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ANTIBACTERIAL ACTIVITY OF *IN VITRO* REGENERATED ROOTS OF *BRYOPHYLLUM PINNATUM* LAM. KURZ

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ABSTRACT: *In vitro* antibacterial activity of *Bryophyllum pinnatum* (*in vivo* generated and *in vitro* regenerated roots) were analysed in this study. Traditional uses for these plants treat specific conditions or diseases. The present study examined the antibacterial activity using the disk diffusion method using Ethanolic & Methanolic extract as part of the process of understanding the chemistry, toxicity and efficacy of these plant extracts. Methanolic and Ethanolic extracts of this plant was examined using a standard antimicrobial disk diffusion method. Extracts were tested against both Gram negative (*Escherichia coli*, *Vibrio cholerae*, *Salmonella typhi* and *Pseudomonas aeruginosa*) & Gram positive (*Bacillus cereus* & *Staphylococcus aureus*) bacteria. Methanolic *in vitro* regenerated roots showed higher inhibition zone against all bacterial species while natural growing plant root extract showed minimum inhibition. *In vitro* regenerated root extract showed moderate inhibition zone against all bacterial species. *S. aureus* showed maximum (25.2 mm) inhibition zone in *in vitro* root methanolic extract while *V. cholerae* showed minimum (14 mm) in natural growing root extract. In ethanolic extract maximum inhibition zone (22.1 mm) was observed against *S. aureus* and another maximum inhibition zone (21.3mm and 21mm) was reported against *B. cereus* and *P. aeruginosa* respectively. These results served to validate our procedures and indicate the need for the present study. Implications of these results for bioactivity and drug discovery potential of plant extracts can be further explored. This study serves as basis for further research on these weed plants.

INTRODUCTION: In the past few decades there has been a resurgence of interest in the study and use of medicinal plants in health care and in recognition of the importance of medicinal plants to the health system. ¹ This awakening has led to a sudden rise in demand for herbal medicines, followed by a belated growth in international awareness about the dwindling supply of the world's medicinal plants. ² Most of the pharmaceutical industry is highly dependent on wild populations for the supply of raw materials for extraction of medicinally important compounds.

India has a rich cultural heritage of traditional medicines which chiefly comprised the widely flourishing systems of treatments i.e. Ayurvedic. ³ The history of herbal remedies is rather old and dates back to the time when the early man became conscious of his environment. Thousands of years' experience, by trial and error had taught people to distinguish between beneficial and non-beneficial plants with their properties as healing agents dawning on him much later. In this era of increasing microbial diseases, Ayurveda (Herbalism) is a better option to get cure from diseases or infections.

Herbalism uses whole plants or plant parts as therapeutic remedies. Plants are autotrophic in nature and thus can synthesize various kinds of compounds which aids in their survival. They beautify the environment, reduce pollution and are

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applicants in almost all walks of life. The natural products today symbolize safety in contrast to the synthetic drugs that are considered as unsafe to human and environment. Although herbs had been valued for their medicinal, flavouring and aromatic properties for centuries, the synthetic products of the modern era surpassed their importance, for a while.

B. pinnatumis the air plant, miracle leaf or life plant is a native to of Tropical Africa, naturalized throughout the tropics of the world. It is a popular houseplant and found in temperate regions of Asia, The pacific and Caribbean, Australia, New-Zealand, West-Indies, Macaronesia, Mascarenes, Galapagos, Melansia, Polynesia and Hawaii.⁴ Genus *Bryophyllum* of the family Crassulaceae is a valuable medicinal as well as ornamental plant. These plants are cultivated as ornamental house plants and rock or succulent garden plants.⁵

The present study has been done to discover the antimicrobial properties of *in vitro* and *in vivo* regenerated roots of *Bryophyllum pinnatum*. The main reason of selection behind this area was that there is no research on this plant. *In vitro* study of antimicrobial activity of weed plant is a global scenario. An antimicrobial is a compound that kills or inhibits the growth of microorganisms such as bacteria, fungi and protozoa etc. This plant was used to check the antibacterial properties against clinical and environmental microbial pathogens like six bacterial strains *Escherichia coli*, *Vibrio cholerae*, *Salmonella typhi*, *Pseudomonas aeruginosa* (Gram negative) *Bacillus cereus*, *Staphylococcus aureus* (Gram positive).

MATERIALS AND METHODS:

Collection of plant materials: *Bryophyllum pinnatum* was collected encompassing the Crassulaceae family was utilized and transferred in department for further searching to microbiological activity. In research laboratory plant was thoroughly washed using water, dried and carefully separated into leaves, stems and roots with suitable equipment. The plant material (*in vitro* regenerated roots) was also collected from plantlets. Roots were thoroughly washed using water and mopped with tissue paper. To avoid microbial contamination from Rhizo-sphere and phyllo-sphere, plants

surface sterilization was done with 70% alcohol with standard microbiological parameters. After this plant was shade dried at room temperature to prevent the decomposition of chemical constituents.⁶ After drying at room temperature the plant materials was allowed to drying in a hot air oven (Vaiometer Sonar) at 20-30°C. The dried plant material was grinded into a fine powder with a mortar and pestle for further processing. The powder was stored in air tight zip lock poly bags until for further investigation at room temperature with properly sealed and scientific labeling of each part of plant.

