Research Article

A Comparative Assessment of Selected Metabolites in *In Vivo* Leaf and *In Vitro* Callus of *Jasminum grandiflorum L*

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ABSTRACT
Phytochemical are naturally present in the all plants and shows biological significance by playing an essential role in the plants to defend themselves against various pathogenic microbes by showing the antibacterial activity by inhibition or killing mechanisms. The secretion of secondary metabolite compounds is varying from plant to plant some produce more and some produce in minimal quantity. Sometimes they can be harmful and sometimes they can be very helpful. There is evidence from laboratory studies that phytochemicals in fruits and vegetables may reduce the risk of cancer, possibly due to dietary fibers, polyphenol antioxidants and anti-inflammatory effects. Pharmacological activities of *Jasminum grandiflorum* reported so far are spasmolytic, antiinflammatory, anti-microbial, antioxidant, antiulcer, cytoprotective, chemoprotective, wound healing and anti-acne activity**.** Fast growing callus was obtainable through leaf explants on MS media supplemented with 1.5 mg/L 2,4-D taken for the analysis. The callus and leaf were used for determination of physicochemical constituents and primary metabolites quantification. Maximum soluble sugars, starch and protein found in callus, maximum amount of chlorophylls and ascorbic acid contents were found in leaf.

Key words: Jasminum grandiflorum, MS media , 2,4-D, callus, primary metabolites

INTRODUCTION

Herbal medicines are promising choice over modern synthetic drugs. They show minimum or no side effects and are considered to be safe. Generally herbal formulations involve the use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Many of these indigenous medicinal plants are used as spices and food. Medicinal herb is considered to be a chemical factory as it contains a multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, locations and oils (essential and fixed). Higher plants produce both primary and secondary chemical metabolites, the former being vitally important in normal development and reproduction of plants. On the other hand, secondary metabolites are known to play important roles in plant survival as defense mechanisms against adverse biotic and a biotic conditions. Secondary metabolites present in plants have been linked with the healing properties of plants. In addition to their active ingredients, plants contain minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavonoids, and other substances that are important in supporting a particular herb's medicinal properties [1]. Jasmines are an important group of flowering plants. They are widely cultivated and esteemed for their attractive fragrant flowers .This genus belongs to the family Oleaceae. Moreover, different parts of the plant such as the leaf, stem, bark, and roots are very useful and important in pharmaceutical industries. *J. grandiflorum L*. (Family: Oleaceae) exhibit a wide ecological range and found extensively all over India. The leaves of *J. grandiflorum* are used in the treatment of odontalgia, fixing loose teeth, ulcerative stomatitis, leprosy, skin diseases, ottorrhoea, otalgia, stangury, dysmenorrhoea, ulcers, wounds and corns. The leaves of this species have a distinction of being used in Indian folk medicine for treating ulcers. Literature suggests the use of this plant as a diuretic and spasmolytic agent, which is given during child birth[2]. Flowers of *J. grandiflorum* are useful to women when used as a tonic as it aids in preventing breast cancer and stopping uterine bleeding [3].
Primary metabolites are substances widely distributed in nature, occurring in one form or another virtually in all organisms. They are of prime importance and essentially required for growth and development of plants by supplementing sugars, proteins, lipids, and starch. Biotechnological approaches, specifically plant tissue culture plays a vital role in search for alternatives to production of desirable medicinal compounds from plants. Tissue culture tool are considered expensive than conventional mass multiplication method when it comes to commercial and large scale production needs [4]. Tissue culture media accounts to large proportion of any *in vitro* mass propagation protocol. The present study was conducted to investigate the comparative physicochemical constituents and primary

metabolites of callus and fresh leaf from *J. grandiflorum*.

Materials and methods

Plant Collection and Identification of Plants

The plants of *J. grandiflorum* L. used in this study was collected from Namakkal District, Tamil Nadu and it was authenticated (No.BSU/SRC/5/23/2011-12/Tech-132) at Botanical Survey of India, South Circle, Coimbatore, Tamil Nadu. The specimens were poisoned, pressed and the herbarium specimens were prepared according to the standard instructions[5].

