

COMPARATIVE STUDIES OF ANTIMICROBIAL PROPERTIES OF BRYOPHYLLUM PINNATUM AND CITRUS LEMON ON PATHOGENIC BACTERIA ISOLATES.

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ABSTRACT

Antimicrobial activities of *Bryophyllum pinnatum* and Lemon Juice extract against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were investigated. Blanched and ethanol extract of the leaves of *Bryophyllum pinnatum* extract were prepared and dilutions were impregnated into filter paper disc. The dilutions were made in 1:10, 1:100, 1:1000 and 1:10000. The lemon juice was also diluted in the concentrations of 1:10, 1:100, 1:1000 and 1:10000 and impregnated into filter paper. Agar dilution method was used for the sensitively testing. The organisms showed resistance to the blanched leaves. The result of the ethanol extract showed that *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* showed

susceptibility to the crude extract of *Bryophyllum pinnatum* and lemon juice with a mean zone diameter of inhibition from 10mm to 15mm. *Pseudomonas aeruginosa* and *Escherichia coli* showed sensitivity at 1:10 at 10-13mm while *Staphylococcus aureus* was resistant. The degree of zone of inhibition however differs, but lemon extract exhibited a higher inhibitory zone for isolates than *Bryophyllum pinnatum* especially on *Pseudomonas aeruginosa* in which it still showed inhibitory effect at 1:1000 dilution. Conclusively, *Bryophyllum pinnatum* extract and lemon extracts are potential antimicrobial agents.

KEYWORDS: Antimicrobial agents, Impregnated, Organisms, Susceptibility, Blanched.

INTRODUCTION

Bryophyllum Pinnatum (Africa never die) is a perennial herb that belongs to the family crassulaceae. It grows up to 3-5 feet with a fleshy dark green leaves that are distinctively

scalloped and trimmed in red. They show close proximity in usage, habitat, preparation and identification with *Kalanchoe -crenata* (Chopra *et al.*, 2002). It is grown widely and used in folkloric Medical practice in Tropical Africa, Tropical America, China, India and Australia. The plant flourishes throughout the Southern part of Nigeria. It is Sour in taste, sweet in the post digestive effect and has effective potency. It is known for its haemostatic and wound healing properties. In traditional medicine, the leaves have been used for antimicrobial, antifungal, anti-ulcer, anti-inflammatory, analgesic, antihypertensive, potent anti histamine and anti-allergic activity (Ojewole, 2002).

Streams of *Bryophyllum* plants are succulent, hallow, 50-200cm tall and rarely branched. They are found producing vegetatively by adventitious shoots from base (Igwe and Akunyili, 2005).

The leaves are succulent. The opposite blades are flat, elliptic, measuring, 5-20 x 2-10cm, margins are cremated and they are at the same time producing plantlets called petioles that are 2-10cm long (Ojewole, 2005).

The leaves and bark are bitter and used for the treatment of diarrhea, vomiting, earache, burns, abscesses, gastric ulcers and insect bites (Ofokansi *et al.*, 2005).

The Juice from fresh leaves is used for treatment of Asthma, Convulsion and general debility (Jain, 2010). The plant is also used in the treatment of Oedema of the leg (Okwu and Nnamdi, 2011).

However, in Southeastern Nigeria and in some part of South-South, the herb is used to facilitate the quick dropping of the placenta of new born babies (Okwu, 2007).

The Citrus Lemon is a small evergreen tree common to Asia. The Juice of lemon is about 5 to 6% citric acid, which gives lemon a sour taste.

Lemon is an important medicinal plant of the family *Rutaceae*. It is used mainly for its alkaloids, which are having anticancer activities and antibacterial potentials in crude extract of leaves, stem against clinically significant bacterial strains (Kawaii *et al.*, 2000).

According to Burt (2004) and Ortuno *et al.*, (2006). Citrus Flavonoids have a broad spectrum of biological activities including anti-bacterial, anti-fungal, anti-diabetic, anti-cancer and anti-viral activities.

