MICROPROPAGATION AND PHYTOCHEMICAL ANALYSIS OF *SALACIA CHINENSIS L*. AN ENDANGERED MEDICINAL PLANT

Abstract

*Salacia chinensis L.*is an endangered medicinal plant of Indian subcontinent mainly used to treat diabetes. Endangered nature of *Salacia chinensis L.* is due to over exploitation for its medicinal uses and poor seed germination. Therefore, it is important to propagate the plants using tissue culture technique. The plants propagated successfully by using micropropagation technique and nodal explants were used in this technique with different combination and concentrations of plant growth hormones but maximum shooting is observed when MS media provided with3mg/l BAP and 1.5mg/l IBA. Successful rooting was observed in MS media supplemented with 1.5mg/l IBA. Qualitative phytochemical analysis also conducted to study the presence of different phytochemical constituents. These studies were useful for conservation of endangered medicinal plant*Salacia chinensisL.*

Keywords: *Salacia ChinensisL.*, micropropagation, shooting, rooting, phytochemical constituents.

Author

P Manjula
Department of Botany and Food & Nutrition
R.B.V.R.R Women's College
Hyderabad, Telangana, India

I. INTRODUCTION

Salacia chinensis, belonging to the Celastraceae family, is a woody climbing plant native to submontane forests in Sri Lanka and India. In the ayurvedic system of traditional Indian medicine, the roots and stems of this plant have been widely employed for the management of diabetes. Additionally, other plant species within the Salacia genus, such as S. prinoides and S. reticulata, have historically been utilized in ayurvedic medicine due to their recognized antidiabetic attributes.[1]

In India and various other countries, Salacia chinensis has been traditionally employed as a tonic, blood purifier, and for addressing conditions like amenorrhea and dysmenorrhea.[2] Its root bark is harnessed for managing conditions such as gonorrhea, rheumatism, and various skin ailments. Research has indicated significant hypoglycemic activity associated with its aqueous extract.

Rheumatism, gonorrhea, itching, asthma, thirst, and ear disorders can all be treated with root bark that has been heated in oil, made into a decoction, or ground into powder.[/3] For thousands of years, traditional treatments have employed several members of the genus, including S. chinensis, as dietary supplements to prevent diabetes and obesity [4]. Salacia spp., which include S. chinensis, have been used extensively in medicine to treat a wide range of illnesses, including bronchitis, fever, rheumatism, inflammation, leucorrhoea, and arthritis [4].

S. chinensis, sometimes referred to as Saptarangi or Saptachakra, is a significant underappreciated plant found throughout the Indian subcontinent, particularly in the Western Ghats' semi-evergreen forests. The edible pulp surrounding the fruit's seeds is delicious, transparent, and jelly-like. In obese human patients, an anti-obesity effect was reported with dietary intake of S. chinensis fruits [5,6].

It has been discovered that this plant's roots and leaves contain certain phytochemicals [5,6]. Numerous S. chinensis extracts have been shown to exhibit a range of biological properties, including as antibacterial, antidiabetic, and anti-obesity properties.One species of plant in the Celastraceae family is Salacia chinensis. In Ayurveda, it is also referred to as saptachakra and is a climbing shrub.extensively dispersed over the world's tropical and subtropical regions, particularly in China, India, and Sri Lanka. The plant's many parts have been widely used to treat a wide range of illnesses. 4, 5. Salacinol6,7, kotalanol, neokotalanol, neosalacinol, salaprinol3,8, mangiferin9, phenolic glycosides10,11, and triterpenes12,13 are among the biologically active substances that have been extracted from the plant. S. chinensis are classified as endangered species because they face serious threats23–25.

One of the greatest advances in biotechnology, plant tissue culture offers a wide range of useful applications, particularly for the bulk production of disease-free, healthy plants and the simple regeneration of rare plant species cultivated using conventional methods.26, 27,. The primary objectives of the current study were to establish an efficient regeneration method for the conservation and mass propagation of S. chinensis and to conduct a preliminary phytochemical investigation for the secondary metabolites.

II. MATERIALS AND METHODS

1. **Materials:** Plant material of *Salacia chinensis L.* was collected from Pocharam wildlife sanctuary, Medak district. The plant materials *Salacia chinensis* have been authenticated by the Department of Botany, Osmania University Hyderabad. The material was deposited in the Department of Botany R.B.V.R.R Women's College, Hyderabad for future reference.

2. ***In Vitro* Propagation Studies:** For the chosen plant species, a micropropagation protocol was created. First, teepol was used to wash the nodal transplants under running water from the faucet. Lastly, these were dipped in 100% alcohol after being surface sterilized for three to five minutes in a freshly made 0.1% (w/v) mercuric chloride solution under aseptic circumstances. After that, there was a five-minute wash with sterile distilled water. The 10-mm-long surface-sterilized explants were inoculated on MS medium (Murashige and Skoog, 1962) supplemented with BAP and IBA (0.5, 1.0, and 2.0 mg/L) at the same concentrations and in different combinations. The medium also contained 3% (w/v) sucrose and 0.8% (w/v) agar-agar.

