. CONCLUSION

Antibacterial activity of *B. pinnatum* leaf extracts against *Staphylococcus* sp. and *Streptococcus* sp. isolated from the respiratory tract of infants have been established in this study. Bacterial growth inhibition, evidenced by MIC and MBC values of the methanol and aqueous leaf extracts, were comparable to, and averagely higher than that observed with selected broad spectrum antibiotics. A stronger antagonistic activity was observed against *Streptococcus* sp. than against *Staphylococcus* sp., and with the methanol extract than the aqueous extract. Antibacterial activity of the plant have been related to the presence of bioactive phytochemicals in parts of the plant. Hence, *B. pinnatum* leaves 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.

ETHICAL APPROVAL

The authors hereby declare that this research has been performed in accordance with laid down standards and that samples were taken with the full understanding and informed consent of parents guided by their physicians.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Marjorie C. Plant products as antimicrobial agents. Clinical Microbiology Review. 1999;12:564-582.
2. Huang WY, Cai YZ, Zhang Y. Natural phenolic compounds from medical herbs and dietary plants; potential use for cancer prevention. Nutrition and Cancer. 2010; 62:1-20.
3. Eleyinmi AF. Chemical composition and antibacterial activity of *Gongronema latifolium*. Journal of Zhejiang University Science B. 2007;8(5):352-358.
4. Ofokansi KC, Esimore CO, Anele CK. Evaluation of the *in vitro* combined antibacterial effects of the leaf extract of *Bryophyllum pinnatum* (Fam. Labiate). Plant Product Resources Journal. 2005; 9:23-27.
5. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology. 2006;5(4):357-361.
6. Iwu MM. Hand-book of African Medicinal Plants. 2nd ed. Florida: CRC Press; 1993.
7. Nwali BU, Okaka ANC, Ibiam UA, Aja PM. Phytochemical composition of *Bryophyllum pinnatum* leaves. International Journal of Advanced Biological Research. 2012;2(4): 614-616.
8. Sofowora A. Medical plants and traditional medicine in Africa. Ibandan: Spectrum; 1993.
9. Oliver-Bever B. Medicinal plants in tropical West Africa. Cambridge: University Press; 1986.
10. Theophil D, Agatha FL, Nguelefack TB, Asongalem EA, Kamtchouing P. Anti-inflammatory activity of leaf extracts of *Kalanchoe crenata*. Indian Journal of Pharmaceuticals. 2006;38:115-117.
11. Cheesbrough M. District laboratory practice in tropical countries. United Kingdom: Cambridge University Press; 2000.
12. Taura DW, Hassan A, Yayo AM, Takalmawa H. Bacterial isolates of the respiratory tract infection and their current sensitivity pattern among patients attending Aminu Kano Teaching Hospital Kano-Nigeria. International Research Journal of Microbiology. 2013;4(9):226–231.
dx.doi.org/10.14303/irjm.2013.048
13. Obi CL, Bassey PO, Memba MNB. Profile of antibiotic sensitivity of bacterial isolates and physicochemical quality of water supply in rural vendor community. South African Water. 2004;30:515-520.
14. Clinical Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests; approved standard. 11th ed. USA: Wayne; 2012.
15. Shafi MS. Determination of antimicrobial MIC by paper diffusion method. Journal of Clinical Pathology. 1975;28:989–992.
16. National Committee for Clinical Laboratory Standard. Methods for determining bacteriocidal activity of antimicrobial agents; Tentative guideline; NCCLS document M26-T. Villa-nova, Pa; 1992.
17. National Committee for Clinical Laboratory Standard. Performance standards for antimicrobial susceptibility testing, 8th Informational Supplement. M100-S12. Villa-nova, Pa; 2002.

18. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in combined medical-surgical intensive care units in the United States. Infection Control and Hospital Epidemiology. 2000;21(8): 510 – 515.
19. Taiwo SS, Okesina AB, Onile BA. *In vitro* antimicrobial susceptibility pattern of bacterial isolates from wound infections in University of Ilorin Teaching Hospital. African Journal of Clinical and Experimental Microbiology. 2002:3(1): 6–10.
Available:http://dx.doi.org/10.4314/ajcem.v3i1.7342
20. Akinpelu DA. Antimicrobial activity of *Bryophyllum pinnatum* leaves. Fitoterapia. 2002;71:193-194.
21. Akinsulire RA, Aibinu IE, Adenipekun T, Adelowotan T, Odugbemi T. *In vitro* antimicrobial activity of crude extracts from plants *Bryophyllum pinnatum* and *Kalanchoe crenata*. African Journal of Traditional, Complementary and Alternative Medicines. 2007;4(3):338–344.
22. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Review. 1999;12:564-582.
23. Okwu DE, Morah FNI. Isolation and characterization of flavanone glycoside 4I,5, 7-trihydroxy flavanone rhamnoglucose from *Garcinia kola* seed. Journal of Applied Science. 2007;7(2):306-309.
24. Adegoke AA, Iberi PA, Akinpelu DA, Aiyegoro OA, Mboto CI. Studies on phytochemical screening and antimicrobial potential of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. International Journal of Applied Research in Natural Products. 2010;3(3):6-12.
25. Okwu DE, Nnamdi FU. Two novel flavonoids from *Bryophyllum pinnatum* and their antimicrobial activity. Journal of Chemical and Pharmaceutical Research. 2011;3(2):1-10.
26. Gill LS. Ethnomedical uses of plants in Nigeria. Benin: University of Benin Press; 1992.

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