Micro-organisms can be described as living things that have the ability to act independently and consist of a single cell. Micro-organisms include bacteria, protozoa, certain algae and fungi. They may be harmless, free living, disease producing or extremely harmful (Madigan *et al.*, 2006). Various bacteriological agents that are pathogenic to man include species of *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Escherichia coli*, *Klebsilla* and *Proteus*. Some of these organisms form normal flora of man but may become opportunistic pathogens when the host is immuno-compromised or when they are found in areas in which they are not normally resident.

This study was carried out to confirm the use of herbs such as *Bryophyllum pinnatum* and citrus lemon as being effective against diseases and ailment and to determine the actual concentrations that can inhibit the effect of common pathogenic bacterial isolates.

MATERIALS AND METHODS

STUDY AREA

The study was carried in Braithwaite Memorial Specialist Hospital in Port Harcourt Metropolis.

Sample Collection

The leaves of *Bryophyllum pinnatum* and lemon fruits purchased in fruit garden market in Port-Harcourt and taken to the Laboratory: Stock Cultures of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained from Braithwaite Memorial Specialist Hospital Laboratory.

SAMPLE ANALYSIS

Nutrient agar plates for the culture and sensitivity testing were prepared in the Laboratory by standard and methods.

PREPARATION OF SENSITIVITY DISC

Whatman no I filter paper was punched into circular shape using a perforator. The paper disc was transferred into a bijou bottle and sterilized using the Autoclave.

BRYOPHYLLUM PINNATUM EXTRACTION

The leaves of *Bryophyllum pinnatum* were plucked, Cleaned and dried using a hot air oven and grounded to powder. About 80 grams of the grinded powder was weight and dispensed into 250ml of 70% ethanol and allowed to ripen for 7 days at room temperature. This was also mixed in warm water (blanched).

LEMON JUICE EXTRACTION

The lemon fruit was cleansed and allowed to dry. A swabbed outer skin in 70% alcohol was removed with sterile surgical blade. This was cut open into equal parts and squeezed into a sterile container.

The whitish yellow juice and sludge that exudes were passed through a muslin cloth to remove particulate matter and filtered into a sterile bottle and further stored at 4⁰C.

DILUTION OF EXTRACTION

The neat samples of the *Bryophyllum pinnatum* and lemon juice were further diluted in normal saline in dilutions of 1:10, (0.1m); 1:100, (0.01m); 1:1000, (0.001m); 1:10000, (0.000ml). The universal bottles contain filter paper disc were impregnated with *Bryophyllum pinnatum* and lemon juice extract at different dilutions by adding 1ml of the neat (undiluted) and diluted extract of different dilutions.

Thereafter well dried nutrient agar plates were seeded by streaking the required organisms separately throughout the entire surface of the plate.

This was followed by aseptically transferring the *Bryophyllum pinnatum* and Citrus lemon impregnated discs on the surface of the inoculated nutrient agar plates with the aid of a flamed forceps.

The inoculated plates with the test disc in place were incubated at 37⁰C. After 24hours incubation, plates showing clear zone of inhibition were noted and zone diameter measured with a meter rule.

RESULT

The study revealed antimicrobial activity of *Bryophyllum pinnatum* ethanol extract as *Escherichia coli* 12mm, *Pseudomonas aeruginosa* 12mm and *Staphylococcus aureus* 15mm.

The blanched leaves of *Bryophyllum pinnatum* showed no inhibitory effect against all the test organisms as showed in figure 4.1.