   The cultures were incubated in a culture environment with 2000 lux of light intensity and a 16h:8h photoperiod at 25 ± 2ºC. For root genesis, the in vitro-regenerated shoots were cut off and implanted on full- and half-strength MS medium with or without growth regulators like IAA, NAA, and IBA at 0.5 and 1.0 mg/L. After removing the rooted plantlets from the medium, sterile distilled water was used to wash away any remaining agar residue. The plantlets were then moved to pots filled with a 3:1 mixture of sand and dirt.

3. **Preparation of Extracts for Phytochemical Analysis:** The collected leaf, stem and root samples were shade dried under room temperature for 7 days andthen milled into coarse powder by a mechanical grinder. The MeOH (Methanol) extract ofeach sample was prepared by soaking 10g dried powder samples in 100 ml of methanol for24 hrs. The extracts were filtered and evaporated under pressure. Powdered leaf, stem and root extracts were further subjected to phytochemical analysis for the presence of alkaloids,flavonoids, phenols, steroids, saponins, tannins and glycosides.

4. **Qualitative Phytochemical Analysis:** The qualitative phytochemical for identifying various constituents were performed by using methanolic extracts of stem,leaf and root of *Salacia chinensis*.

   - **Alkaloids:** The presence of alkaloids in the methanolic extracts of *Salacia chinensis*stem,leaf and root weretested by Mayer and Dragendorffs reagents.

     - **Mayer:** Dragendorffs Reagent Test: HCl (2%) solution was used to treat Salacia chinensis methanolic extracts. The Mayers reagent (potassium mercuric iodide solution) was then applied in 1 to 2 drops, and the precipitation of yellow color was watched for.

   - **Flavonoids:** The presence of flavonoids in the methanolic extracts was tested by ferric chloride test and alkaline reagent test.

- **Ferric Chloride Test:** After combining the extracts with a few drops of ferric chloride solution, the mixture was tested for the development of a blackish-red hue.

- **Alkaline Reagent Test:** After the extracts were combined with sodium hydroxide solution, it was noted that the extracts' yellow color intensified and eventually turned colorless when a few drops of diluted hydrochloric acid were added.

- **Phenols:** The extracts were mixed with 1ml of FeCl 3 (1%) and observed for the fresh radish blue color.

- **Steroids:** The presence of steroids in the extracts was tested by the Salkowski test.

  - **Salkowski Test:** After combining the extracts with a few drops of strong sulfuric acid along the test tube's sides, the extracts were examined to see if a brown ring formed where two layers met.

- **Saponins:** This was performed by foam test.

  - Foam Test – The extracts were combined with water, agitated, and the production of froth—which is stable for 15 minutes—was monitored for a favorable outcome.

- **Tannins:** The presence of tannins was done by gelatin test.

  - **Gelatin Test:** The extracts were mixed with gelatin solution and observed for the white precipitate for the presence of tannins

- **Glycosides:** The presence of glycosides was done by using Keller Killiani test.

  - **Keller Killiani Test:** A small amount of glacial acetic acid and ferric chloride solution were combined with the extracts. Conc. H2SO4 was added, and the creation of distinct strata was monitored.

- **Terpenoids- *Liberman-Burchard Test:*** Acetic anhydride is combined with the extract. Conc. sulfuric acid is added to this mixture. Two layers form, joining in a brown spot. Steroids are represented by the upper layer, which is green, and terpenoid red by the lower layer.

III. RESULT

In the present study nodal explants cultured on MS medium supplemented with 3.0 mg/LBAP and 1.0 mg/l shows high response to shoot initiation after 15 to 20days. The elongated shoots weretransferred to half strength MS medium with 0.5 mg/L IBA shows highest percentage of rootinitiation (table-1 &2). The preliminary phytochemical analysis of ethanol extracts of leaves and stem and roots of *Salacia chinensis* showed the presenceof alkaloids,steroids and tannins (Table-3). Multiple shoots were found on half strength MS mediumcontaining 3.0 µM/l of BAP in an average of 2-3 shoots per explant. These shoots

wereisolated and transferred to Half strength, full-strength MS Medium and Mc Cown &Lyodmedium supplemented with different phytohormones. But the number of roots were more andalso root induction was observed after 10 days time in full-strength MS medium with 1.5µM/l of IBA (6 roots/explant) .Where as in Mc Cown and Lyod medium with 5.7 µM/l ofIAA+4.9µM/l of IBA resulted in 3 roots/explant and root induction was observed after 4weeks.Half strength MS medium did not show any response with IAA,IBA and NAA individually and in combination. Rooted plants were transferred to pots in green house and later shifted to the field.

Numerous categories of metabolites, including phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids, were found in the preliminary qualitative phytochemical screening of S. chinensis extracts (Table-1, 2&3). The existence of these secondary metabolites is known to have a number of therapeutic benefits [9]. The primary source of important phytochemicals in this investigation was determined to be a methanolic extract of the S. chinensis root and leaves. Terpenoids are found in every section of S. chinensis and constitute a significant category in the pharmaceutical industry.