Callus induction

The leaf explants from four-week old plants were collected and first thoroughly washed in running tap water for 30 min[6]. The sterilization procedure was performed [7] and explants were inoculated in Murashige and Skoog (MS) medium containing 1.5 mg/L 2,4-D for callus culture. Callus cultures were maintained on solid MS medium (1.5 mg/L 2,4-D), sub cultured every 30 days at 28±2ºC with a photoperiod of 16 hours. The developed undifferentiated homogenous cell mass was then used for further investigation.

Determination of Physicochemical Constituents

The callus were selected and collected on the 20th day after inoculation [8] and dried under shade avoiding contamination. These dried materials were mechanically powdered, sieved using 80 mm meshes and stored in airtight containersand used for further physicochemical and biochemical analysis[9]. Loss on drying, total ash, acid insoluble ash and water soluble ash were determined [10].

Primary metabolite estimation

Callus and fresh leaf parts of *J. grandiflorum* were evaluated quantitatively to estimate the total levels of Carbohydrates, Reducing Sugars , Starch, Proteins, Total Amino acids, Chlorophyll, Ascorbic acid [11]. All experiments were replicated three times for precision and values were expressed in mean ± standard deviation [12].

Results and discussion

The study included collection of plant materials and processing it for the studies. Identified plant samples were used for the callus induction and phytochemical analysis. The plant samples were collected from same plant in same time for the analyses.

Callus induction

The plant material inoculated into the standard media used in the experiment successfully produced callus required for the analyses. They were produced with the intention to assess the phytochemical constituents produced by the tissue in *in vitro* condition. It helps for the mass production and acquiring required quantity of the phytocomponent of the plant without destructing the vegetation in nature [13].

Table 1: Physico Chemical Analysis of Powdered of *Jasminum grandiflorum* Leaf and Callus

Parameters	% (W/W)	
	Leaf	Callus
Loss on drying	12.45±0.16	15.20±0.52
Total Ash Value	6.35±0.11	7.24±0.10
Water soluble Ash Value	7.31±0.07	8.24±0.09
Acid insoluble Ash Value	2.10±0.05	3.04±0.03

Data are expressed as mean ± SD of three replicates

Analyses of phytochemical component in *in vitro* and *in vivo* plant tissues

The plant material was freshly collected from naturally grown plant and used for the analyses. It gives the amount of phytocomponents in plants in *in vitro* condition. For *in vitro* analyses the callus induced/produced in the lab were used.The total ash value of the material gives an idea of the earthy matter or the inorganic composition and other impurities present along with the plant sample. The percentage of loss on drying, total ash, acid insoluble ash and water soluble ash were carried out and the consequences were as tabulated in the Table 1. The fresh leaves and callus of *J. grandiflorum* were analyzed for loss on drying, in this higher water content observed in callus (15.20±0.52) compared to leaf (12.45±0.16) powder. Similarly ash content also high in callus compare to leaf. It was understood that the *J. grandiflorum* callus contained more of carbonless substances in the ash compared to leaf sample [14]. This could be due to the tissue nature of callus. The present study was designed to determine the physicochemical parameters, phytochemical screening, organoleptic identification and fluorescence analysis on dried powder of *Dendrobium macrostachyum L*. The detailed pharmacognostic or phytochemical evaluation of this plant has not been reported earlier. Total ash, water soluble ash, acid insoluble ash were observed to be respectively 6.5%, 4.12%, 0.83% for stem and 3.80%, 1.69%, 0.67% for leaves. Preliminary phytochemical analysis revealed the presence of alkaloids, leaf flavonoids, glycosides, sterols, tannins and phenols [15]. Preliminary phytochemical screening showed the presence or absence of alkaloids, flavonoids, phenols, steroids, saponins, terpenoids, tannins, anthraquinones, anthocyanin, quinones, volatile oils, proteins and carbohydrates. HPTLC of aqueous extract of Rotula aquatica was carried out to assess the further assess the presence of phenol, tannin and saponins[16].

