



GROWTH STUDIES AND PHYTOCHEMICAL ANALYSIS OF *LEPTADENIA RETICULATA* CELL SUSPENSION CULTURES

Sudipta KM¹, Balasubramanya S³, Anuradha M^{1,2}

¹Department of Biotechnology, Acharya Nagarjuna University, Nagarjunanagar, Guntur, India.

²Padmashree Institute of Management and Sciences, Kommagatta, Bangalore- 560060, India.

³Rishi Foundation, #234, 10th C main, 1st Block, Jayanagar, Bangalore- 560011, India.

*Corresponding author's E-mail: su_sudepta@yahoo.co.in

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ABSTRACT

Leptadenia reticulata (Asclepiadaceae) callus and suspension cultures were initiated on MS medium fortified auxins. Among all the auxins tested leaf explants cultured on picloram at 2 mg/l resulted in friable callus. Growth pattern and secondary metabolite profile was studied in suspension cultures. Phytochemical screening study indicated both exogenous and endogenous production of secondary metabolites. Spent media and cells are positive for the presence of alkaloids, glycosides, saponins, flavonoids, terpenoids, reducing sugars and tannins. However steroids are produced endogenously and absent in the media. Anthraquinone test is negative both in cells and media. This is the first report on study of suspension culture and secondary metabolite production in *Leptadenia reticulata*.

Keywords: *Leptadenia reticulata*, suspension culture, phytochemical analysis, growth studies.

INTRODUCTION

Leptadenia reticulata (Retz) Wight & Arn. belongs to family Asclepiadaceae is an important medicinal plant. This plant is commonly known as Jivanti, Dori or Swarn Jivanti is used in traditional and other Indian systems of medicine. *Leptadenia* is distributed in Asia, Africa, Burma, Sri Lanka and Philippines. In India it grows in Gujarat, Punjab, Himalayan ranges, Konkan, Nilgiris and in Southern parts. This has many pharmacological applications and is reported to be used in the treatment of hematopoiesis, emaciation, cough, dyspnoea, fever, burning sensation, night blindness. The whole plant is used for tuberculosis, enhancement of lactation¹, skin infection and eye diseases. Several important properties such as vasodilation², antidepressant³, anti-abortion⁴, oligospermic effect⁵ have been well studied in this plant. Due to its pharmacological activity the herbal drug industry is exploiting the wild resources, as there is no cultivation practice of this plant. The exploitation is making the plant vulnerable as whole herb or roots are being used for pharmacological applications. Therefore, now an urgent need to evolve a sound strategy for the management, conservation and for the production of active principles of *Leptadenia reticulata*. Though micropropagation of this plant is reported^{6,7} there is no evidence of publication in cell suspension cultures. Hence the present study has been undertaken to initiate preliminary investigations on suspension cultures and secondary metabolite production.

MATERIALS AND METHODS

Plant material

Cuttings of *Leptadenia reticulata* were procured from wild and the same were rooted and being maintained in the garden of Rishi foundation, Bangalore. The plants grown

in the herbal garden were the source of explants for the initiation of callus cultures.

Callus Initiation and establishment

Different explants such as leaves, petioles, internodes and nodes were screened for initiation of friable callus. Murashige and Skoog⁸ medium with 3% sucrose was fortified with various auxins viz. 2,4-dichlorophenoxy acetic acid (2,4-D), naphthalene acetic acid (NAA), Indole-3 acetic acid (IAA), picloram of different concentration (0.25, 0.5, 1.0, 2.0 and 5.0 mg/l) and screened for the ability to induce friable callus. The pH of the media was adjusted to 5.6-5.8 and autoclaved at 1.1 kg/cm² for 20 min at 121°C. The cultures were incubated at 25±2°C in complete darkness unless otherwise mentioned.

Initiation of cell suspension culture

Approximately 5 grams of callus was added to 75 ml of MS liquid medium in 250 ml Erlenmeyer flask. The liquid medium was supplemented with 2 mg/l picloram and the cultures were incubated on an orbital shaker and allowed for continuous shaking at 120 rpm.

