# Antimicrobial Activity of Aqueous and Ethanolic Extracts of *Bryophyllum pinnatum*

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**Abstract:** *Bryophyllum pinnatum* is a crassulescent herb with wide distribution and documented antimicrobial efficacies. Inconsistence in its activities has however, been suggestively linked with some factors. Hence, this study was carried out to establish the effect of some extrinsic factors on the antibacterial activities of *Bryophyllum pinnatum* against some disease causing microorganisms. Antibacterial activity of Bryophyllum pinnatum was determined by standard agar-diffusion method. Results from this study showed a significantly higher zone of microbial inhibition with ethanolic extract when compared with aqueous extracts ($P<0.05$). Conclusion from this study have shown that solvents and concentration of extract are some of the extrinsic factors that influences the antibacterial activities of *Bryophyllum pinnatum*

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## Introduction

The increasing occurrence of antibiotic resistant organisms coupled with the non availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality (Williams, 2000). This observation have led to the search for alternative therapy with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs (Pretorius *et al.,* 2003, Moreillion *et al.,* 2005). The plant, *Bryophyllum pinnatum* (Crassulaceae) is commonly known as air plant, love plant, miracle leaf, life plant, Zakham-e-hyat, panfutti, Ghayamari [26], has been accepted as a herbal remedy in almost all parts of the world [52, 57, 25].

It is a crassulescent herb of about 1 metre in height, with opposite, glabrous leaves (with 3–5 deeply crenulated, fleshy leaflets) [53], distributed worldwide but growing primarily in the rain forest [76, 71]. It grows widely and used as folk medicine in tropical Africa, India, China, Australia and tropical America, Madagascar, Asia and Hawaii [51, 40]. It is astringent, sour in taste, sweet in the post digestive effect and has hot potency. It is well known for its haemostatic and wound healing properties. The plant have considerable attention for their medicinal properties and find application in folklore medicine, as well as in the contemporary medicine [33,62].

The antibacterial activity of the leaf juice of B. pinnatum was reported by Obaseiki-Ebor (1985). Flavonoids, polyphenols, and triterpenoids have been identified from the leaves of B. pinnatum (Ojewole, 2005). Quecetin-3-α-L-rhu-β- D-xyl; a flavonoid (Cao et al., 2005), Bryophyllin B [1], a novel potent cytotoxic bufadienolide (Yamagishi et al., 1989) and Malic acid (Sutton et al., 1972) were isolated from the leaves of B. pinnatum. Yet, there is paucity of information on the efficacy of this plant on the strains of microorganisms commonly encountered in our environment. This study was therefore aimed at filling this existing gap in our vicinity.

## Materials And Methods

### Source of plants

*Aloe vera* specimens was obtained from the Agronomy Unit of Dagwon farm (National Veterinary Research Institute, Vom). The plants were authenticated by the taxonomist at the Department of Botany and Biochemistry, University of Jos, Nigeria.

### Preparation of plant materials and extracts

The plucked leaves were washed with running tap water and thoroughly washed with distilled water several times. They were then disinfected and weighed using metler's balance. The leaves were further sliced longitudinally. One kilogram of the fresh leaves was air dried (27°C) on the laboratory table after which they were shredded and preserved in airtight cellophane bags. The shredded leaves were milled into powder form using a warring commercial blender to give 600g. Hundred grams of each of the powdered plant materials was soaked in 500 ml of ethanol and water for 6h using soxhlet apparatus.

**Phytochemical studies**

Phytochemical tests were carried out to determine the presence of flavonoids, tannins, alkaloids, saponins and anthraquinones using the methods described by Odebiyi and Sofowora (1978).

**Test organisms**

*Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shigella spp, Proteus vulgaris, Pseudomonas aeroginosa, Klebsiella aerogens, Trichophyton mentagraphytes, Candida albican and Cryptococcus neoformans* were obtained from Bacteriology and Dermatophilosis Unit, Division of NVRI, Vom. The isolates identities were further confirmed in our laboratory using standard procedures (Cheesborough, 2005). The isolates were maintained on Tryptone Soy agar (TSA) (Oxoid) and Sabouraud dextrose agar (oxoid) at 4°C for bacteria and fungi respectively.

