

## Research Article

## AN IN VITRO ANTIMICROBIAL ACTIVITY OF CALLUS AND ROOT EXTRACTS OF *PLUMBAGO ZEYLANICA* LINN. IN VARIOUS TEST MICROORGANISM

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### ABSTRACT

*Plumbago zeylanica* Linn. (Plumbaginaceae) is a perennial subscandant shrub and is listed as threatened medicinal plant. It is commonly known as 'Chitrak' and the roots of the plant were used traditionally as a germicidal, abortifacient, and in treatment of cancer, liver disease, fever, body pain, and inflammation. Since it is a threatened and potential medicinal plant therefore it is of great interest to evaluate the anti microbial activity of callus developed by nodal explant and to compare its action with respect to root extract of parent plant. Dried callus and roots from parent plant were powdered and extracted with ethanol. The callus extract and root extract at the concentration of 5mg/ml and 10mg/ml were evaluated for anti microbial activity by cup plate method against some selected gram-ve and Gram+ve microorganism. The minimum inhibitory concentration (MIC) against test microorganism studied by turbidity method. The Root extract and callus extract show the maximum zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus* (Gram+ve bacteria) and the MIC of root extract against *S. aureus* and *M. luteus* is 1250 and 2500 µg/ml. whereas the MIC of callus extract against these microorganisms is 5000 µg/ml. In conclusion, a massive light creamish brown and granular callus formed with MS medium supplemented with naphthalene acetic acid (1.5 ppm) and kinetin (0.25 ppm) and it possess a significant anti microbial activity against the test microorganism which might be due to the naphthoquinones present in the callus extract which need to be further analyzed.

**Keywords:** *Plumbago zeylanica*, Plant tissue culture, anti microbial activity.

### INTRODUCTION

*Plumbago zeylanica* Linn. (Plumbaginaceae) commonly called chitrak, is a perennial, subscandant shrub found wild in South India and West Bengal. It is also cultivated in gardens throughout India<sup>1,2</sup>. Its roots are used in traditional system of medicine to cure various ailments like body pain, headache, fever and inflammation<sup>3</sup>. *Plumbago zeylanica* roots were reported to possess antioxidant, hypolipidemic, anti artherosclerotic, central nervous system stimulant and anti fertility properties<sup>4-7</sup>. It is a threatened plant<sup>8</sup> with potential medicinal value hence it was considered worthwhile to evaluate the anti microbial activity of callus obtained from nodal explant and to compare its activity with respect to root extract from parent plant.

### MATERIALS AND METHODS

#### Identification of Plant material

*Plumbago zeylanica* roots were procured from kharibawri market, Delhi. They were identified and authenticated by the Dr. H.B. Singh, Head, Raw material, Herbarium and Museum division, National Institute of Science Communication And Information Resources (NISCAIR), New Delhi. The stem twigs for tissue culture study were collected from Medicinal and Aromatic Plant Garden, CCS Haryana Agricultural University and were identified by Dr. C. S. Tyagi, Head, Medicinal Aromatic and Under Utilized Plant Section, Department of Plant Breeding, CCS HAU,

Hisar. A voucher specimen is preserved in the Department for the ready reference (0432).

#### Tissue Culture Study

Plant material collected was thoroughly washed in running tap water followed by treatment with Teepol solution 2% (v/v) to remove the adhere dust particles. The stem segments were further cut into 3-4 cm pieces with sterile blade having one node, used as the explant. The explants were treated with fungicide 'Tagstin' 2% (w/v) for 7-8 minutes. The explants were surface sterilized with 0.1% (w/v) aqueous mercuric chloride solution for 10-15 minutes followed by three to four washing with sterile water to remove last traces of sterilizing agent<sup>9</sup>. Nodal explants were inoculated in culture bottles with sterile murashige and skoog nutrient medium<sup>10</sup> with different concentration of phytohormones naphthalene acetic acid (0.25-2.00 ppm) and Kinetin (0.05-0.50 ppm) in five batches. Each batch of the experiment was started with 20 cultures and follow strict aseptic conditions. All the cultures were maintained at 26±2°C for 16 hours photoperiod per day provided by white fluorescent tubes.

#### Preparation of extracts

Dried roots (100gm) and callus (12 gm) were mechanically pulverized to a coarse powder and extracted with ethanol 95% in soxhlet extractor for 72 hours. After exhaustive extraction, the root extract (PRE) and callus extract (PCE)



were filtered and concentrated over boiling water bath to recover the solvent.

### Experimental microorganism

#### Gram negative Strain:

- i. *Escherichia coli*

#### Gram Positive Strain:

- i. *Staphylococcus aureus* (NCIM 2901)
- ii. *Bacillus subtilis* (NCIM 2106)
- iii. *Micrococcus luteus* (MTCC 1541)

### Preparation of test inoculum

#### (a) Seeded Broth preparation

The various strains of microorganisms were procured from National Chemical Laboratory, Pune and were inoculated in sterile nutrient broth (about 100ml). This medium was incubated at 37°C ± 1°C for 24 hours and termed as seeded broth.

