

Antimicrobial effect of Callus and Natural plant extracts of *Premna serratifolia* L.

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Abstract:

Premna serratifolia L. is an important medicinal shrub used in traditional system of medicines as cardio tonic, antibiotic, anti-coagulant, carminative, hepatoprotective, anti tumor etc. The present study aims in the evaluation of antimicrobial activity against the selected human pathogens (*Bacillus sp*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Non-haemolytic Streptococci*, *Streptococcus epidermidis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) by using natural leaves, roots and its respective calli induced with help of various plant growth regulators. Result revealed that, increased inhibitory activities of callus extracts were found to be the best when compared to the natural plant material extracts. The study suggests the plant *Premna serratifolia* L. is an potent source for phytomedicine development in future.

Key Words: BAP: Benzyl amino purine, KI: Kinetin, NAA: Naphthalene acetic acid, 2,4-D: 2,4-dichlorophenoxy acetic acid etc.

Introduction:

Nature has provided an important source of remedies to cure all the ailments of mankind. In recent years all the medicines used were from natural source, especially from plants.

Premna serratifolia L. (Verbenaceae) is an important medicinal shrub having tremendous medicinal values like cardiac stimulant activity[1], anticoagulant activity[2], decoction of *P.serratifolia* exhibited anti inflammatory and anti arthritic activity[3], antibacterial activity from root extracts[4], antimicrobial activities from bark and wood[5]. Due to the medicinal importance of this species *Premna serratifolia* L. the present investigation designed to establish the protocol for callus culture using various growth regulators and screening the anti bacterial effect of ethanolic extracts of both natural plant materials(Leaf and root) and callus derived from leaves and root extracts.

Material and method:

This method is used to test the susceptibility of microorganisms against the different extracts of *Premna serratifolia*. The leaf, leaf callus, root and root callus extracts were tested for their antimicrobial activity against 10 bacterial cultures viz., *Bacillus sp*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Non-haemolytic Streptococci*, *Streptococcus epidermidis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*. Cultures used for the present study were collected from the Microbial culture collection maintained in the Department of Microbiology J.J. College of Arts and Science, Pudukkottai, Tamil Nadu India.

Callus induction and proliferation

The leaf and root explants were excised into 0.5 – 1.5 cm long segments and small incision was made on the surface of each explant using a sharp sterile blade. All the explants were cultured on MS basal medium supplemented with various concentrations of different auxins ranging from 1.0 – 7.0 mg/l of 2,4-D, NAA, IAA and IBA for callus induction. The twenty-eight days old callus was collected and sub cultured on fresh medium with same growth regulator combinations for further proliferation. Callus was

sub cultured twice in 4 week time interval. All the cultures were incubated at $25 \pm 1^{\circ}\text{C}$ under a relative humidity of 50 to 60% and 12/12 – hour photoperiod.

Extraction of samples

The organic constituents from dried plant tissue (leaf and root) and callus materials from leaf and root explants obtained by continuously extracting the powdered materials in Soxhlet apparatus with ethanol as solvent. The extracts were concentrated to one third of their original volume and used for testing the chemical constituents. After completion of extraction, the extract was filtered and concentrated to dryness under hot air oven at 55°C . The residue appeared as a dark brown powder.

Procedure

Nutrient agar was used as the culture medium for this assay. The molten nutrient agar was dispensed in pre sterilized Petri dishes (25 ml each) and allowed to cool. These agar plates were homogenously inoculated with the test bacterium previously suspended in tryptone broth (106cells/ml). The plates were allowed to solidify and after solidification holes/wells (cups) of 6 mm diameter were punched into the agar with the help of flamed cork borer. Five wells were prepared in each plate. Of these five, first and second well were filled with 0.2 ml of the leaf and leaf callus extract, the third and fourth well were filled with 0.2 ml of root and root callus extracts and the fifth one was filled with Ethanol (Extracting solvent alone). The Petri dishes were incubated at 37°C for 24 h. After this incubation period the diameter of the inhibition zone formed around each is well/cup was measured and the values were recorded. The antimicrobial activity was expressed as the ratio of the inhibition zone produced by the plant extract and the inhibition zone caused by the standard. Testing was carried out for each bacterium in quadruplicates

Result and Discussion:

Effect of different auxins on biomass production from root explants

Root explants from *Premna serratifolia* L. were cultured on medium supplemented with various auxins (IAA, IBA, 2,4-D and NAA 1.0 – 7.0 mg/l each) the best biomass (80mg) was obtained from 5.0 mg/l of IAA and 60 % callusing response was observed. The least biomass production (22mg) was obtained from 7.0 mg/l of IBA. But there was no callusing response observed in 1.0 to 3.0 mg/l of IBA and 1.0 mg/l of 2,4-D.

Effect of different auxins on biomass production from leaf explants

Leaf explants of *Premna serratifolia* L. were cultured on MS medium fortified with all the tested hormone concentrations. Among this highest biomass (178 mg/l) was observed in the medium supplemented with 5.0 mg/l IAA on 25th day. The callus obtained from this supplementation condition was yellow and friable. Relatively higher biomass (147mg) of green nodular callus was obtained in 7.0 mg/l of NAA, and least biomass (84 mg) white friable calli was obtained with 7.0 mg/l of 2, 4-D.

