In vitro root induction and studies on antibacterial activity of root extract of *Costus igneus* on clinically important human pathogens

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**ABSTRACT**

In vitro root induction and its antibacterial activity of Costus igneus was conducted. The plant *C. igneus* was well known for its anti-diabetic property. Traditionally the plant was used to treat fever, rash, asthma and intestinal worms. Two growth regulator IAA (Indole 3-acetic acid) and IBA (Indole butyric acid) in combinations were applied to MS medium for direct root induction. MS medium supplemented with 0.5mg/ml IBA, induced maximum amount of root culture (fresh weight was 1.566 g and dry weight 0.102 g) after 30 days. In vitro raised roots were subjected to its antimicrobial activity. Acetone, chloroform and methanol were used as solvents to extract plant materials from IBA and IAA derived roots and the extracts were subjected to antimicrobial activity against four gram negative bacteria namely *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella sp*, *Proteus vulgaris*. In which *Klebsiella pneumonia* was found to be most susceptible to both IBA and IAA derived roots. Whose zone of clearance was found to be 25 mm, which was almost equal to that of commercially available antibiotic Gentamycin.

**Keywords**: *Costus igneus*, *in vitro*, direct root, antimicrobial activity.

**INTRODUCTION**

Innumerable biologically active compounds that are found in plants [1, 4, 15] possess antibacterial properties [3, 14]. Plant produced compounds are of interest as sources of safer or more effective substitutes for synthetically produced antimicrobial agents [2]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world’s population [5].

*Costus igneus* (*C. igneus*) commonly known as insulin plant in India, belongs to the *Costaceae* family. The whole plant *C. igneus* were used for it anti-diabetic property and prevents the body from disease, protects mind and which prolongs the longevity of life. The rhizome has been used...
to treat fever, rash, asthma, bronchitis, intestinal worms, ailments of eyes, stomach, neck, jaws, tongue, mouth and also be used for curing fever, edema, wheezing (dyspnoea), haemorrhoids, spermaturia. In siddha medicine system *C. igneus* root has been used as in the form of powder (chooranam), decoction (kudineer) and oil (thylam). Until now, *C. igneus* has been reported to contain resinoids, essential oil, and alkaloid named saussurine, inulin and resin [6].

Adventitious root culture is the unique technique which renders the secondary metabolites in huge amount and it fulfils the global demand in field of medicine, agriculture, drug production, pigment production, dye production and so on. Root cultures can be used in many ways including studies of carbohydrate metabolism, mineral nutrients requirements, essential need for of vitamins, growth regulators, differentiation of the root apex and gravitropism. The advantage of using root cultures is that they grow rapidly, relatively easy to prepare and maintain, show a low level of variability and can be easily cloned to produce a large supply of experimental tissues. However, there is no previous report for the adventitious root culture for *C. igneus*. Hence the present study is aimed for the induction and culture of adventitious roots of *C. igneus* and the analysis of the induced *C. igneus* root for its antimicrobial activity in different microorganisms.

**MATERIALS AND METHODS**

**2.1 Mother plant**
The explants used for this study were collected from Dates India Nursery, Trichy and then transferred to Bharathidasan University shade house. The Mother plant were grown in plastic pots which contain 2 part perlite and 1 part peat. The plant materials were watered twice a day using sprinkler system to maintain the moisture condition of the soil. The explants were collected from six months old, healthy and diseases free plant. Juvenile leaf segments were used as explants.

**2.2 Preparation of culture medium**
The basal medium for the culture of *C. igneus* was MS medium enriched with 3% (w/v) sucrose and 0.2% (w/v) phytagel. Before autoclaving at 121°C for 15 minutes the pH of MS medium was adjusted to 5.8 by adding 0.1 N NaOH (Sodium hydroxide) or HCl (Hydrochloric acid). For induction of adventitious root, growth regulators were supplemented with the culture medium.

Analytical chemicals and growth regulators were used for the preparation of stock solutions of different media. Salts, growth regulators and gelling agent (Agar, Phytagel) for tissue culture were purchased from Sigma, St. Louis, USA. Tissue culture grade sucrose (SRL) was used as carbohydrate source.