Primary Metabolites

A primary metabolite is directly involved in the normal growth, maturation and reproduction. Plants are rich sources of high value metabolites like proteins, phenols, sugars, starch, lipids, amino acids

and ascorbic acids are useful in flavoring, fragrances, insecticides, sweeteners and natural dyes. India has a century's old tradition of using medicinal plants and herbal medicines for the alleviation of various diseases and ailments[17]. About 500 plants with medicinal uses are mentioned in ancient literature, and around 800 plants have been used in indigenous systems of medicine. In recent decades there is manifold increase in medicinal plant based industries due to the increase in the interest of the use of medicinal plants throughout the world which are growing at a rate of 7-15% annually [18]. The evaluation of new drugs especially phytochemically obtained materials has opened a vast area for research and development. Therefore, the evaluation of the rich heritage of traditional medicine is essential. Many medicinal plants contain a broad range of natural antioxidants such as phenolic acids, flavonoids and tannins, which possess remarkable antioxidant activity[19]. Several investigations indicate that these compounds are of great value in preventing the onset and/or progression of many human diseases [20]. Quantitative estimation of total carbohydrates (Table 2) showed that the content of carbohydrate is more in callus i.e., 51.60±0.31mg/g dw in callus and a minimum amount of 45.42±0.24 mg/gdw in leaf. The content of reducing sugar was also observed to be more in callus i.e. 22.52±0.20mg/g dw and minimum in leaf (18.47±0.21 mg/gdw) (Table 2). Plant sugars can be used as artificial baits and they can even help diabetics by supporting physical structure of the body and development [21]. They are increasingly being considered as an eco-friendly alternative to the use of synthetic additives in many products, including plastics, detergents, pharmaceutical products, pesticides, cosmetics and even oil-drilling fluids [22]. Starch is biodegradable and renewable in nature. The highest amount of starch was observed in callus (36.19±0.32 mg/gdw) compared to leaf (29.54±0.37mg/gdw).In present work, total levels of protein were found to be higher in leaf (40.74±0.21mg/gdw) than in callus (36.73±0.17mg/gdw). Proteins perform a vast array of functions within living organisms. Proteins are the primary components of living things. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base bioactive compound could also be isolated in future [23].

Amino acids play central roles both as building blocks of proteins and as intermediates in metabolism. Amino acids are used for a variety of applications in industry, but their main use is as additives to animal feed. These are commonly used in food technology and industry. The maximum amount of amino acid was observed in the leaf (8.64±0.22mg/gdw) of *J. grandiflorum* compared to callus (7.68±0.18mg/gdw). Very often in plants during diseases conditions, the free amino acid composition exhibits a change and hence, the measurement of the total free amino acids gives the physiological and health status of the plants[22]. Total levels of Chlorophylls were found to be high amount in leaf (7.29±0.36mg/g) and lowest in callus (4.19±0.15mg/g). Chlorophyll is the molecule that traps this 'most elusive of all powers' - and is called a photoreceptor. Chlorophylls (Table 2) is the most indispensable class of primary compounds as they are the only substances that capture sunlight and make it available to plant system for its cultivation by photosynthesis. Chlorophyll itself is bound to proteins and can transfer the absorbed energy in the required direction [24]. As the callus is grown in *in vitro* conditions they develop comparatively less chlorophylls. Ascorbic acid (vitamin C) is a familiar molecule because of its dietary significance, most aspects of its metabolism and some aspects of its function in plants are very poorly understood [25]. There are many different biosynthesis pathways for ascorbic acid in plants and it's used for preventing tissue damage. Total levels of ascorbic acid were found to be higher in leaf i.e. 4.32±0.21mg/gdw than callus (2.43±0.27mg/gdw). However, there was a decrease in chlorophylls and ascorbic acid content and an increase in sugar in callus primary metabolite analysis due to effect of phytohormones. *In vitro* cells accumulate more sugar and other metabolites due to its easy availability in culture medium and these cells are in highly proliferating stage so they accumulate more primary metabolites [4]. Similar result was observed in *Catharanthus roseus in vivo* plant and *in vitro* callus[26]. In vitro cells accumulate more sugar due to its easy availability in culture medium and these cells are in highly proliferating stage so they accumulate more primary metabolites than storage metabolites (starch, lipid) and secondary metabolites (phenolic contents). The phytochemical screening of the C. odorata and citrus sinensis (unripe)extracts observed that both extracts contain alkaloids, cardiac glycosides, flavonoids, tannins and cyanogenic glycosides[1].The World health organization (WHO) estimates that 4 billion people (80%) of the world's population presently use herbal medicine for one form of primary health care or another. Its history is inextricably intertwined with that of modem medicine, but pharmacologists, rather than use a whole plant identify, isolate, extract and synthesize individual components, thus capturing the active properties as against the herbalist who considers that the power of a plant lies in the interaction of all its ingredients [27].