**Determination of antimicrobial activity**

The medium used was Mueller Hinton agar (Oxoid, U. K). The bacterial inoculum were adjusted to 0.5 McFarland turbidimetric standard and inoculated onto the medium using sterile swabs. For each extract, three replicate plates were prepared against the test organisms. Antimicrobial activity of the ethanolic and aqueous extracts of the plant samples was evaluated by the agar well diffusion method. Using sterile cork-borer of 6 mm diameter, equidistant wells were cut in each of the agar plates, while different concentrations of the extracts, 1000, 750, 250, and 100 mg/ml were introduced into the wells. The plates were left for 2 h at room temperature to allow the extract to diffuse. The solvents used for extraction served as control and was introduced into a separate well as appropriate. Ciprofloxacin /Fluconazole (250/150 µg/ml) was used as standard antimicrobial agent for comparison. The plates were then incubated at 37°C for 24 h. Antimicrobial activity was determined by measurement of zone of inhibition around each well using a pair of calipers (in mm) and read on a meter rule.

**Serial dilution of *Aloe vera* juices**

Twelve sterile tubes were arranged in the rack and 1ml of sabouraud dextrose broth/nutrient broth was added to tubes 2 to 12 (except tubes 1 and 11) and 2.0ml of *Aloe vera* juice were put into tube 1. Then, 1.0ml of *Aloe vera* juice was transferred from tube1 to tube 2. Serial doubling dilution was made from tubes 2 to 10 by transferring 1.0ml of the homogeneous tube 2 content to tube 3, and from 3 to 4 and so on to 10 and the remaining 1ml was discarded. Then, 1ml of the *Aloe vera* juice was added to tube 11 (negative control) and 1ml of sabouraud dextrose broth/nutrient broth was added to tube 12 (positive control).

**Determination of minimum inhibitory dilution (MID) of *Aloe vera* juice**

1.0 ml of 0.5 McFarland turbidity of each of the organisms was added to the contents of all the tubes and incubated at 37°C for 7days. The highest dilution showing no turbidity was defined as the MID.

Table 2. Effect of solvent of extraction on the antibacterial activity of Tapinanthus bangwensis

| | Zones of inhibition | |
|---|---|---|
| Solvents of extraction | n | Mean ± SEM(mm) |
| Methanol | 10 | 15.64 ± 1.20 |
| Chloroform | 10 | 19.00 ± 1.50 |

$P < 0.05$.

Table 3. Effect of concentrations on the antibacterial activity of Tapinanthus bangwensis

| | Zones of inhibition | |
|---|---|---|
| Concentrations | n | Mean ± SEM(mm) |
| 100mg/ml | 10 | 12.05 ± 2.06 |
| 150mg/ml | 10 | 14.30 ± 1.99 |
| 200mg/ml | 10 | 16.00 ± 1.90 |
| 50mg/ml | 10 | 19.50 ± 2.45 |

$F = 2.23$, $P > 0.05$.

The effect of host plants on the antibacterial activities of T. bangwensis was determined in table1. Although, the zones of inhibition (mm) varieds with different host plants, but the difference was insignificant (F=1.06, P>0.05). In table 2, when the mean of zones of inhibition of T. bangwensis concentrates from different solvents were compared, a significant difference was observed (F=10.13, P0.05) in table 5. Comparison of percentage weight yields (%) of crude T. bangwensis concentrate regarding the type of solvents of extraction demonstrated that the highest yield of 4.63±1.99% was with chloroform (F = 448.17, P).

**Discussion**

Result of this study have shown that the antibacterial activities of T. bangwensis were independent of host plant factor. This observation supported the null hypothesis which says that host plant does not influence the antibacterial activities of T. bangwensis. The significantly higher percentage yield of crude concentrate of T. bangwensis by chloroform in comparison with other solvents demonstrated higher extraction strength with chloroform (P Steam method of extraction of T. bangwensis has been shown to produce bioactive

compound with better antibacterial activities than cold and hot methods of extraction (P0.05). This indicated that T. bangwensis exhibited antibacterial activities almost at equal level at concentration range of 50-200mg/ml. Equilibrium of antibacterial activities between the lesser and higher concentrations of T. bangwensis clearly shows that the antibacterial quality of the active constituents of the plant is largely dependent on its molecular weight and diffusion rates through agar rather than its concentration. Since higher zones of inhibition in agar diffusion antibiotic susceptibility tests is an attribute of faster rates of drug diffusion and low molecular weight, it can then be inferred that the active antibacterial constituent of T. bangwensis might be among compounds with lower molecular weight. In conclusion, the outcome of this study has demonstrated that antibacterial activities of concentrates from T. bangwensis could be best enhanced by methods that include airdrying and chloroform extraction of the plant via steaming.

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