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RESEARCH ARTICLE

BIOCHEMISTRY

BIOCHEMICAL PROFILE OF *IN VIVO* AND *IN VITRO* DEVELOPED *PAEDERIA FOETIDA* L- A RERE MEDICINAL PLANT

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ABSTRACT

Paederia foetida L. (Skunk vine) belongs to Rubiaceae is an important medicinal climbing herb, used in the treatment of Diarrhea and Dysentery. Plant tissue culture approach has been found to be advantageous as it provides a continuous and reliable source of natural product year round with out the destruction of entire plant. In the present study, reported biochemical profile of *in vivo* and *in vitro* produced metabolites of *P. foetida* in dry and fresh materials of leaf and callus respectively. The results revealed that the amount of the sugar was notably less in *in vitro*, while proteins, starch, amino acids, phenol, DNA and RNA showed slight variation but did not significantly for both dry and fresh materials. Enzymatic activities like peroxidase, IAA oxidase and volatile oil were higher in *in vitro* callus comparatively with leaf material *in vivo*.

KEYWORDS

Paederia foetida, medicinal plant, Callus and Biochemical compounds.

INTRODUCTION

Plants have been offering valuable and safe natural sources of medicines and agents of therapethitic ecological and environmental utilities across the varied cultures and civilizations. *Paederia foetida* is an important perennial climbing herb having very rich medicinal value. It is known in Hindi-Ghandali, Assam-Beololita, Telugu- Gabbuteega, Tamil-Mudiyar and Malayalam-Talanili. It is usually found in Himalayas from Dehradoon eastwards up to an altitude of 1800m. and also in Assam, Bihar, Bengal, Orissa and Andhra Pradesh[1]. This plant having anti inflammatory[2] and anti diarrheal and dysentery[3] activities. *P. foetida* extracts have been earlier studied for the presence of anti tussive compound and its effects on non- anaesthetized cats[4]. The present work has taken up to evaluate the biochemical profile of *in vivo* and *in vitro* produced materials of *P. foetida.* Tissue culture technique could play an important role in the production of pharmacognastical and phytochemical[5] substances. Plant cells grown in culture have potential to produce and accumulate chemicals similar to the parent plant from which they were derived. There are numerous reports describing the production of diverse metabolites through cell line selection and or addition of precursor in to the production medium[6,7] . The plant having bitter taste with foul smell. It also used in gout, vesicle calculi, piles, liver and emetic and enters into the preparation of Dasmularishta.

MATERIALS AND METHODS

Plants of *P. foetida* were grown in Botanical garden of Kakatiya University, Warangal, Andhra Pradesh. Leaves were used as explant material for tissue culture studies. Explants were soaked in 4% of Bavistin solution for 20-30min. and washed thoroughly under running tap water up to 30min. then followed by distilled water. Further the plant material was washed with the series of Teepol solution / Tween 20 solution for 15min. followed by distilled water for three times. The washed explants were surface sterilized for 2min. with the mixture of 0.1% mercuric chloride + 5% sodium hypo chlorate + cetrimine and rinsed five times of with double sterile distilled water to remove the traces of sterilants.

Analytical grade chemicals obtained from Himedia laboratories and Hormones from Sigma chemicals were used for preparing the stock solutions and subsequent media preparation. Sterilized explants were excised into pieces of 0.5 - 1cm^2 and carefully inoculated on MS medium[8] supplemented with different combinations of auxins and cytokinins for callus induction. All the cultures maintained at a temperature 25 ± 1^0 C, relative humidity 60 - 80 % and 16 : 8h photoperiod at photon flux density provided by day light fluorescent tubes. They were observed regularly for any sign of contamination, swelling and initiation of result. The callus obtained was harvested at the end of 2, 4, 6 and 8 weeks.

The biochemical profile of *P. foetida* has been carried out in *in vitro* (callus) and *in vivo* (leaf) at different age of 2, 4, 6 and 8 weeks. Estimation of Proteins[9], starch[10], sugars[11], phenols[12], amino acids[13], DNA and RNA[14] (from fresh and dry materials) and enzymatic activities like peroxidase[15], IAA oxidase[16] and fatty acid volatile oil[17] were analyzed from

cytoplasmic as well as wall bound fractions of the fresh material using the following standard methods.