**2.3 Surface sterilization protocols**
Explants were collected from healthy and actively growing mother plant (Shade house, Bharathidasan University). Young leaf segments were used as explants. The explants were rinsed under running tap water for 10 minutes to remove visual contaminants. Then the explants were subjected to ethanol, sodium hypochlorite and mercuric chloride treatments to obtain aseptic culture. In treatment 1, the explants rinsed with 70% ethanol for 0.5 minutes followed by 3% sodium hypochlorite for 6 minutes. Whereas in treatment 2, the explants were treated with 70% ethanol for 0.5 minutes and followed by 3% sodium hypochlorite for 1 minutes and 0.1% mercuric chloride for 3 minutes. In treatment 3, explants were rinsed in 70% ethanol for 1.5 minutes and followed by 3% sodium chloride for 1.5 minutes and 0.1% mercuric chloride for 4 minutes. Following each treatments explants were washed with sterile distilled water. All the 3
treatments were repeated thrice (3 replicates, with 30 explants / replicate). The surface sterilized explants were inoculated aseptically.

2.4 Culturing of explants
The edges of surface sterilized explants were trimmed and dried in sterile tissue paper before transferring onto the culture medium. The explants were separately inoculated in culture tubes containing 10ml MS medium to avoid cross contamination. The tubes were capped and sealed with parafilm to avoid contamination risk.

The cultures were observed daily to identify any contamination, growth of explants and mortality of explants. Contamination-free cultures were used for sub-culturing on medium containing growth hormones (auxins). Sub culturing was done every two weeks onto the same medium composition. The growth and incidence of adventitious root was noted after 30 days of culturing. All cultures were incubated in a culture room under dark condition.

2.5 Adventitious Root Culture
The sterilized explants (0.5 – 1 cm) were inoculated on MS solid medium containing 3.0 % (w/v) sucrose, 0.2 % phytagel (w/v) along with various concentration of auxin such as IAA and IBA.

2.5.1 Effect of IBA and IAA on Adventitious root culture
The explants were inoculated on various concentrations of auxin such as IAA and IBA in the range of (0.05 – 2.0 mg/L) and incubated under total darkness at 24°C±2 °C for adventitious root formation. The cultures were monitored at 7 days interval and results were assessed at the end of 30th day. After 30 days of inoculation the roots produced from the cut end of the leaves, were separated from the explants and the growth in different auxin regimes was assessed in terms of fresh and dry weight. Fresh weight of roots was determined by blotting the harvested root on filter paper after the gentle wash in distilled water. Dry weight was obtained after drying them at room temperature for 48hrs. The roots were powdered using mortar and pestle and about 100mg of root powder was used for studying its antimicrobial activity.

2.6 Antibacterial Activity
2.6.1 Materials and media preparation
Gram negative Bacterial cultures such as *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumonia* (*K. pneumonia*), *Salmonella sp*, *Proteus vulgaris* (*P. vulgaris*) were used in the present study to determine the antibacterial activity of the *C. igneus*, *in vitro* raised root extracts. Above mentioned bacterial strains are laboratory stains. Nutrient Agar medium is one of the most commonly used medium for several route lines of bacteriological purposes.

2.6.2 Root sample preparation
The shade dried and powdered IBA and IAA derived root materials were used for the sample preparation. About 10 grams of the IBA and IAA derived root materials was extracted with 5ml of acetone, chloroform and methanol using soxhlet apparatus at 800°C. Further, the solvent was evaporated using a rotary vacuum evaporator. The residues were dissolved with dimethyl sulfoxide (DMSO) and used for antimicrobial activity.

2.6.3 Preparation of inoculum
About 100 µl of overnight culture of *P. aeruginosa*, *K. pneumoniae*, *Salmonella spp*, *P. vulgaris* were taken. These cultures were spread plated over the petriplates containing agar medium.
2.6.4 Disc diffusion method
The anti-bacterial activities of the test samples were carried out by disc diffusion method. Size of disc in the present study is 0.5 mm. *Gentamycin* were used as positive control and the concentration of each disc was 10 µg. Two different concentrations of samples (40 and 60 µg/ml) of both IBA and IAA derived roots extracts were loaded on discs (Prepared from whatman filter paper No.1) and placed on the plates. Solvent (DMSO) is maintained as negative control. Later plates were incubated at 37°C for 24 hours. Results were later observed as inhibition zones and were expressed in millimetres.

