Investigation of Plumbagin Content in *Plumbago indica* Linn. Grown under Different Growing Systems

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Abstract: Background: *Plumbago indica* Linn. (Family: Plumbaginaceae) is a shrubby perennial herb native to the South Asia and at present it is widely cultivated throughout India and Sri Lanka. Plumbagin (5-hydroxy-2-methyl-1,4-napthoquinone) is a therapeutically important natural napthoquinone occurs mainly in the roots of *P. indica*. Materials and Methods: Compared the chemical profile (in terms of qualitative analysis of phytochemicals and TLC fingerprints) and quantified the plumbagin content in *P. indica* grown under different growing systems: using roots of (a) conventionally field grown, (b) tissue cultured field grown, (c) hydroponically grown plants and (d) in vitro developed callus from leaf explants. Results: The maximum plumbagin content was observed in roots of tissue cultured field grown plants followed by roots of conventionally field grown hydroponically grown plants and callus samples. Conclusion: Among the different growing systems of *P. indica* maximum plumbagin content was observed in roots of tissue cultured field grown plants.

Key words: *Plumbago indica* Linn, plumbagin, growing techniques, phytochemicals

INTRODUCTION

*Plumbago indica* Linn. (Family: Plumbaginaceae) is a shrubby perennial herb, native to the South Asia and at present it is widely cultivated throughout India and Sri Lanka. The regional names of *P. indica* is called in Sinhala as Ratnetol or Ratnitul, in Tamil as Akkini, in Hindi as Chitra (Jayaweera, 1982). *P. indica* is heavily used in Sri Lankan traditional systems of medicine for the preparation of formulations used to treat a variety of disease conditions. The juice of the leaves and roots mixed with oil is employed as an application for rheumatism paralysis and leprosy (Jayaweera, 1982). The roots are used in dyspepsia, colic, inflammations, cough bronchitis helminthiasis, haemorrhoids, elephantiasis chronic intermittent fever, ring worm, hepatosplenomegaly amencorrhoea, odontalgia, anaemia, skin diseases diarrhoea, piles anascarca and as an abortifacient (Satyavathi et al., 1987).

Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these chemically active constituents of plants are flavonoids tannins alkaloids saponins and phenolic compounds (Chandur et al., 2011). Plumbagin (5-hydroxy-2-methyl-1,4-napthoquinone) is a therapeutically important natural napthoquinone, occurs mainly in the roots of *P. indica* (Jayaweera, 1982). Studies on the pharmacological activities of plumbagin have shown the presence of antitumor (Hazra et al., 2008) antimicrobial (Didry et al., 1994, 1998) anticancer (Kuo et al., 2006) antifertility (Phargava, 1984) antileishmanial (Chan-Baab and Pena-Rodriguez, 2001) and antiallergic (Dai et al., 2004) properties. Therefore, *P. indica* has increased market demand in both domestic and international level.

The slow growth rate absence of seeds and lack of fruiting stage of the *P. indica* in traditional agricultural methods necessitate the search for an alternative and effective source to meet with enhanced commercial demand (Gangopadhyay et al., 2011). According to Gontier et al. (2002), hydroponic culture technique also can be used to produce medicinal plants in large scale.

The aim of the present investigation was to compare the chemical profile and quantify the plumbagin content in *P. indica* grown under different growing systems using roots of (a) conventionally field grown, (b) tissue cultured field grown, (c) hydroponically grown plants and (d) in vitro developed callus from leaf explants.

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MATERIALS AND METHODS

Conventionally field grown plants: Healthy shoots of *P. indica* were cut and immersed in a bucket of water. The cuttings were separated into single nodes from shoot tips. Then the cuttings were established in polythene bag (5”×7”) filled with soil: compost: coir dust in a ratio of 1:1:1. Watering was done daily. After 6 weeks when the new shoots were immerged they were transferred into the field plots. These plants were maintained for 1 year.

Tissue cultured field grown plants: Tissue cultured *P. indica* were established in polythene bag (5”×7”) filled with coir dust: sand (1:1) potting mixture. Then they were transplanted into pots filled with soil: coir dust: compost in a ratio of 1:1:1 and maintained under green house for 1 year.

Hydroponically grown plants: Healthy shoots of *P. indica* were cut and immersed in a bucket of water. The cuttings were separated into single nodes from shoot tips. Then the cuttings were established in polythene bag (5”×7”) filled with soil: compost: coir dust (1:1:1). Watering was done daily. After 6 weeks, when the new shoots were immerged, they were transferred into the hydroponic system as follows. *P. indica* were transplanted in pots filled with coir dust and transferred to the regrowth box (18”×15”×8”) containing Albert solution.