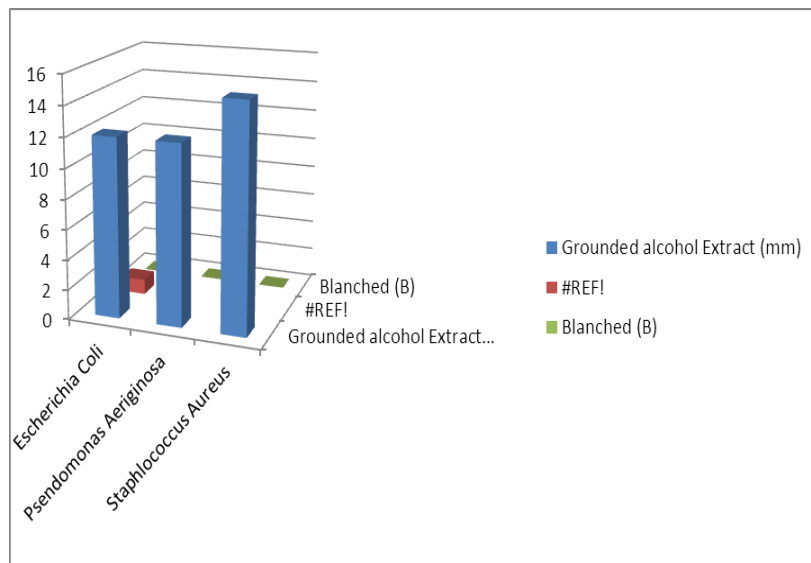


Figure 1: Antimicrobial Sensitivity of Extracts and Leaves of *Bryophyllum pinnatum*.

The inhibitory effect of the neat (100%) grounded extract of *Bryophyllum pinnatum* leaf on the test organism had *Staphylococcus aureus* showing the highest inhibition rate of 15mm while *Escherichia coli* and *Pseudomonas aeruginosa* showed inhibition rate of 12mm. The diluted leaf showed inhibition at 1:10 as *Escherichia coli* 11mm, *Pseudomonas aeruginosa* 10mm while *Staphylococcus aureus* showed resistance. Dilutions of 1:100, 1:1000 and 1:10000 were all resistant as shown in table 1.

Table 1: Antimicrobial sensitivity of Dry grounded ethanol extract of *Bryophyllum Pinnatum*.

Test Organisms	Size of Inhibition		Zone(mm)		
	Neat	1:10	1:100	1:1000	1:10000
Escherichia coli	12	11	Resistant	Resistant	Resistant
Pseudomonas aeruginosa	12	10	Resistant	Resistant	Resistant
Staphylococcus aereus	15	Resistant	Resistant	Resistant	Resistant

Table 2: Shows the inhibitory effect of the neat (100%) extract of lemon juice
Escherichia coli showed inhibition of 12mm as neat and resistant at all other dilutions
Pseudomonas aeruginosa showed inhibition rate of 15mm at neat, 13mm at 1:10, 11mm at 1:100 and 10mm at 1:1000 while *Staphylococcus aureus* showed inhibition of 15mm at neat and resistant at all other dilutions.

Antimicrobial sensitivity of lemon juice extract

Test Organisms	Size of Inhibition Zone(mm)				
	Neat	1:10	1:100	1:1000	1:1000
Escherichia Coli	12	Resistant	Resistant	Resistant	Resistant
Pseudomonas aeruginosa	15	13	11	10	Resistant
Staphylococcus aureus	15	Resistant	Resistant	Resistant	Resistant

DISCUSSION

The study had revealed that *Bryophyllum pinnatum* and lemon (Citrus Lemon) Juice have varied antimicrobial action against pathogenic clinical isolate, such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The blanched leaves of *Bryophyllum pinnatum* showed no inhibitory effects against all the test organisms.

The inhibitory effect of the neat (100%) of ethanol extract of *Bryophyllum pinnatum* leaf on the entire organisms tested showed that *Staphylococcus aureus* exhibited the highest level of susceptibility with an inhibitory zone of 15mm while the inhibition effect of the neat (100%) extract of Lemon Juice on all the entire organisms tested showed that *Pseudomonas aeruginosa* and *Staphylococcus aureus* exhibited the highest level of susceptibility with inhibition zone of 15mm.

The susceptibility of these organisms to the extracts explains their use on native medicine for the treatment of infection such as sour throat, boil, wound, cough and dysentery. This study further reveals that these plants are broad spectrum in their activities since they are effective against gram positive and gram negative bacteria. The Study then correlates with the previous workers that plants contain substances that are antimicrobial (Olukoya *et al.*, 1986). The Zone of inhibition observed in the Gram positive organisms agrees with work of Ofokansi (2005) which showed strong activities of methanol extract of *Bryophyllum pinnatum* against some Gram positive organisms. The leaf juice of this plant is commonly used in treating ear infection and navel of newborn babies which not only heals fast but present infection at the site. This practice is common among women in the Southern part of Nigeria. Other extracts

of *Bryophyllum pinnatum* showed a moderate to weak activity against tested organisms. This may be as a result of loss of some of the plant's active principle during dilutions or the inability of the solvent to dissolve some of the active principles of the plant (Ellof, 1998).