RESULTS

3.1 Sterilization of explants
The sterilization results of the leaf explants using 3 different treatments are presented in Table 1. Treatment 3 was found to be the best; it reduces the contamination level up to 10%.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sterilants and concentration</th>
<th>Duration (Min)</th>
<th>% of Contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70% Ethanol, 3% NaOCl</td>
<td>0.5, 6.0</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>70% Ethanol, 3% NaOCl, 0.1% HgCl⁻</td>
<td>0.5, 1.0, 3.0</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>70% Ethanol, 3% NaOCl, 0.1% HgCl⁻</td>
<td>1.5, 1.5, 4.0</td>
<td>10</td>
</tr>
</tbody>
</table>

(Note: Each treatment consisted of 2 replicates with 30 explants in each)

3.2 Adventitious root culture
3.2.1 Nature of Explants
In the present study well established normal roots were produced from matured leaf explants in all the concentrations of IBA and IAA after three weeks. The leaf exhibited higher response for root induction when supplemented with different concentration of IBA, when compared to IAA.

3.2.2 Effect of exogenous auxins on root production
3.2.2.1 IAA
In medium supplemented with IAA growth hormone, roots initiated in the medium after 10 days. Of various concentrations tested (0.1-2.0 mg/l), IAA at 0.1 mg/l was proven to be good. The mean fresh weight and dry weight are 1.200 and 0.117 g respectively (Table 2).

<table>
<thead>
<tr>
<th>S.No</th>
<th>IAA mg/l</th>
<th>Response (%)</th>
<th>Fresh weight of roots (g)</th>
<th>Dry weight of roots (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.05</td>
<td>31.25</td>
<td>0.291</td>
<td>0.025</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td><strong>66.25</strong></td>
<td><strong>1.200</strong></td>
<td><strong>0.117</strong></td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>40.00</td>
<td>0.817</td>
<td>0.083</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>28.75</td>
<td>0.437</td>
<td>0.042</td>
</tr>
<tr>
<td>5</td>
<td>1.5</td>
<td>21.25</td>
<td>0.290</td>
<td>0.027</td>
</tr>
<tr>
<td>6</td>
<td>2.0</td>
<td>11.25</td>
<td>0.177</td>
<td>0.017</td>
</tr>
</tbody>
</table>

(Note: Each treatment consisted of 4 replicates with 30 explants in each; Response %: Mean value of all the 4 replicates)

3.2.2.2 IBA
When compared to IAA, IBA was effective in producing adventitious roots. The roots initiated in the medium after 10 days of inoculation (Figure 1). IBA at 0.5 mg/l was found to be best with mean fresh weight and dry weight of 1.566 and 0.102 g respectively (Table 3).

Available online at www.scholarsresearchlibrary.com
Table 3: Effect of IBA on induction of adventitious root from *C. igneus*

<table>
<thead>
<tr>
<th>S.No</th>
<th>IBA mg/l</th>
<th>Response (%)</th>
<th>Fresh weight of roots (g)</th>
<th>Dry weight of roots (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.05</td>
<td>31.25</td>
<td>0.296</td>
<td>0.025</td>
</tr>
<tr>
<td>2.</td>
<td>0.1</td>
<td>48.75</td>
<td>0.973</td>
<td>0.094</td>
</tr>
<tr>
<td>3.</td>
<td>0.5</td>
<td>76.25</td>
<td>1.566</td>
<td><strong>0.102</strong></td>
</tr>
<tr>
<td>4.</td>
<td>1.0</td>
<td>47.50</td>
<td>0.983</td>
<td>0.096</td>
</tr>
<tr>
<td>5.</td>
<td>1.5</td>
<td>28.75</td>
<td>0.463</td>
<td>0.045</td>
</tr>
<tr>
<td>6.</td>
<td>2.0</td>
<td>21.25</td>
<td>0.263</td>
<td>0.026</td>
</tr>
</tbody>
</table>

(Note: Each treatment consisted of 4 replicates with 30 explants in each; Response %: Mean value of all the 4 replicates)

**Figure 1**

*Figure 1: A. Field grown mother plant; B. Explants were surface sterilized and inoculated in MS Medium containing growth hormone; C. Observation of root initiation after 20 days of incubation.*
3.3 Antibacterial activity

3.3.1 Pseudomonas aeruginosa
Of different root extracts used, the best result was observed in root extracted with chloroform (Figure 2). The maximum zone of inhibition was about 17 mm (IBA derived root). Zone of clearance in positive control is 25 mm (Table 4).

