Indian J. Innovations Dev., Vol. 1, No. 2 (Feb 2012) ISSN 2277 - 5390

In vitro regeneration and phytochemical analysis of *Justicia gendarussa*

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Abstract

Justicia gendarussa is a rare medicinal plant belonging to the family Acanthaceae. An efficient protocol for *in-vitro* propagation of *Justicia gendarussa* has been developed. The shoot initiation, callus development and phytochemical analysis were studied. MS medium supplemented with BAP (2 mg/l) induced shoot formation from nodal explants. Thick friable calli were producecd at lower concentrations of NAA (0.5 mg/l) and BAP (2 mg/l). The dried powder of leaf, stem and root of *Justicia gendarussa* were analysed for phytochemical screening.

Keywords: *Justicia gendarussa*, Phytochemistry; In-vitro regeneration; Medicinal plants.

Introduction

Today's health care systems rely largely on plant material. In spite of recent development in the synthetic drug, chemistry and production of antibiotic, plants still occupy an important role in the modern and traditional system in all over the world. Modern medicines are primarily from synthetic or plant origin while synthetic origin may have toxic effects, the plant medicines have less toxicity, and their importance is being realized in both developed and developing countries, plants are an important source of medicines and play a key role in world health (Constabel, 1990).

Use of plant-based remedies is also widespread in many industrialized countries and numerous pharmaceuticals are based on or derived from plant compounds. Plant secondary metabolites were found to be sources of various phytochemicals that could be used directly or as intermediates for the production of pharmaceuticals, as additives in cosmetic, food or drink supplements (Ong Poh Liang, 2007).

The plant selected for the present study is *Justicia gendarussa* Burm F., (family: Acanthaceae). It is a native plant of China but is found wild or cultivated in Sri Lanka, India, Malaya (Malaysia) and Philippines. The leaves are simple, opposite, lanceolate or linear-lanceolate, acute at base, tapering into rounded apex and glabrous and shining leaves (8-12.5 cm long, 1.2-2 cm broad) with prominent purple veins beneath.

In-situ conservation techniques are an effective way to improve, maintain and use traditional or native varieties of medicinal plants. The conservation of genetic resources in natural population of plant species, such as forest genetic resources in natural populations of tree species and is being increasingly being applied to conservation of agricultural biodiversity in agro-ecosystems by formers, especially those using unconventional farming practices (Nguyen and Luu, 2007).

Ex-situ conservation may also be used on some or all of the population, when *in-situ* conservation is too difficult or impossible. Ex-situ conservation methods include live plant collections in botanic gardens, seed banks, *in-vitro* banks, cryo-banks and DNA libraries (Nalawade, 2007). *Ex-situ* conservation helps to provide the flexibility to respond to unforeseen environmental changes and

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Table 1. Composition of Murashige and Skoog (MS) Medium (1962)

Group	Components	Final concentration (mg/l)	Stock Solution (g/500ml)	Volume of stock solution to be taken (ml/l)
1	Ammonium Nitrate	1650.00	-	-
2	Potassium Nitrate	1900.00	-	-
A	Magnesium Sulphate	370.00	18.05	10
	Manganese Sulphate	16.09	0.845	
	Zinc Sulphate	8.06	0.43	
	Copper Sulphate	0.025	0.00125	
B	Calcium Chloride	440.00	22	10
	Potassium Iodide	0.83	0.0415	
	Cobalt Chloride	0.025	0.00125	
C	Potassium dihydrogen- ortho phosphate	170.00	8.5	10
	Boric Acid	6.02	0.31	
	Disodium Molybdate	0.25	0.0125	
4	Ferrous Sulphate	27.84	2.784/100ml	2
5	Sodium EDTA	37.24	3.724/100ml	
D	Thiamine – Hcl	1.00	0.05	10
	Niacin	0.50	0.025	
	Pyridoxine – Hcl	0.50	0.025	
	Glycine	2.00	0.1	
6	Myo-inositol	100.00		
7	Sucrose	25g		
8	Agar – 0.6%, pH – 5.8 ±0.05			

consequent impacts on habitat conservation and utilization of wild plant species.

In vitro propagation refers to the true-to-type propagation of selected genotypes using *in-vitro* culture techniques. Single cells, plant cells without cell walls (protoplasts), pieces of leaves, or roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones (Akin-Idowu *et al*., 2009). The potential for developing antimicrobial drugs from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Therefore, such screening experiments form a primary platform for further phytochemical and pharmacological studies that may open the possibility of finding new clinically effective antibacterial compounds (Mohamed *et al.,* 2010). In Sri Lanka traditional medicine, the leaves of *J. gendarussa* are used as an analgesic to treat hemiplegia, rheumatism, arthritis, headache and earache (Albuquerque *et al.,* 2007). In Brazil, it is used to treat pains, fever, and for the treatment of diseases of magical-religious origin (Arokiyaraj *et al.,* 2007).