Quantitative analysis is very essential for identifying the compounds present in the medicinal plants. The results obtained from the present study provides evidence that aqueous hot extract of *E. uniflora* leaves contains various primary and secondary metabolites and this justifies the use of plant species

as traditional medicine for treatment of various diseases. The finding of this study suggests that this plant leaves could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing various diseases. The results are very much encouraging but scientific validation is necessary before being put into practice [28]. India has a rich, vibrant and diverse cultural history. An important component of this culture and tradition is that of health and healing. India is the largest producer of medicinal herbs and is rightly called the botanical garden of the world. There are very few medicinal herbs of commercial importance, which are not found in this country. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk, and herbal medicine, representing about 75% of the medicinal needs of the third world countries.

Table 2: Biochemical (Primary Metabolites) Analysis of *Jasminum grandiflorum* Leaf and Callus

Constituents/ Experiments	Primary metabolites mg/gdw	
	Leaf	Callus
Carbohydrates	45.42±0.24	51.60±0.31
Reducing sugars	18.47±0.21	22.52±0.20
Starch	29.54±0.37	36.19±0.32
Proteins	40.74±0.21	36.73±0.17
Amino acids	8.64±0.22	7.68±0.18
Chlorophylls	7.29±0.36	4.19±0.15
Ascorbic acid	4.32±0.21	2.43±0.27

mg/gdw = milli gram per gram dry weight, Data are expressed as mean ± SD of three replicates.

Conclusion

In conclusion, the present study indicated the ability to utilize tissue culture techniques towards development of desired bioactive metabolites in *in vitro* culture as an alternative way to avoid exploitation of endangered or rare wild plants for pharmaceutical purposes. This study also suggests the plant *Jasminum grandiflorum* as a potent source for phytomedicine development in future.