#### (b) Standardization of seeded broth (viable count):

**(i) Dilution:** In 99 ml of sterile water containing 0.05% Tween 80, 1ml of seeded broth is added. From this 1ml was taken and diluted to 10ml with sterile water and seeded broth is further diluted up to 10<sup>-10</sup> dilution.

**(ii) Inoculation into nutrient agar petridishes:** 0.2ml of seeded broth dilutions were inoculated into solidified nutrient agar medium by spread plate method. Number of colonies of microorganisms formed after incubation at 37°C ± 1°C for 24 hours. The seeded broth was suitably diluted to contain 10<sup>6</sup>-10<sup>7</sup> colony forming unit/ml (cfu/ml). It is the working stock and used for microbiological evaluation.

### Preparation of test compound solution

The standard and test compound (root and callus extract) solution were prepared at the concentration of 10 mg/ml and 5 mg/ml respectively in dimethyl sulphoxide (DMSO). Standard drugs used in study were ampicillin trihydrate for bacterial assay prepared at concentration of 1 mg/ml in DMSO.

### Screening of antimicrobial activity

The seeded broth 0.2 ml containing 10<sup>6</sup>-10<sup>7</sup> cfu/ml of the test organism was inoculated on solidified agar plate with the help of micropipette and spreaded. Two or three

wells or cavity were made in agar layer of each petridish by a steel borer. To these cavities standard and test compound solution were added. All the work was carried out strictly under aseptic conditions for bacterial assay. The plates for bacterial assay were incubated at 37°C ± 1°C for 18 hours. The antimicrobial potential of test compound was determined on the basis of diameter of zone of inhibition around the wells. As appreciable results in form of significant zone of inhibition was observed so minimum inhibitory concentration of various test compounds is also screened<sup>11,12,13</sup>.

### Screening of minimum inhibitory concentration (MIC)

MIC of extracts was determined using turbidity method in nutrient broth medium. The experiment was conducted according to serial dilution method. The suspension of seeded broth was made by transferring the 2 ml of the seeded broth to the 100 ml of the 0.9% w/v of the sterilized saline solution. The stock solution of test compounds were prepared at concentration of 10 mg/ml in nutrient broth and serially diluted to the 5 assay test tubes (containing 1 ml nutrient broth) to give concentration of 5, 2.5, 1.25, 0.625 and 0.3125 mg/ml. 0.1 ml of the normal saline suspension is added to each assay tube. The procedures were conducted under strict aseptic conditions. The inoculated tubes were kept at 37°C ± 1°C for 24 hours for bacterial assay. After incubation period, tubes were removed and observed for any deposits and shaken to suspend bacteria that might have been settle down. MIC values were determined by checking for the absence of visual turbidity<sup>14</sup>.

### RESULTS AND DISCUSSION

A massive, light creamish brown and granular callus formed in 90 percent cultures in MS medium supplemented with NAA (1.5 ppm) and Kinetin (0.25 ppm) (Table 1), (Figure 1,2).

The ethanolic extracts of roots and callus of *Plumbago zeylanica* Linn were screened for their antimicrobial activity against different strains of bacteria and it was found that the root extract show zone of inhibition against all microorganism whereas callus extract show maximum zone of inhibition against the *S. aureus* and *M. luteus*. The diameter of zone of inhibition for the ethanolic extracts of root and callus was shown in Table 2, figure (3-7).

**Table 1:** Effect of hormones (alone and in combination) on callus induction

Batch No.	NAA (ppm)	Kinetin (ppm)	Callus initiation time (days)	Explant showing callusing (%)	Nature of callus
1	0.25	-	-	-	-
2	0.50	0.05	09-12	30	GY, G
3	1.00	0.10	08-10	60	LCB, N
4	1.50	0.25	08-11	90	LCB, G, C
5	2.00	0.50	10-12	50	LCB, C

C- Compact; N- Nodular; GY- Greenish Yellow; LCB- Light Creamish Brown; G- Granular



**Table 2:** Data showing the diameter of the zone of inhibition for the Ethanol extract of root and callus against various microorganisms

