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Original Article

Comparative qualitative analysis of callus extracts of *in-vitro* and *in-vivo* plants of Jasminum angustifolium, a wild and medicinal plant

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ABSTRACT

Plant tissue plays an important role in the production and conservation of medicinal plants. The present study reveals that callus was raised from the leaf explants of Jasminum angustifolium. The leaf and stem explants of Jasminum angustifolium were used for callus regeneration on MS medium supplemented with 3% sucrose with different concentrations of 2, 4-D and BAP. A maximum callus was obtained from leaf explants when cultured on MS medium supplemented with 2, 4-D and BAP at 2.0mg/litre respectively. The callus obtained in 2, 4-D concentration was best and shows 73% of callus growth than BAP. The callus used in this study was taken after eight to nine weeks of growth. The qualitative analysis of this study reveals that the secondary metabolites have the ability to produce the changes in the human body. The qualitative analysis shows the presence of tannins, saponins, terpenoids and quinones was observed in leaf callus. The wild leaf extracts shows the presence of tannins, saponins, terpenoids, and quinones.

Key Words: 2, 4-D, BAP, secondary metabolites, phytochemical analysis, qualitative, callus regeneration.

INTRODUCTION

Jasminum angustifolium (wild.) Linn., belongs to the Family: Oleaceae. It is commonly known as 'Kattu malligai' and it is distributed throughout India, Srilanka and Andaman islands. In South India Jasminum angustifolium was distributed in kerela and Karnataka on the hills of lower elevation [2]. Leaves are simple ovate lanceolate, acute, glabrous [5] and flowers are either solitary or usually in three. Petals are linear, obtuse and acute [6]. Jasminum is scientifically known as wild jasmine in English and people use these jasmine flowers as religious offerings to the Gods like Lord Shiva and Lord Vishnu. This plant is traditionally used to treat skin diseases, ulcers, stomatitis, eye diseases and leprosy [13]. The juice of the leaves is given as an emetic in case of poisoning [12]. The plant extracts were reported to the biological activities such as anti-inflammatory, astringent, diuretic, skin diseases [7]. The plant tissue culture are considered expensive than conventional mass multiplication method. In the present study, the callus was raised from the leaf explants of Jasminum angustifolium. The leaf extracts of wild plant was compared with invitro leaf callus. The present study is justified that it is planned to propagate the medicinal plant of Jasminum angustifolium in invitro conditions of various plant growth hormones with various concentrations and compare the qualitative analysis of invitro callus with leaves of wild plant.

MATERIALS AND METHODS

Plant Material: The healthy plants of Jasminum angustifolium were collected from the herbal garden of Irula Tribal Women's Welfare Society, Thandarai, Chenglepet. The collected plant leaves was shade dried. The powdered samples were subjected to sequential extraction using Hexane, Chloroform and ethanol solvents. The extracts were filtered using Whatman filter paper No. 1 and concentrated with rotary evaporator. The leaf explants and stem explants were excised to 1 cm long segments and were washed with running tap water for 20 to 30 minutes and were washed with liquid detergent teepol for 5 to 7 minutes and rinsed with double distilled water for 3 to 5 times. Then followed by 0.1% mercuric chloride for 1 to 2 minutes. Finally washed with sterile water for 5 to 7 times under aseptic conditions and inoculated on MS medium [8]. The laboratory conditions recommended by Purvis [9] were followed for the preparation of media, inoculating the plant explants and maintenance of the cultures.

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Callus Induction: The leaf explants and the stem explants were inoculated on the MS medium supplemented with various concentrations of 2, 4-D and BAP for callus induction 2.0 mg/ l is the best concentration for callus induction. MS medium [8] containing 3% sucrose solidified with 1% agar (Tissue culture grade, Hi-media, India) was used. The pH of the medium was adjusted to 5.6 - 5.8 by adding sodium hydroxide and hydrochloric acid [3] and agar was added before autoclaving at 121°C for 15 minutes under 15 lb pressure. The cultures were incubated in growth room at temperature of 25±2°C and 16h-photoperiod provided with white fluorescent lights. 30 replicate cultures were established and each experiment was repeated thrice and the cultures were observed at regular intervals.

Extraction from Callus Cultures: About 8 to 9 week old calli obtained from leaf extracts were collected for further work. The calli obtained from leaf extracts were shade dried and was homogenized to a fine powder and stored in airtight container.

PHYTOCHEMICAL ANALYSIS

The leaf and invitro callus extracts of Jasminum angustifolium were analysed for the presence of tannins, saponins, terpenoids, sterols, phenolic compounds, alkaloids and flavonoids. The tests were based on the visual observation of a change in colour or formation of precipitate after the addition of specific reagents by following the standard phytochemical methods [11, 4].

Test for Carbohydrates: To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.

Test for Tannins: To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for Saponins: To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for Flavonoids: To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for Alkaloids: To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for Quinones: To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

Test for Glycosides: To 2ml of plant extract, 3ml of choloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for Cardiac Glycosides: To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides.

Test for Terpenoids: To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids.

Test for Triterpenoids: To 1.5ml of extract, 1ml of Libemann –Buchard Reagent (aectic anhydride + concentrated sulphuric acid) was added. Formation of blue green color indicates presence of triterpenoids.

Test for Phenols: To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

Test for Coumarins: To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins.

Test for Steroids and Phytosteroids: To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

Test for Phlobatannins: To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins.

Test for Anthraquinones: To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones.

RESULTS AND DISCUSSION

The present study was carried out on the plant sample reveals the presence of medicinally important bioactive compounds. The wild leaf extracts contained phytochemicals like carbohydrates, tannins present in all the three solvents, saponins in hexane extract, cardiac glycosides and phenol present in ethanol and hexane extracts and was absent in chloroform extract. In the past few decades the secondary metabolites plays an important role in the pharmacognosy field.