References

1. Kelechi Obi Robert, Chiegboka Nneamaka. Ethanolic extraction and phytochemicals screening of two Nigerian herbs n pathogens isolated from wound infections. International Journal of Comprehensive Pharmacy 2011; 2(10):1-5.
2. Mahajan Nilesh, Sakarkar Dinesh, Sanghai Dhirendra. Evaluation of anti-ulcer potential of leaves of *Jasminum grandiflorum* L. International Journal of Pharmaceutical Science 2009;1(2):247-249.
3. John De Britto A, Herin Sheeba Gracelin D. Efficacy of fruits of *Jasminum grandiflorum* linn. against plant and animal pathogens. Asian J Pharm Clin Res. 2011;4(4):74-75.
4. Satyapal S, Babeet S T and Moinuddin K. Callus induction and *in vivo* and *in vitro* comparative study of primary metabolites of *Withania Somnifera*. Advances in Applied Science Research 2011;2(3):47-52.
5. Rao, R.R and Sharma B.D. A manual of Herbarium collections, Botanical Survey of India, Calcutta, 1990, 1-184.
6. Bhattacharya P, Dev S, Das N and Bhattacharya B.S. Rapid mass propagation of *Chrysanthemum morifolium* by callus derived from stem and leaf explants. Plant Cell Rep.1990; 9:439-442.
7. Thenmozhi M., Rajeshwari Sivaraj and Kalaiselvi K. Antimicrobial activity of *Chrysanthemum parthenium* leaf and callus extracts. Journal of Pharmacy Research. 2011;4(10):3763-3765.
8. Martre P, Dominique Lacan, Daniel Just, Claude Teisson. Physiological effects of temporary immersion on *Hevea brasiliensis* callus. Plant Cell, Tissue and Organ Culture 2001;67:25-35.
9. Harborne JB. Phytochemical methods. In: A guide to modern techniques of plant analysis. J.B. Harborne (ed.), Chapman and Hall, London. 1973; 279.
10. Bele A.A and Khale A. Standardization of herbal drugs: A review. International Journal of Pharmacy. 2011;2(12): 56-60.
11. Abhimanyu Sharma and Amla Batra, 2016, Primary Metabolite Profiling of Tinospora cordifolia. Nat Prod Chem Res 2016, 4:4 1-7.
12. Seth R and R. Sarin, 2012. *In Vivo* and *in Vitro* Biochemical Estimation of Primary Metabolites from *Jatropha curcas*: An Important Biodiesel Plant. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(1):81-84.
13. Rajkumar D and R. Murugesan, 2014, Hairy root induction of *Psoralea corylifolia* for enhanced production of antifungal compound against red rots pathogen *Colletotrichum falcatum*. African Journal of Agricultural Res. 2014;9(8):755-765.
14. Shobana V and Rajalakshmi K. Quantitative analysis of primary metabolites in *Mangifera indica* (unripe mango). Rasayan Journal of Chemistry. 2010;3(3):597-599.
15. Nimisha.P.S and Hiranmai Yadav.R. Proximate and physicochemical analysis of *Dendrobium macrostachyum* LINDL International Journal of Pharmacy and Pharmaceutical Sciences2012;4(1): 385-386.
16. Vijayakumari Bhavaniamma, Venkatachalam Sasikala, Singanallur Ramu Radha and Hiranmai Yadav Rameshwar. HPTLC finger printing of aqueous root extract of *Rotula aquatica* for phenol, tannin and saponin contents. Der Pharmacia Lettre, 2016, 8 (9):299-306.
17. Mohan V.R, Rajesh A, Athiperumalsami T and Sutha S. Ethnomedicinal plants of the Tirunelveli District, Tamil Nadu, India. Ethnomedicinal Leaf 2008;12:79-95.
18. Padmaa MP. *Nigella sativa* L. - A comprehensive review. Indian J Nat Prod Res 2010;1(4):409-29.

19. Wong SP, Leong LP, Koh JH. Antioxidant activities of aqueous extracts of selected plants. Food Chem 2006; 99(4):775-83.
20. Halliwell B, Gutteridge J.M and Cross C.E. Free radicals, antioxidants and human disease: Where are we now? J Lab Clin Med 1992; 119:598-620.
21. Freeze H. Disorders in protein glycosylation and protein therapy. The Journal of Pediatrics. 1998;133(5):543-600.
22. Savita Sagwan, D.V.Rao and R.A. Sharma. Biochemical Estimation of Primary Metabolites from *Pongamia Pinnata* (L.): An Important Biodiesel Plant. International Journal of Pharmaceutical Sciences Review and Research 2010; 5(1):146-149.
23. Thomsen S, Handen HS and Nyman V. Ribosome inhibiting proteins from *in vitro* cultures of *Phytolacea dodecandra*. Planta. Med. 1991;57:232-236.
24. Murray A.P, Gibbs C.F and Longmore A.R. Determination of chlorophyll in marine waters: inter comparison of a rapid HPLC method with full HPLC, spectrophotometric and fluorometric methods. Marine chemistry. 1986;19:211-227.
25. Nicholas. The function and metabolism of ascorbic acid in plants. Annals of Botany. 1996;78:661- 669.
26. Anshul K. Jain , Meena and Dinesh Kumar . *In vivo* and *in vitro* comparative study of primary metabolites and antioxidant activity of *Catharanthus roseus*. Asian Journal of Plant Science and Research, 2014, 4(2):42-46.
27. Rios J L and Recio M C; Medicinal plants and antimicrobial activity. J Ethanopharmacol. 2005; 100: 80-84.
28. Geedhu Daniel and Krishnakumari S. Quantitative Analysis of Primary and Secondary Metabolites in Aqueous Hot Extract of *Eugenia Uniflora* (L.) Leaves. Asian J Pharm Clin Res, 2015; 8(1):334-338.