Preparation of crude extracts of roots:

The root extract of *Bryophyllum pinnatum* was prepared using methanol solvent. For the methanolic extraction, dried power put in the soxhlet thimble using Whatman filter paper No. 1 and 300 ml of methanol and ethanol (50%) in soxhlet flask. After that sample were extracted at 50-60°C. After this samples were allowed for extraction at 20-30°C. Following this the extracts were concentrated under pressure using rotary vacuum evaporator. The concentrated extract was weighed and labeled appropriately. All residues were kept in tightly stoppered bottle until used for the anti-microbial tests.

Bacterial strains and antibiotics:

The antimicrobial activity was assayed utilizing two groups of well-known microorganisms. One group of MTCC gram negative, pathogenic strains: *Escherichia coli* 1692; *Vibrio cholerae* 3906; *Salmonella typhi* 0733; *Pseudomonas aeruginosa* 4676; other the gram positive *Bacillus cereus* 1272; and *Staphylococcus aureus* 7443. The microorganisms were maintained in nutrient agar at 4°C until the assays were carried out. Different (Himedia) antibacterial antibiotics (Streptomycin, Ampicillin) were used in work.

Antimicrobial susceptibility test:

The antimicrobial screening of the bio extracts were carried out by determining the zone of inhibition using disc diffusion method.⁷ The sterilized Mueller-Hinton agar (MHA) plates were prepared and labeled appropriately with the name of the bacterial strains and the plant extracts. Using sterile forceps, sterile 6mm discs (cut from

Whatman No. 1 filter paper with a paper punch device and sterilized before use) were picked and submerged in concentrated extracts. Overnight bacterial culture 1×10^6 CFU/mL viable count inoculums were used and applying the bio extract impregnated discs (6mm). Commercially prepared antibiotic (Himedia) disc were used as a positive control and discs soaked in distilled water as a negative control in each agar plate.

The plates were allowed to stand for 30 minutes and then incubated at 37°C for 24 hours. Antimicrobial activity of each extract against the test organisms were indicated by a growth-free zone around the respective discs and the diameters of the zones of inhibition to the nearest millimeter with a ruler were obtained by measuring the distance from one end of the inhibition zone, across the disc to the other end, as reported by Nwanebu et al.⁸

RESULTS & DISCUSSION:

Plants are a potential source of therapeutic activities due to the presence of bioactive components. Many reports are available on the antibacterial, antifungal, antiviral, antihelminic, antimolluscal and anti-inflammatory properties of plants.⁹ Plants contain substances that are antimicrobial.¹⁰ In this study methanolic and ethanolic extract of *in vivo* and *in vitro* regenerated roots was assayed *in vitro* by disc diffusion for antimicrobial activity against bacterial strains. Samples were extracted with methanol and ethanol solvent. Some Gram-positive and Gram-negative pathogenic micro-organisms were used against these extracts. Several *in vitro* experiments were conducted and observed with respect to inhibition zone. Inhibition zone was measured in mm. Standard antibiotics discs of streptomycin (10 mcg) and ampicillin (10 mcg) were also used as standard comparison purpose.

Methanolic Extract:

Methanolic *in vitro* regenerated roots showed higher inhibition zone against all bacterial species while natural growing plant root extract showed minimum inhibition. *In vitro* regenerated root extract showed moderate inhibition zone against all bacterial species. *S. aureus* showed maximum (25.2 mm) inhibition zone in *in vitro* root methanolic

extract while *V. cholerae* showed minimum (14 mm) in natural growing root extract (**Table 1** and **Fig.1**). Dey SK et al reported that Methanol extract of *Psidium guajava* and *Terminalia arjuna* showed pronounced activity against all the tested gram positive and gram negative microorganisms except *Pseudomonas aeruginosa*.¹¹ Natural growing root extract showed maximum inhibition zone (21 mm) against *B. cereus* and minimum zone (14 mm) was obtained against *V. cholerae*. Akinpelu showed strong activities of methanol extract of leaves explant of *Bryophyllum pinnatum* against some Gram-positive organisms.¹²

The antimicrobial effect of methanol extract against these organisms may be due to the ability of the methanol 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.¹³ The methanolic extract showed considerably more activity than the aqueous extract. In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant.^{14, 15}

Ethanolic Extract:

In ethanolic extract maximum inhibition zone (22.1 mm) was observed against *S. aureus* and another maximum inhibition zone (21.3mm and 21mm) was reported against *B. cereus* and *P. aeruginosa* respectively (**Table 2** & **Fig. 2**). The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality.¹⁶ Therefore, there is a need to look for substances from other sources with proven antimicrobial activity.

Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs.¹⁷ This study does not only show the scientific basis for some of the therapeutic uses of this plant in traditional medicine, but also confirms the fact that ethno botanical approach should be considered when investigating antimicrobial properties of plants.^{18, 19} It also explains why these

plants give similar therapeutic result when they are used interchangeably in spite of their uniqueness.²⁰ In the case of standard antibiotics (Streptomycin & Ampicillin) maximum inhibition zone was reported

against *S. aureus* in both Streptomycin (21.3 mm) & Ampicillin (33.3 mm). Minimum inhibition zone was obtained against *V. cholerae* in Ampicillin (Table 3).

TABLE 1: SHOWING ANTIMICROBIAL ACTIVITY OF *BRYOPHYLLUM PINNATUM* USING METHANOL EXTRACT AGAINST GRAM POSITIVE AND NEGATIVE BACTERIAL STRAINS

Microorganisms	50% Methanol	<i>In vivo</i> roots (mm)	<i>In vitro</i> roots (mm)	Streptomycin 10mcg	Ampicillin (10mcg)
Gram Negative Bacteria					
<i>E. coli</i>	0±0	15±1.21	20.5±0.80	18.3±.95	21±.52
<i>V. cholerae</i>	0±0	14±0.58	22.2±0.72	17.6±.56	13.3±.57
<i>S. typhi</i>	0±0	18.1±1.10	22.1±0.35	21±1.55	29±.90
<i>P. aeruginosa</i>	0±0	20.2±0.66	24±1.40	-----	-----
Gram Positive Bacteria					
<i>B. cereus</i>	0±0	21±0.24	23.6±0.33	16.6±.38	25.6±1.20
<i>S. aureus</i>	0±0	20.5±1.04	25.2±0.98	21.3±.92	33.3±.66

Result as per shown in Mean±S.E; ----- No inhibition

TABLE 2: SHOWING ANTIMICROBIAL ACTIVITY OF *BRYOPHYLLUM PINNATUM* USING ETHANOL EXTRACT AGAINST GRAM POSITIVE AND NEGATIVE BACTERIAL STRAINS

Microorganisms	50% Ethanol	<i>In vivo</i> roots (mm)	<i>In vitro</i> roots (mm)	Streptomycin 10mcg	Ampicillin (10mcg)
Gram Negative Bacteria					
<i>E. coli</i>	0±0	15±1.21	17.8±0.80	18.3±.95	21±.52
<i>V. cholerae</i>	0±0	14±0.58	16.2±0.72	17.6±.56	13.3±.57
<i>S. typhi</i>	0±0	18.1±1.10	19.2±0.35	21±1.55	29±.90
<i>P. aeruginosa</i>	0±0	20.2±0.66	21±1.40	-----	-----
Gram Positive Bacteria					
<i>B. cereus</i>	0±0	21±0.24	21.3±0.33	16.6±.38	25.6±1.20
<i>S. aureus</i>	0±0	20.5±1.04	22.1±0.98	21.3±.92	33.3±.66