3.3.2 Klebsiella pneumonia
The extract of roots initiated on IBA and IAA using acetone as solvent recorded the maximum result. The zone of inhibition was about 25 mm (Figure 3). It was as efficient as that of positive control (Table 4).

3.3.3 Proteus vulgaris
Extract of IBA derived root using chloroform as solvent showed maximum zone of inhibition. The highest zone of inhibition was about 13 mm (Figure 2). The antibiotic failed to control the growth of *P. Vulgaris* (Table 4).

3.3.4 Salmonella sp
Acetone derived plant extract of both IBA and IAA derived roots recorded the best of about 20 mm zone of inhibition respectively (Figure 3). In this the antibiotics didn’t showed any effect on *Salmonella sp*.

The plant extract derived from both the IBA and IAA induced roots showed the significant results against all the four gram negative bacteria (Table 4).

<table>
<thead>
<tr>
<th>Microbes</th>
<th>Solvents</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IAA 40µl</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>A Chloroform</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>B Acetone</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>C Methanol</td>
<td>4</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>A Chloroform</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>B Acetone</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>C Methanol</td>
<td>11</td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td>A Chloroform</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>B Acetone</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>C Methanol</td>
<td>5</td>
</tr>
<tr>
<td><em>Salmonella sp</em></td>
<td>A Chloroform</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>B Acetone</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>C Methanol</td>
<td>7</td>
</tr>
</tbody>
</table>
DISCUSSION

Plant tissue culture work requires strict maintenance of asepsis in all the operations. In the present study, among three types of treatments, treatment 1 & 2 were found to have 100% and
40% percentage fungal contaminations in the cultured vessels respectively while treatment 3 (70% alcohol- 1.5 minutes, 3% sodium hypochlorite- 1.5 minutes, 0.1% mercuric chloride 4 minutes) was found to have only 10% of contamination in the cultured vessels. Hence, it was found to be the best when compare to others and chosen for further studies.

Adventitious roots induced by in vitro methods showed high rate of proliferation and active secondary metabolism [8, 19]. Adventitious roots are natural, grow vigorously in phytohormone supplemented medium and have shown tremendous potentialities of accumulation of valuable secondary metabolites.

In the system described here young leaf explants exhibited good response for root induction. This result goes in accordance with the results of Nandagopal [13] in Cichorium intybus (L.) cv. Focus, [16] in Decalepis arayalpathra Venter. As [16] reported, the incubation of the explants under total darkness showed good result.

Previous reports revealed that supplementation of exogenous (IAA and IBA) auxin to the medium resulted in adventitious roots formation [10, 17]. In our study, IAA at 0.1mg/l, IBA at 0.5 mg/l responded well in adventitious root culture formation.

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world population. There are about 45,000 plant species in India with capacity to produce a large number or organic chemicals concentrated hotspot in the region of Eastern Himalayas, of high structural diversity [18, 11]. In the present work different extract of C. igneus showed higher activity to P. aeruginosa, K. pneumonia, Salmonella sp, P. vulgaris. The results confirmed that antimicrobial potential in plant material (root and stem) shows more potential against the Klebsiella pneumonia as the zone of inhibition is more when compare to other organism tested. Gothandam [7] established the antimicrobial activity of C. igneus with good zone of inhibition in all the organisms tested. The zone of inhibition of the present study goes in accordance with the results of Gothandam [7]. However, the extracts of the present study were prepared from the IBA and IAA induced roots.

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials [9]. Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy.

However, the present study of in vitro antimicrobial evaluation of the IBA and IAA induced roots of C. igneus forms a primary platform for further phytochemical and pharmacological studies. In the present study, we have found that IBA and IAA induced roots of C. igneus possess anti microbial activity which may be due to the presence of phytochemicals. Further studies are required to isolate the active components from the IBA and IAA induced roots of C. igneus.
Acknowledgement
I would like to sincerely thank Dr. A. Ganapathi, Head of the Department, Department of Biotechnology, Bharathidasan University, Trichy for allowing me to work in his reputed laboratory.

REFERENCES