RESULTS AND DISCUSSION

The different concentrations and combinations of different auxins and cytokinins showed varied results. However, media containing 2.5 mg/l 2, 4-D and 1 mg/l IBA each was most effective for callus culture (Plate-1). Changes in biochemical profile of the leaf and callus during the growth at different intervals of callus (2,4,6 and 8 weeks) of *P. foetida* revealed certain interesting features. Reducing sugar was higher in dry materials of the leaf, which increased with increase age of callus in both fresh and dry materials (Fig 1a). Amount of proteins in callus was higher in both *in vivo* and *in vitro* materials at the age of 4 weeks, it is decreased with increase of age of callus in dry material (Fig 1b). Starch content was maximum at 2 week old callus (Fig 1c), amino acid concentration increased linear in order from leaf to 2,4,6 and 8 week callus in both dry and fresh materials (Fig 1d). Nucleic acid compound content gradually increasing in fresh material with increasing the age of callus 2,4 and 6th week (Fig 1f, Fig 1g). Enzymatic pattern peroxidase increased with increasing age of callus and was maximum in wall bound fractions of 6th week callus (Fig 1h). IAA oxidase activity was quite related among 2, 4 and 6 week old callus. Maximum amount was noted in cytoplasmic fraction of the leaf and the wall bound fraction of 6th week callus (Fig 1i). Increase in the phenol activity was identified in the 2nd and 6th week of dry callus showed high amount of phenol (Fig 1e). The relationship between the enzyme activity with relevance to exhibit of phenol compounds and protein[18] phenolic content of the *P. foetida* [19], total phenolic content for extract decreased in dry material. The amount of total phenolic compounds varied widely between 48 and 40 mg/g sample weight and the volatile oil content was mostly showed in leaf and as well as 4th week of callus in cytoplasmic fractions, in wall bound fractions decreasing the content with increase the age of callus (Fig 1j). The previous investigation with regard to total phenolic contents are low to compare in fruit[20] in Jatropha and biochemical aspects in *Myristica malabarica*[21] numerous endogenous phenolic compounds are recognized as protein precipitants and enzyme inhibitors. Rathod and Saxena studied biochemical analysis and enzyme activity in *Boganvilea*[22] during callus induction, starch and amylase, protein protease, phenol polyphenol, oxidase enzyme protein in cytoplasmic and wall bound fractions was isolated from callus cultures from *Boganvilli*. Similarly studied wall bound enzyme activity in *cotton*[23] and the variations in the composition of membrane lipids in relations to changes in *Aegle mermoloes*[24] callus culture. The volatile oils isolated by vacuum distillation from *Syzygium*[25] species contain high percentage of terpenoides and re-terpene. Earlier reports noted the iridiod glycosides, paederolone, paederone, paederine and paederinine were the phytochemicals identified in this plant *P. foetida*[26]. As a result of these metabolic reactions, different products are formed, out of which some products are further needed in growth (carbohydrates, amino acids, proteins, nucleic acids, lipids and vitamins etc.,) their large diversity in nature, permit the identification of lead molecules of great interest for the development of new pharmacognostical and phytochemical agents, as well as to understand the biochemical and molecular mechanism of action involved in physiological and pathological process. The biological functions of plants are also due to their diverse chemical properties.

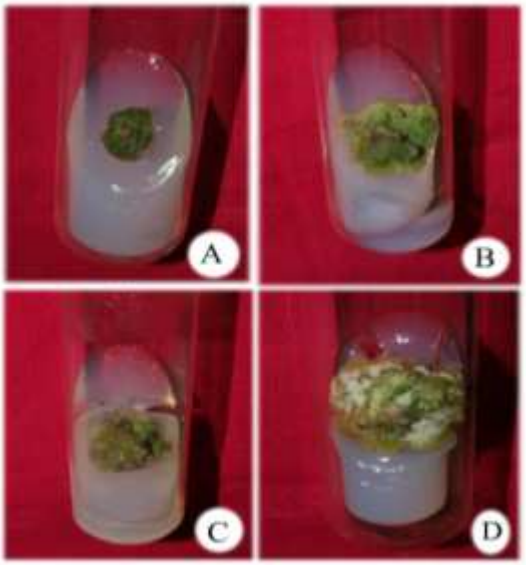

Plate. 1
A)Two weeks old callus. B) Four weeks old callus.
C) Six weeks old callus D) Eight weeks old callus.