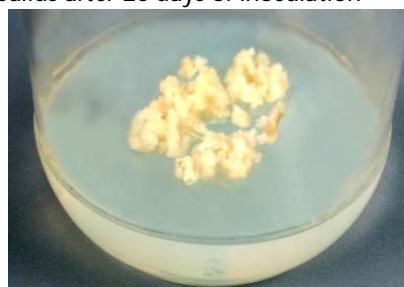
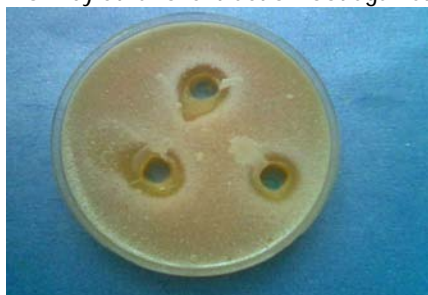
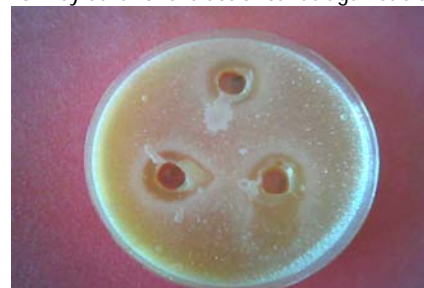
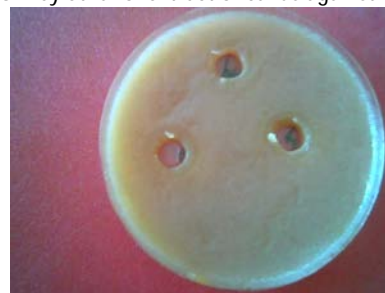
Sr. No.	Name of microorganism	Diameter of zone of inhibition (mm)					
		Ethanol extract (Roots)		Ethanol extract (callus)		Ampicillin Trihydrate	DMSO
		5 mg/ml	10 mg/ml	5 mg/ml	10 mg/ml		
1.	<i>S. aureus</i>	12	15	10	12	30	–
2.	<i>B. subtilis</i>	09	13	03	05	34	–
3.	<i>M. luteus</i>	11	14	07	09	25	–
4.	<i>E. coli</i>	10	12	04	06	22	–

*S. aureus* – *Staphylococcus aureus*; *B. subtilis* – *Bacillus subtilis*; *M. luteus* – *Micrococcus luteus* ; *E. coli* – *Escherichia coli*.

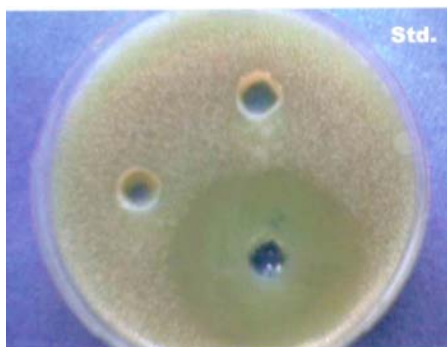
**Table 3:** Data showing the minimum inhibitory concentration for the ethanol extract of root and callus against various microorganisms

Sr. No.	Name of the microorganism	Minimum Inhibitory Concentration ( MIC ) ( $\mu\text{g/ml}$ )			
		Ethanol extract (Roots)	Ethanol extract (callus)	Ampicillin trihydrate	DMSO
1.	<i>S. aureus</i>	1250	5000	312.5	–
2.	<i>B. subtilis</i>	5000	10000	312.5	–
3.	<i>M. luteus</i>	2500	5000	312.5	–
4.	<i>E. coli</i>	10000	10000	312.5	–

*S. aureus* – *Staphylococcus aureus*; *B. subtilis* – *Bacillus subtilis*; *M. luteus* – *Micrococcus luteus* ; *E. coli* – *Escherichia coli*.

**Figure 1:** Callus after 08 days of inoculation**Figure 2:** Callus after 25 days of inoculation**Figure 3:** Z.O.I. by ethanol extract of root against *S. aureus***Figure 4:** Z.O.I. by ethanol extract of root against *M. luteus***Figure 5:** Z.O.I. by ethanol extract of callus against *S. aureus***Figure 6:** Z.O.I. by ethanol extract of callus against *M. luteus*

**Figure 7:** Z.O.I. by std. drug against *S.aureus* and no zone by DMSO



The MIC of root extract against *S. aureus* and *M. luteus* is 1250 and 2500 µg/ml. whereas the MIC of callus extract against these microorganisms is 5000 µg/ml (Table 3).

The naphthoquinones are reported to possess the anti microbial activity<sup>15</sup> and roots of *Plumbago zeylanica* also reported to possess the naphthoquinones<sup>16</sup> which is responsible for the anti microbial activity of roots. From the present study it was revealed that callus grown from the nodal explants of *Plumbago zeylanica* show the significant anti microbial activity against certain microorganism which might be due to the naphthoquinones present in the callus extract which need to be further analysed.

In conclusion, a massive light creamish brown and granular callus formed with MS medium supplemented with naphthalene acetic acid (1.5 ppm) and kinetin (0.25 ppm) and it possess a significant anti microbial activity against the test microorganism which might be due to the naphthoquinones present in the callus.

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