Preparation of albert solution: Albert solution was prepared by adding 10 g of mineral elements and 25 g of Ca(NO₃)₂ to the 20 mL of water. Albert solution was replaced in every two months. Hydroponically grown plants were maintained under green house condition for 1 year.

Induction of callus: The leaf explants of well grown *P. indica* (age: five months) were collected from hydroponically grown plants. They were washed with running tap water for 1 h. Subsequently explants were cleaned with 0.2% (v/v) aqueous “Teepol” to remove the dirt. Then it was treated with 1% (w/v) fungicide solution (Antracol) for 1½ h. Then, explants were washed with distilled water for five repeated times. After that, explants were dipped in Clorox solution 10% (v/v) prepared under aseptic conditions and these were washed with sterilized double distilled water for five repeated times. Then sterilized explants were cut into appropriate size (1×1 cm) and inoculated on Murashige and Skoog (MS) supplemented with optimized concentration of 2 mg L⁻¹ Benzyl Adenine (BA) and 3 mg L⁻¹ Indole 3-Butyric Acid (IBA).

Sample preparation for chemical analysis: Root samples of *P. indica* (age: 1 year) were collected from the plants maintained under different growing systems: (a) conventionally field grown, (b) tissue cultured field grown (c) hydroponically grown plants and (d) *in vitro* developed callus from leaf explants (age: 6 weeks) at premises of Industrial Technology Institute. Samples were cut into pieces and air dried for 3 days at room temperature (28±2°C) and subsequently in oven at 50°C for 4 h. Two grams of each sample was refluxed with methanol (50 mL) separately for 1 h. The extracts were filtered separately and filtrates were concentrated under reduced pressure at 50°C using a rotavapour. The dried extracts were dissolved in methanol (10 mL) and stored at 4°C until use. This experiment was done in triplicate.

Phytochemical screening studies of *Plumbago indica* grown under different growing systems: roots of (a) conventionally field grown (b) tissue cultured field grown and (c) hydroponically grown plants and (d) callus samples: The phytochemical screening tests were performed according to methods described by Farnsworth (1966) with some modifications. Test for tannins: Few drops of 0.1% ferric chloride was added to the methanolic extract (2 mL) and observed for brownish green or a blue-black colouration.

Test for saponins: The combined froth and emulsion test was used to test for presence of saponin in the sample. Methanolic extract (10 mL) was mixed with distilled water (5 mL) and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion.

Test for steroid glycosides: Extract was dissolved in equal volumes of acetic anhydride and CHCl₃. The mixture was transferred to a dry test tube and con. H₂SO₄ acid was introduced to the bottom of the tube. Formation of a reddish brown or violet-brown ring at the interface of the two liquids indicate the presence of steroids. Test for flavonoids: A drop of bench ammonia solution was added to 3 mL of the methanolic extract of the sample in a test tube. The formation of yellow coloration which clears on the addition of concentrated acid solution was taken as a positive result for the test.

Test for Alkaloids: Alkaloids form a white precipitate or turbidity with Mayer’s reagent. 1 mL portions of each extract was acidified with 2-3 drops of 1M HCl and treated with 4-5 drops of Mayer’s reagent.
Comparison of Thin Layer Chromatography (TLC) fingerprints of Plumbago indica grown under different growing systems: roots of (a) conventionally field grown (b) tissue cultured field grown and (c) hydroponically grown plants and (d) callus samples: The silica gel GF254 adsorbent was used in a layer thickness of 0.25 mm. The running distance of the solvent front was 8 cm. Dried methanolic extracts were dissolved separately in 10 mL of methanol and 8 μL of each extract was spotted on a precoated silica gel GF254 TLC plate and air dried. The mobile phase consists of methanol: ethyl acetate: cyclohexane (0.1: 3.9: 1). After air drying the TLC plate detection was done under UV light at 254 nm. Vanillin-Sulfuric acid was sprayed to the TLC plate and heated at 105 °C for 5 min.

Comparison of plumbagin content in Plumbago indica grown under different growing systems: roots of (a) conventionally field grown (b) tissue cultured field grown and (c) hydroponically grown plants and (d) callus samples:

- **Test solutions:** Test solutions were prepared separately by dissolving dried methanolic extracts of *P. indica* grown under different growing systems in 10 mL of methanol
- **Standard solution:** Standard solution was prepared by dissolving 10 mg of plumbagin in 10 mL of acetone
- **Calibration curve and quantification of plumbagin content:** The calibration curve for plumbagin was drawn with 6 data points. Different volumes of the standard solution (5, 10, 15, 20, 25, 30 μL) and test solutions (10 μL from each) were applied on the pre-coated TLC plate of uniform thickness of 0.25 mm. The plate was developed in the solvent system consisting of dichloromethane and hexane in a ratio of 1:1 to a distance of 80 mm. The plate was scanned densitometrically (CS-9301PC, Shimadzu, Japan) at 254 nm. The peak area under the curve was recorded and the calibration curve for plumbagin was established (callus samples). Among the tested samples, maximum plumbagin content was present in the roots of tissue cultured field grown plants. Further, plumbagin content was decreased plotted. By using the calibration curve plumbagin content was determined in the above samples.