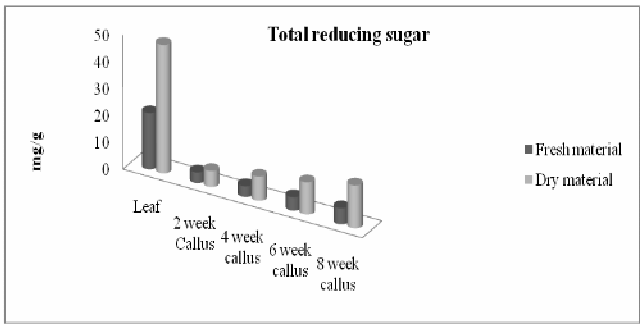

Fig 1a
Total reducing sugar

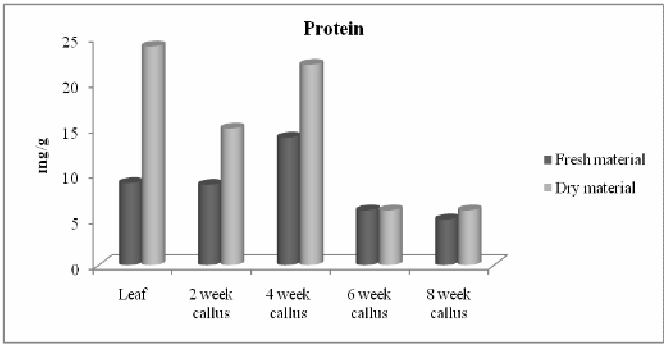

Fig 1b
Protein

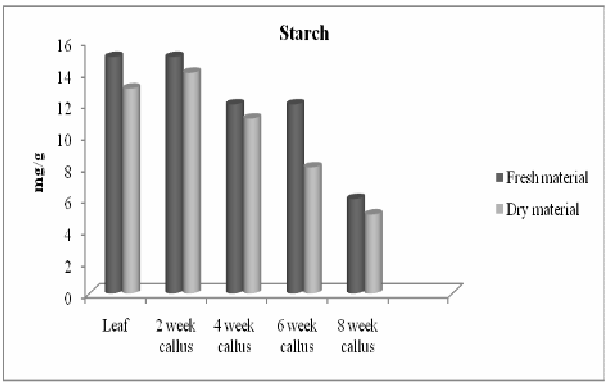

Fig 1c
Starch

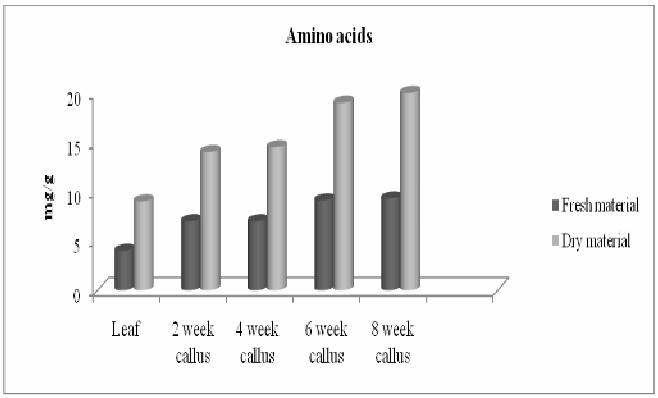

Fig 1d
Amino acids

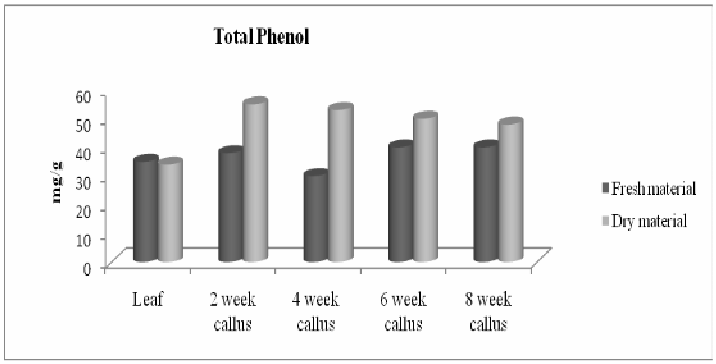

Fig 1e
Total Protein