IV. DISCUSSION

In recent in vitro propagation research, the efficacy of BAP in initiating shoot growth was observed in nodal explants of Salacia chinensis (Phulwaria et al., 2013). The most favorable rooting results were achieved when utilizing half-strength MS media supplemented with 0.5mg/L IBA, resulting in an average of 10 roots per shoot. IBA has been widely recognized for its role in promoting root development and influencing the number of roots (Pokhriyal, 1990). In contrast, Senapati et al. (2013) documented the highest rooting percentage (73.3%) with IAA when using nodal explants in the Salacia chinensis species.

V. CONCLUSION

In vitro propagation proves to be a viable method for the large-scale multiplication and establishment of this endangered medicinal plant, offering an economical approach to extensive micropropagation and restoration within a brief timeframe and limited explants. This investigation has demonstrated that S. chinensis is a valuable source of phenolic compounds and saponins. Nevertheless, different parts of S. chinensis exhibit varying levels of phytochemicals and diverse antioxidant properties. Among these, the S. chinensis root displayed the highest concentrations of phenolic compounds, flavonoids, and saponins, followed by the stem and the leaf. It is advisable that future research endeavors focus on the extraction and isolation of major bioactive compounds from the root, stem, and leaf of S. chinensis to further explore their biological attributes.

Table 1: Methanolic Root Extract of *Salacia chinensis L.*

S. No	Name of the Phytochemical	Present/ absent
1	Alkaloids	--
2	Flavonoides	++
3	Phenols	++
4	Steroids	++

5	Saponins	--
6	Tannins	++
7	Glycosides	++
8	Terpenoids	++

Note: "++" indicates presence and "–" indicates absence of phytochemicals

Table 2: Methanalic Stem Extract of *Salacia chinensis*

S. No	Name of the Phytochemical	Present/ absent
1	Alkaloids	--
2	Flavonoides	++
3	Phenols	++
4	Steroids	++
5	Saponins	++
6	Tannins	++
7	Glycosides	++
8	Terpenoids	++

Note: "++" indicates presence and "–" indicates absence of phytochemicals

Table 3: Methanalic Leaf Extract of *Salacia chinensis*

S. No	Name of the Phytochemical	Present/ absent
1	Alkaloids	--
2	Flavonoides	++
3	Phenols	++
4	Steroids	++
5	Saponins	--
6	Tannins	++
7	Glycosides	++
8	Terpenoids	++

Note: "++" indicates presence and "–" indicates absence of phytochemicals

VI. ACKNOWLEDGMENTS

The author is thankful to the Principal, R.B.V.R. R Women's College, Narayanaguda, Hyderabad for financial assistance.

REFERENCES

[1] Nomura H, Kimura Y, Okamoto O, Shiraishi G. Effects of antihyperlipidemic drugs and diet plus exercise therapy in the treatment of patients with moderate Hypercholesterolemia. *Clin Ther.* 1996;18:196. [PubMed] [Google Scholar]
[2] Inman WD, Reed MJ. In-ventors; Shaman Pharmaceuticals, assignee Triterpenoid compound for the treatment of diabetes. US Patent 5, 691, 386. 1997 Nov 25; [Google Scholar]

[3] Gopalakrishnan S, Ismail ST, Begum HV, Elango V. Anti-inflammatory activity of SalaciaoblongaWall.AndAzimatetracantha Lam. *J Ethnopharmacol.* 1997;56:145–52. [PubMed] [Google Scholar]
[4] Y. Li, T.S. Huang, J. Yamahara, Salacia root, a unique Ayurvedic medicine, meets multiple targets in diabetes and obesity, Life Sci. 82 (2008) 1045–1049.
[5] K.N. Reddy, C. Pattanaik, C.S. Reddy, V.S. Raju, Traditional knowledge on wild food plant in Andhra Pradesh, Ind. J. Trad. Knowl. 6 (2007) 223–229.
[6] D. Ramakrishna, A.T. Shashank, K. Shinomol-George, S. Kiran, G.A. Gavishankar, Salacia Sps– a potent source of herbal drug for antidiabetic and antiobesity ailments: a detailed treatise, Int. J. Pharm. Phytochem. Res. 7 (2015) 374–382.
[7] M. Yoshikawa, N. Nishida, H. Shimoda, M. Takada, Y. Kawahara, H. Matsuda, Polyphenol constituents from Salacia species: quantitative analysis of mangiferin with alpha-glucosidase and aldose reductase inhibitory activities, YakugakuZasshi. Biochemistry and Biophysics Reports 12 (2017) 79–90 89 121 (2001) 371–378.
[8] T.M. Tran, T.H.A. Nguyen, D.T. Vu, V.S. Tran, Study on chemical constituents and cytotoxic activities of Salacia chinensis growing in Vietnam, Z. Nat. 65b (2010) 1284–1288.
[9] M.K. Julsing, A. Koulman, H.J. Woerdenbag, Combinatorial biosynthesis of medicinal plant secondary metabolites, Biomol. Eng. 23 (2006) 265–279.