**Statistical analysis:** Data are given as Mean±SEM Statistical comparisons were made using one way ANOVA followed by Duncan's Multiple Range Test. A p-value < 0.05 was considered as significant.

**RESULTS AND DISCUSSION**

The secondary metabolites are responsible for the therapeutic properties of plants and the composition of these secondary metabolites varies from plant species to species. The composition of these compounds with the same species of plant can vary with the nutrient composition of the soil climatic season development stage of the plant natural association with other plants (Menkovic et al., 2000). Therefore, in this study an attempt was taken to evaluate the phytochemicals present in *P. indica* roots and callus samples. Phytochemical evaluation on methanolic extracts of *P. indica* roots (taken from conventionally field grown, tissue cultured field grown and hydroponically grown plants) and callus revealed the presence of tannins, steroid glycosides, flavonoids and alkaloids. Further, above extracts did not show the presence of saponins. However, Ajayi et al. (2011) have reported the presence of saponins in ethanolic extract of *P. zeylanica* roots which is a close relative of *P. indica*. TLC fingerprint profiles were developed for roots and callus samples of *P. indica* and phytochemical constituents were compared in terms of Rf values. Four prominent spots (Rf 0.16, 0.35, 0.55, 0.93) were observed in conventionally field grown and tissue cultured field grown *P. indica* root samples. An additional spot (Rf 0.73) observed in hydroponically grown *P. indica* root samples while callus samples consist of only 3 spots (Rf 0.16, 0.55, 0.93). There was no significant differences in chemical constituents of *P. indica* grown under different growing systems. However, the intensity of the spots present in the callus samples were lower than other tested samples.

Quantitative estimation of plumbagin showed a significance (p<0.05) difference in root samples of *P. indica* grown under different growing systems.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Conventionally field grown sample</th>
<th>Tissue cultured field grown sample</th>
<th>Hydroponically grown sample</th>
<th>Callus samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Steroid glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
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<td>Alkaloids</td>
<td>+</td>
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</table>

*: Presence of phytochemical constituent; -: Absence of phytochemical constituent.
Table 2: Plumbagin content in *Plumbago indica* grown under different growing systems: using roots of conventionally field grown, tissue cultured field grown, hydroponically grown and callus callus samples (Mean±SEM, n = 3)

<table>
<thead>
<tr>
<th>Growing systems</th>
<th>Plumbagin content (g of plumbagin in 100 of raw material, dry wt basis)</th>
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<tbody>
<tr>
<td>conventionally field grown</td>
<td>1.33±0.15</td>
</tr>
<tr>
<td>Tissue cultured field grown</td>
<td>1.80±0.25</td>
</tr>
<tr>
<td>Hydroponically grown</td>
<td>1.08±0.01</td>
</tr>
<tr>
<td>Callus samples</td>
<td>0.25±0.02</td>
</tr>
</tbody>
</table>

The values marked with the different letters are significantly (p<0.05) different with each other.

(Conventionally field grown, tissue cultured field grown, hydroponically grown) and in vitro developed callus from leaf explants. Among the tested samples maximum plumbagin content was present in the roots of tissue cultured field grown plants. Further, plumbagin content was decreased in the following order: conventionally field grown > hydroponically grown > callus samples (Table 1). Plumbagin is mainly accumulated in the roots of *P. indica* (Evans, 1996). The callus cultures were established using the leaf explants of hydroponically grown *P. indica*, because the survival rate is very low in the root explants due to high contamination and slow growth (Panichayupakaranant and Tewtrakul, 2002). This may be the reason for the minimum plumbagin content observed in the callus samples of *P. indica*. Though the plumbagin content in the roots of hydroponically grown *P. indica* was not as much as roots of tissue cultured field grown plants, it also has considerable amount of plumbagin. Therefore, as an alternative growing technique for the conventionally field grown plants, hydroponics can be used.

**CONCLUSION**

Among the different growing systems of *P. indica* maximum plumbagin content was observed in roots of tissue cultured field grown plants.

**REFERENCES**