Growth studies in suspension cultures

Growth of the cells in suspension culture was measured in terms of packed cell volume (PCV), fresh weight (FW) and dry weight (DW). The PCV was measured by taking 20 ml of suspension in centrifuge tubes of 50 ml and centrifuged at 8000 RPM for 15 minutes. Fresh weight was determined by separating the cells by filtration through a pre-weighed Whatman No.1 filter paper. Similarly the dry weight measurement was taken after the filter papers along with the cells were dried in a hot air oven (60-80 degree) until there is no further weight change⁹. Each reading was taken with a set of three flasks



harvested at 3-days intervals from the day of first subculture (Day 0) up to 21 days, for every parameter (PCV, FW, and DW).

Phytochemical analysis

The spent medium and the dried cells were analysed by standard biochemical procedure¹⁰⁻¹² for the presence of important phytochemicals such as alkaloids, glycosides, terpenoides, saponins, tannins, anthroquinone, flavones and reducing sugar.

RESULTS AND DISCUSSION

Among the various media tested for initiation of callus and suspension cultures, MS medium fortified with 2mg/l picloram and MS medium with 2 mg/l 2,4-D proved to be best in term of providing response and biomass production. Similarly, the increase in biomass of callus was also reported by various authors, Pradhan *et al.*¹³ in *Dalbergia sissoo*; Pattnaik *et al.*¹⁴, Rao *et al.*¹⁵ in *Gossipium*, and Salman¹⁶ by using 2,4-D. Young and immature leaves found to be efficient as compared to other explant such as nodal segment, roots and inter nodes for callus induction. The major problem of contamination and the sterilization process was skipped as all the explants used were taken from clean cultures ready for multiplication. The growth studies and yield of biomass was performed by comparing three parameters i.e. fresh weight, dry weight and packed cell volume (PCV). The results are presented in Fig 1, 2 and 3. The period of maximum biomass yield was noticed during 12th to 15th day after that the biomass started to decrease. The cultures remain healthy up to 26th day then black coloured cells were observed indicating the start of death phase.

The phytochemicals were characterized and represented in table 1. The preliminary phytochemical screening revealed the presence of alkaloid, steroid, tannin, flavonoid, glycoside, terpenoid and reducing sugar. However anthroquinone was not detected in either spent medium or in cells. Steroid was found to be absent in spent medium but present in cell extract. The presence of various active ingredients such as alkaloids, steroid, tannin, saponin, flavonoids known to have many important medicinal properties and suggest the use of this plant in curing many diseases. Tannin is found to having the wounds healing properties Kozioc *et. al.*¹⁷. Similarly flavonoid and tanins are also reported to be responsible for antidiarrheal activity¹⁸. The antibacterial activity of tannin was also studied by Elmarie and Johan¹⁹. These findings validate the report of Sathyanarayana *et al.*²⁰ that the metanolic extract of *L.reticulata* is having strong antibacterial activity. Parabia *et al.*²¹ described this plant as wound healer and having antidiarrheal activities. The presence of terpenoid has been reported to decrease the blood sugar level in animal studies²². The flavonoids having free radical scavenging activity that prevent the cells from reactive oxygen species and have strong anticancer activity²³.

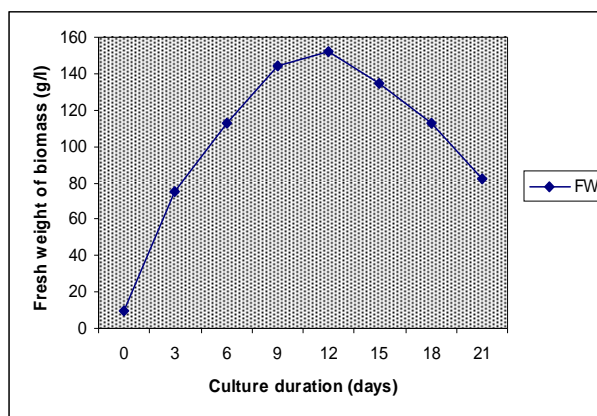


Figure 1: Growth of callus/cell biomass (FW) of *Leptadenia reticulata* in suspension culture at different age.