Result as per shown in Mean±S.E; ----- No inhibition

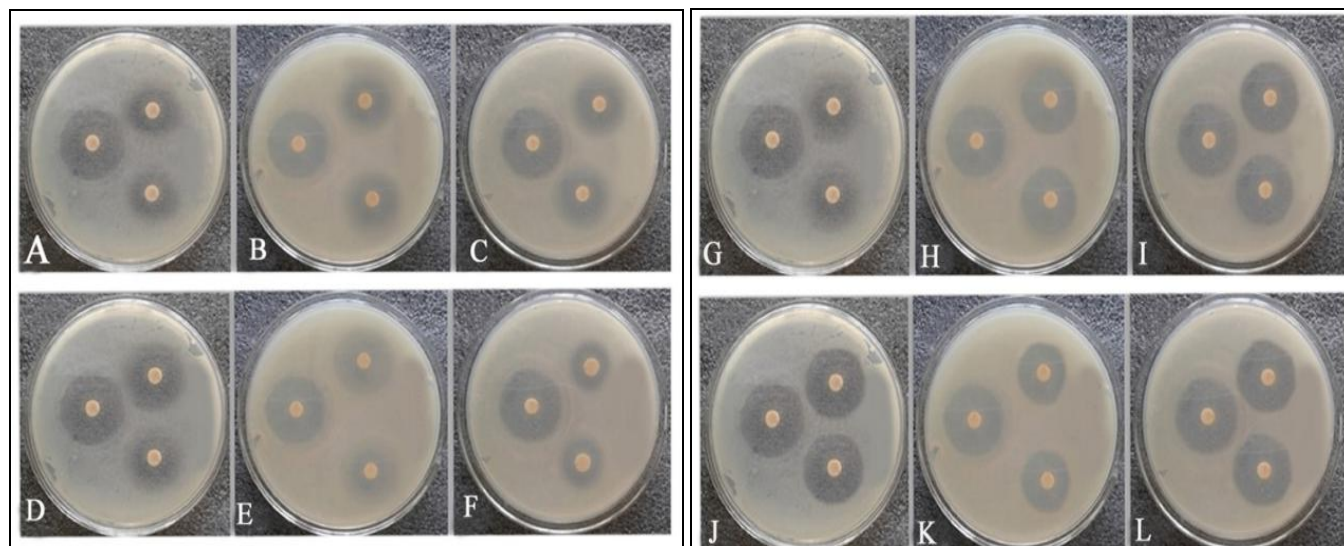


FIG.1: SHOWING ANTIMICROBIAL ACTIVITY OF *B. PINNATUM* USING METHANOL EXTRACT AGAINST GRAM POSITIVE AND NEGATIVE STRAINS

A-F: Antimicrobial activity of *in vivo* roots; G-L: Antimicrobial activity of *in vitro* roots

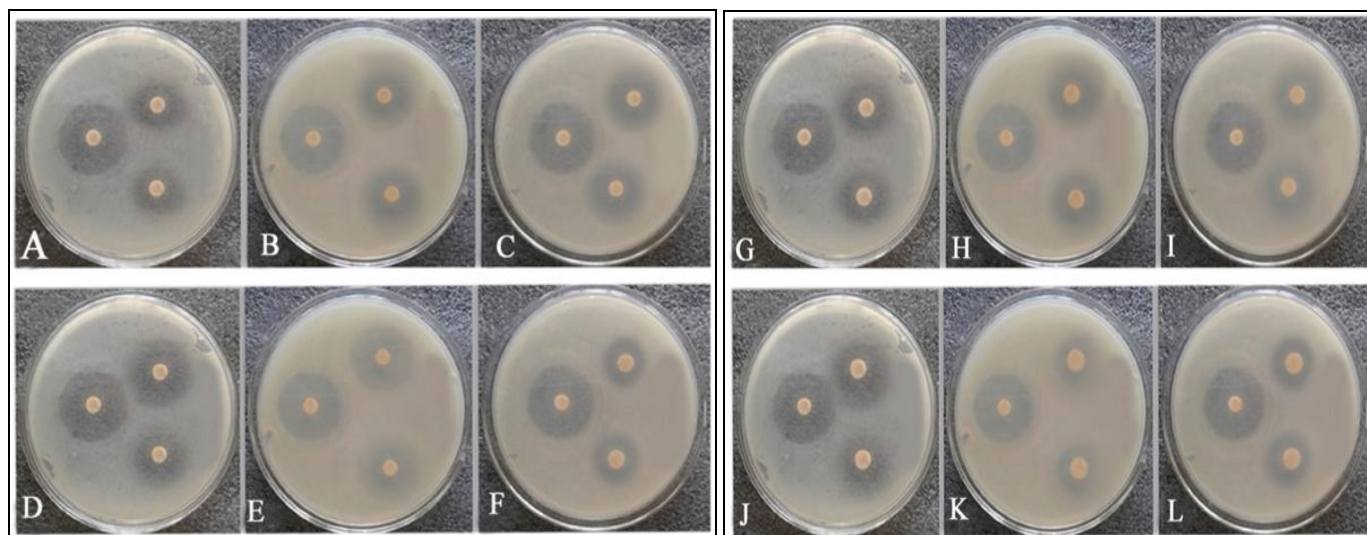


FIG.2 SHOWING ANTIMICROBIAL ACTIVITY OF *B. PINNATUM* USING ETHANOL EXTRACT AGAINST GRAM POSITIVE AND NEGATIVE STRAINS

A-F: Antimicrobial activity of *in vivo* roots; G-L: Antimicrobial activity of *in vitro* roots

CONCLUSION: *In vitro* regenerated roots showed higher inhibition zone against all bacterial species while natural growing plant root extract showed minimum inhibition. *S. aureus* was very susceptible for this extract and showed higher inhibition. So it can be concluded that auxin and cytokinin improves the plant regeneration parameters and substances from this plant prove antimicrobial activity. This study does not only show the scientific basis for some of the therapeutic uses of this plant in traditional medicine, but also confirms the fact that ethno-botanical approach can be applied in investigating in antimicrobial properties of plant. So this plant could be potential medicine for many diseases. It is necessary to carry out screening of these plants in order to reveal the active principles by isolation and characterization of their antimicrobial constituents. It can be used as more effective antimicrobial agents among materials of plant origin, with potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs.

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