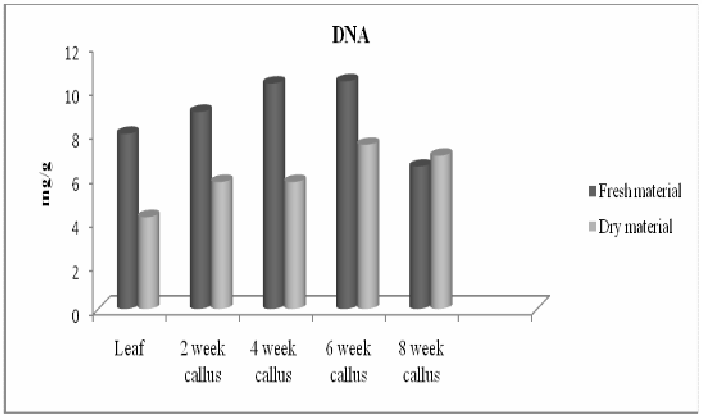

Fig 1f
DNA

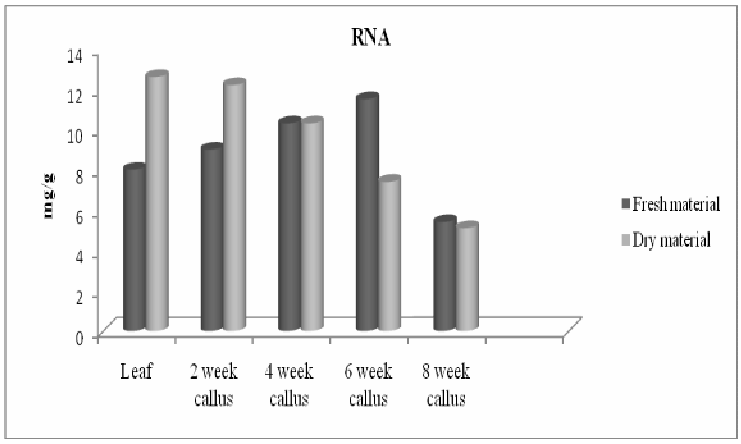

Fig 1g
RNA

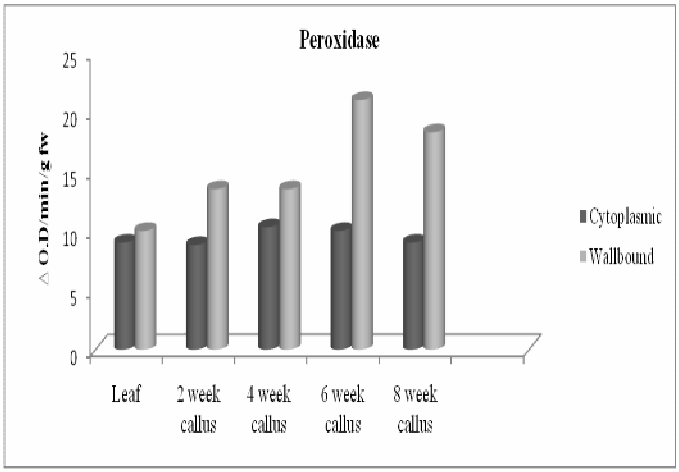

Fig 1h
Peroxidase

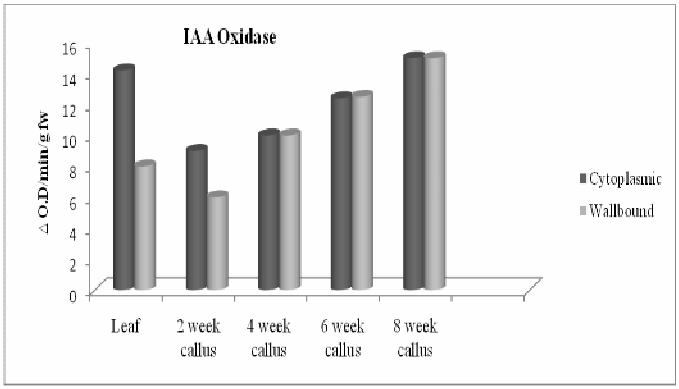

Fig 1i
IAA Oxidase.

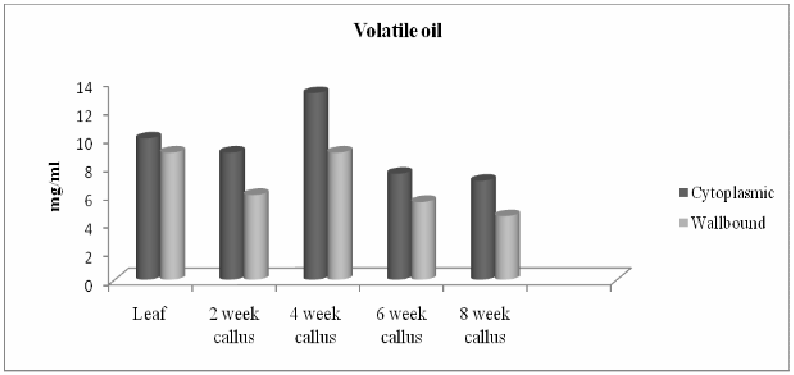

Fig 1j
Volatile oil

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