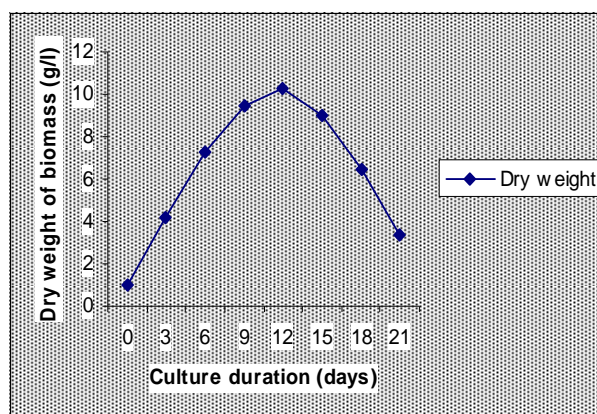


Figure 2: Growth of callus/cell biomass (DW) of *Leptadenia reticulata* in suspension culture at different age.

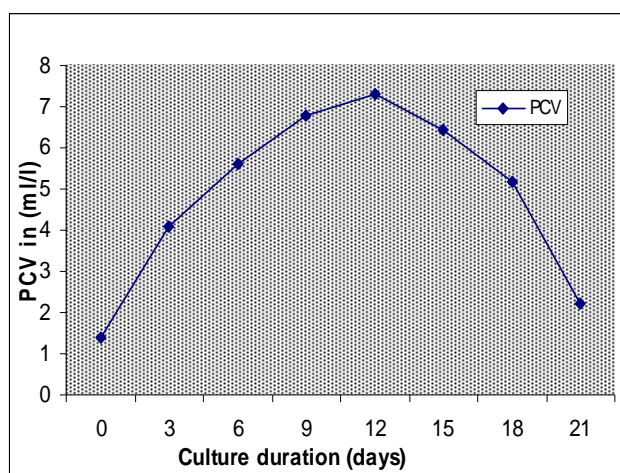


Figure 3: Growth of callus/cell biomass (PCV) of *Leptadenia reticulata* in suspension culture at different age.

It has been studied that saponin is having cytotoxic effect and anticancer properties on a range of animal cells. Akindahuni and Salawu²⁴ also reported the anti tumour activity of saponin. Thus the presence of saponin in this study supports the earlier finding of Sathyanarayanan *et al.*²⁵ that the extract of *L. reticulata* is highly effective against Dalton's Ascitic Lymphoma (DAL) in Swiss Albino mice and causes significant decrease in the cancer cell number and tumour weight. Saponins also act as strong antifungal agent²⁶⁻²⁸. This may be the reason that the

leaves and roots of *L. reticulata* are useful in various skin diseases and against fungal infections. Presence of Steroidal compounds is of special importance in

pharmaceutics due to their contribution towards formulation of synthetic sex hormone²⁹.

Table 1: Qualitative analysis of the phytochemicals in the cells and spent medium of *Leptadenia reticulata*

Phytochemicals	Test performed	Spent medium	Dried cells
Alkaloid	Dragendorff's test	+	+
Glycoside	Keller –killani test	+	+
Saponin	Froth formation	+	+
Steroid	Liebermann-Burchard reagent	-	+
Flavonoid	Shinoda test	+	+
Terpenoid	Salkowski test	+	+
Anthroquinone	Ammonia test	-	-
Reducing sugar	Fehling's reagent test	+	+
Tannin	Neutral FeCl ₃	+	+

CONCLUSION

Thus the present study reveals the usefulness of *L. reticulata* for several medicinal purposes and the presence of various important phytochemicals indicates the potential of this plant as a source of potent drugs.

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