CONCLUSION

The result obtained has shown that antimicrobial activity exhibited by these extracts compares favourably with the action of most antibiotics on bacteria.

Similarly, in Nigeria, it is observed that a lot of the rural populace still rely on medicinal plants and most tribes rely mainly on herbs for their healthcare needs which is readily available, obtained at anytime of the year, cheap and administered easily. More so, because Third World Countries are faced with the high cost of Western drugs (synthetic) for treatment of ailments in developing countries, there is every need to think out the box and look inwards to develop possible biomarkers from local content that could be acceptable to the world over.

Consequently pharmaceuticals and allied companies should avail themselves of this rare opportunity and incorporate *Bryophyllum pinnatum* and *Citrus lemon* as part of their active ingredients in order to get their synergistic actions against the aetiologic agents that infects humans.

REFERENCES

1. Burt, S. A (2004). Essential oils; Their antibacterial properties and potential applications in foods, *International journal of food Microbiology*, 94: 233 – 253.
2. Chopra, R. N., Nayar, S. L and Chopra, I. L (2002). Glossary of Indian Medicinal Plants NISCIR (CSIR). New Delhi, 42: 12 – 39.
3. Ellof, J. N. (1998). Which extractant should be used for the Screening and Isolation of Antimicrobial Components from plants? *Journal of Ethnopharmacology*, 60: 1-8.
4. Igwe, S. A. and Akunyili, D. N (2005). Analgesic Effects of aqueous extracts of the leaves of *Bryophyllum pinnatum*. *Journal of Pharmaceutical Biology*, 43(8): 658-661.
5. Jain, V. C. (2010). Antioxidant and antimicrobial activities of *Bryophyllum Calycinum* Salish leaf. *Pharmacology online*, 1: 393-405.
6. Madigan, M.T., Martinko, J and Parker J. (2006). Molecular analysis of Micro-organisms. *Brook Biology of Micro-organisms*, Prentice Hall, USA P75-276.

7. Ofokansi, K. C., Esimone, C. O and Anele, C.R.(2005). Evaluation of the Invitro combinal antibacterial effect of the leaf extracts of Bryophyllum pinnatum and Ocimum gratissimum. *Plant products Research Journal*, 9: 23 – 27.
8. Ojewole, J. A. O. (2002). Anti-hypertensive properties of Bryophyllum pinnatum leaf extracts *American Journal of Hypertension*, 15(4): A34 – A39.
9. Ojewole, J. A. O. (2005). Antinogceptive, anti-inflammatory and anti-diabetic effects of Bryophyllum pinnatum (Crassu Taceae) leaf aq extract. *Journal of Ethno Pharmacology*, 99: 13 – 19.
10. Okwu, D. E (2007). Nigerian Medical Pant II Medicinal and Aromatic Plant Science and Biotechnology, 1(1): 97-102.
11. Okwu, D. E and Nnamdi, F. U. (2011). Two novel flavnoids from Bryophyllum pinnatum and their antimicrobial activity. *Pharmaceutical chemistry journal*, 3(2): 1-10.
12. Olukoya, D. K; Odughemi, T. O and Barngbose, S. O. A. (1986). Some aspects of traditional therapy of Gonorrhoea in Lagos, *Nigeria Journal of Research in Ethno medicine*, 1: 26-29.
13. Ortuno, A., Baidez, A., Gomez, P, Arcas, M.C Parras, I., Garcia-Lidon, A and Del-Rio, J.A (2006). Citrus Paradisi and Citrus Sinensis Flavonoids: Their influence in the defence mechanism against penicillum digitatum. *Journal of food chemistry*, 98: 351-358.