Callus Induction: The leaf and stem explants of Jasminum angustifolium were collected and inoculated in MS medium containing with different

concentrations of 2, 4-D and BAP, within two weeks of culture maximum callus yield was obtained from 2.0 mg/ l of 2, 4-D (figure 1). After five weeks the callus was sub cultured in the same medium at regular interval of times (figure 2). It was noticed that up to 2.0mg/l of 2, 4 -D shows the best growth of callus in Jasminum angustifolium (figure 3). The explants fail to produce callus in MS medium without plant growth regulators. These results were close to the agreements of Reeta [10].

Comparison of phytochemical analysis of the leaf, stem and callus extracts of Jasminum angustifolium: Preliminary phytochemical analysis of leaf and callus extracts was compared in Table 2. The leaf extract of Jasminum angustifolium contains carbohydrates, tannins present in all the three solvents, saponins in hexane extract, cardiac glycosides and phenol present in ethanol and hexane extracts and was absent in chloroform extract. Flavonoids, alkaloids, quinones, glycosides terpenoids, coumarins, steroids, phlobatannins, anthraquinones were not identified. Significant antioxidant and diuretic property was noticed in leaf extracts [1]. The phytochemical analysis of leaf callus extracts exhibits phenols in all the three solvents, carbohydrates present only in ethanol, tannins revealed in hexane and chloroform, saponins and coumarins reveals only in hexane, quinones revealed in ethanol and chloroform, cardiac glycosides appeared only in chloroform extract. Flavonoids, alkaloids, quinones, glycosides, terpenoids, coumarins, steroids, phlobatannins, and anthraquinones were not detected. For the first time we reported that the leaf callus of phytochemical analysis with the leaf explants of wild plant, Jasminum angustifolium.

CONCLUSION

The present study showed the callus production on MS media supplemented with 2.0mg/l 2, 4-D concentration. Preliminary phytochemical analysis of invitro callus with in vivo leaf extracts was compared. This study will help in bioactive compounds and purification of lead antioxidant molecules from the wild leaf with invitro leaf callus of Jasminum angustifolium.

Table 1: Effect of plant growth regulator for callus induction.

S.No	Plant growth regulator(mg/l)	No of explants Inoculated	Percentage of response(%)	Nature of callus
1	2,4 –D	10	73	Friable bulky callus
2	BAP	10	68	Very slow growth

Table 2: Comparison table of Invivo Leaf extracts of Jasminum angustifolium with Invitro leaf callus extracts of Jasminum angustifolium.

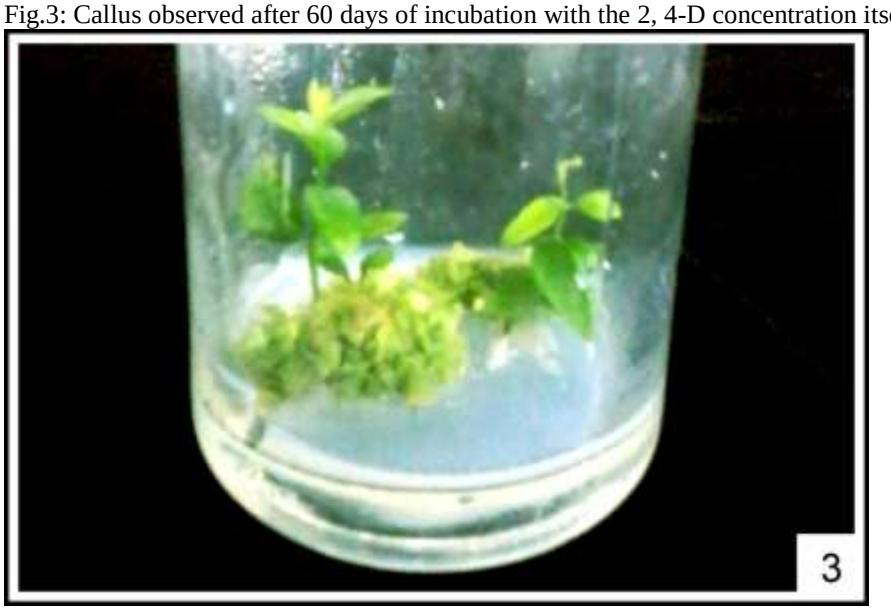
PhytochemicalTests	Results: Invivo leaf extracts			Results: Invitro leaf callus extracts		
	Ethanol	Hexane	Chloroform	Ethanol	Hexane	Chloroform
Carbohydrates test	W+	W+	W+	W+	W+	+
Tannins test	W+	w+	w+	_	W+	+
Saponins test	_	W+	_	_	_	_
Flavonoids test	_	_	_	_	_	_
Alkaloid test	_	_	_	_	_	_
Quinones test	_	_	_	W+	_	W+
Glycosides test	_	_	_	_	_	_
Cardiac glycosides test	+	+	_	_	_	+
Terpenoids test	_	_	_	_	+	_
Phenols test	+	+	-	+	+	+
Coumarins test	_	_	_	_	_	_
Steroids and Phytosteroids test	_	_	_	_	_	_
Phlobatannins test	_	_	_	_	_	_
Anthraquinones test	_	_	_	_	_	_

W+ weekly present; + Present ; _ Absent

Fig.1: Callus produced from leaves of Jasminum angustifolium in MS+2.0 mg/l of 2, 4-D.

Fig.2: Callus observed after 40 days of incubation 2, 4-D of 2.0 mg/L concentration.

Fig.3: Callus observed after 60 days of incubation with the 2, 4-D concentration itself.



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