Antibacterial activity of Natural root and leaf ethanol extracts;

Root and Leaf derived callus ethanol extracts of *Premna serratifolia*:

Plant substances continue to serve as exclusive source of drugs for the majority of the world population and several plant based drugs are in extensive clinical use [6]. The antibacterial activity of these extracts against bacteria revealed an increase in inhibitory activity of root derived callus, compared to the root and other extracts. The order of antimicrobial activity of *Premna serratifolia* extracts was found to be Root callus extract > Natural root extract > Leaf callus extract > Natural leaf extract. From the results it was evident that *Streptococcus epidermidis* was most susceptible and *Micrococcus sp* was the least susceptible to root derived callus extract. *Klebsiella pneumoniae* was the most susceptible and *Micrococcus sp* was the least susceptible to natural root extract. *Pseudomonas aeruginosa* was the most susceptible and

Bacillus sp was found to be the least susceptible to leaf derived callus extract. *Klebsiella pneumoniae* was found to be the most susceptible (Table.1) whereas *Salmonella typhimurium* was the least susceptible to natural leaf extract (Fig. 1).

The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes [7,8]. Antimicrobial activity of crude extract of *Premna serratifolia* L. reported by [9]. The appeal of using natural products for medicinal purposes is increasing, and at present, researchers aim to produce substances with anti-tumor, anti-viral, hypoglycaemic, anti-inflammatory, anti-parasite, antimicrobial, tranquilizer and immunomodulating activities through tissue culture technology. Advances in the area of cell cultures for the production of medicinal compounds has made possible the production of a wide variety of pharmaceuticals like alkaloids, terpenoids, steroids, saponins, phenolics, flavonoids, and amino acids.

Table-1 Antibacterial activity of leaf, root and their callus of *Premna serratifolia* L. (Diameter of zone of inhibition in mm including well diameter of 6mm).

S.No	Name of the micro organism	Zone of inhibition (mm)				
		Leaf	Leaf Callus	Root	Root Callus	Ethanol
1	<i>Bacillus sp</i>	6.5	7.0	9.0	11.0	-
2	<i>Enterococcus faecalis</i>	7.0	8.0	9.0	10.0	-
3	<i>Escherichia coli</i>	7.5	8.5	9.0	12.0	-
4	<i>Klebsiella pneumoniae</i>	8.0	10.0	11.5	13.0	-
5	<i>Micrococcus sp</i>	6.5	8.0	8.5	10.0	-
6	<i>Non-hemolytic streptococcus</i>	7.0	8.5	9.5	11.0	-
7.	<i>S.epidermidis</i>	8.0	9.0	10.5	14.0	-
8.	<i>Pseudomonas aeruginosa</i>	8.0	10.0	11.0	14.0	-
9.	<i>Salmonella typhimurium</i>	6.5	7.5	9.0	11.0	-

Values are means of three replicates.

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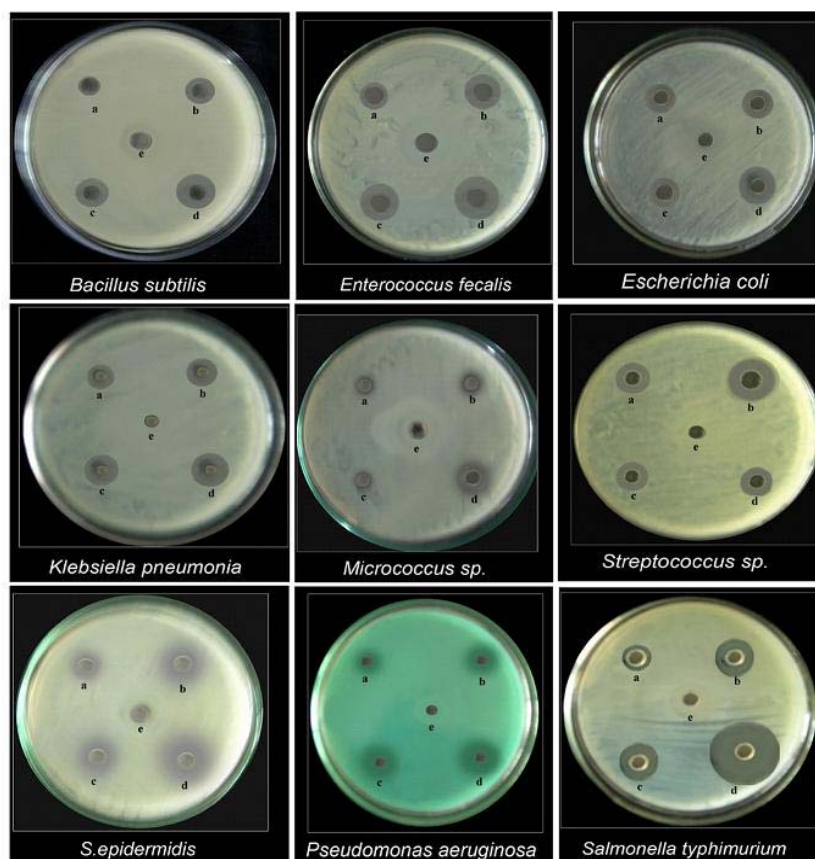


Fig-1 Anti-bacterial activity of field grown leaf, root and leaf, root, derived callus extracts of *Premna serratifolia* L.

a. Field grown leaf extract, b. Leaf callus extract, c. Field grown root extract,
d. Root callus extract e. Solvent (Ethanol)