Table 2. Preparation of Hormonal stock

Name of the hormones	Abbreviations	Concentration in 100ml	Preparation	Stock concentration
Auxin			Dissolve 1ml of 0.1N NaoH and Made up the the volume of 100ml by SMF	1mg/ml
Indole-3 Butyric acid	IBA	100mg		
Kinetin	KIN	100mg		
Napthlalene acetic acid	NAA	100mg		
2-4-Dichlorophenoxy acetic acid	2-4-D	100mg		
Cytokinin			Dissolved 1ml of 0.1N Hcl and made up the volume to 100ml by SMF	1mg/ml
N- Benzyl-9-(2-tetrahydropyranyl) adenine	BPA	100mg		
Benzyl Amino purine	BAP	100mg		

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Materials and Methods

The plant selected for the present study is *Justicia gendarussa,* which was collected from herbal garden of Sathyabama University.

Preparations of media

The stock for nutrient and hormone solutions were prepared as described in the table 1 and 2. The required volume of macronutrients, micronutrients, irons and vitamins were taken from the stock solutions, which is already prepared and mixed well with the appropriate volume of double distilled water. Then, carbon source like inositol and 30% of sucrose were weighted and dissolved wherever need. The final volume in each case was made up to the required volume by adding distilled water.

Selection and sterilization of explants

The explants were collected from the selected mother plants without any visible disease symptoms and with minimum damage to the plant parts. Various parts viz., node, shoot tip, leaf and seeds are used as explants.

The explants were washed well in running tap water for 15 minutes to remove the soil or sand particles. Then the explants were treated with fungicide (Bavistin) and rinsed in distilled water for 15 minutes. Then the explants were treated with detergent (Tween -20) and rinsed in distilled water for 15 minutes. Finally, explants were treated with 70% ethanol for 20 seconds and washed with sterile distilled water for 4 times.

Inoculation and incubation of Explants

The surface sterilized explants were transferred to sterile petriplates, excised into appropriate size (1.0-1.5 cm length), and then inoculated. The explants were transferred aseptically on M.S media supplemented with various concentration and combination of plant growth regulators. The cultures were incubated in the culture room at 25±2^{0}C and 16 hrs photoperiod with 3,000 lux-intensity. The results were observed and tabulated at regular time intervals.

Phytochemical screening

The leaf, stem and root extract of *Justicia gendarussa* were analyzed for the presence of tannins, saponins, terpenoids, glycosides, steroids, phenolic compounds, flavonoids, and starch.

One gram of sample was weighed and dissolved with various solvents such as ethanol, methanol and water. Then the sample was allowed to stay overnight for 24 hours. After overnight incubation the sample was filtered by Whattman filter paper, the filterate was centrifuged at 25,000rpm for 10 minutes, and the supernatant was used for Pytochemical screening.

a) Test for tannins

To the two ml of the extract 2ml of 5% ferric chloride was added. Formations of yellow brown precipitate indicate the presence of tannins.

b) Test of Saponins

To the 0.5 ml of filtrate 5 ml of distilled water was added, frothing indicate the presence of saponins.

Table 3. Effect of PGR on the formation shootlets from *Justicia gendarussa* nodal explants cultured on MS media

Medium	No. of explants Inoculated	Percentage of shoot response (%)	Number of shoot per explant	Mean length of shoot-lets
Basal	20	65	1.8±1.0	3.2±0.4
BAP-2.0	20	86	3.2±1.3	7.8±1.2
BAP-2.0 NAA-0.5	20	70	2.8±0.8	3.6±0.5

Fig. 1. Shoot initiation

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c) Test for Terpenoids

To the 2 ml of extract 5ml of chloroform added followed by addition of 3ml concentrated sulphuric acid. Reddish brown colour indicates the presence of terpenoids.

d)Test for Glycosides

About 1ml of extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered with 1 ml of concentrated sulphuric acid. A brown ring obtained indicated the presence of glycosides.

e)Test for Steroids

To the 0.5 ml of filterate, 2ml acetic anhydride was added followed by then addition of 3ml concentrated sulphuric acid.Blue green ring indicate the presence of Steroids.

f) Test for Phenolics

To the 2ml of extract, 1ml of 1% ferric chloride was added, blue or green colour indicates the presence of phenolics.

g) Test for Flavonoids

To one ml of the extract, a few drops of dilute sodium hydroxide was added. An intense yellow colour was produced in the plant extract, which become colourless on addition of a few drops of dilute acid indicates the presence of flavonoids.

h) Test for Starch

To the extract drops of iodine solution was added formation of blue colour indicated the presence of starch.

Results & Discussion

Micro-propagation of *Justica gendarussa*

Shoot Initiation

The nodal segments of *justicia gendarussaa* were collected and inoculated on MS media fortified with different concentration and combination of cytokinins. After 6 days of incubation, the nodal explants start to break and initiate shooting (Table 3). Maximum number of shoot-lets was obtained on MS medium supplemented with BAP (2.0 mg/l). The number of shoot-lets and length of shoots proliferated from the cultures were used as a better source of explants for further multiplication. Node from the *in vitro* derived shoots were excised properly and cultured on MS media with same and different combination of cytokinin (Fig.1).

Table 4. Effect of plant growth regulator on the callusing, 0f *Justicia gendarussa* cultured on MS medium

S.No	Plant Growth regulator (mg/l)	No explant Inoculated	Percentage of response (%)	Nature of callus
1	NAA – 0.5 2,4 D – 2.0	10	68	Very slow growth, browning occur at the the starting phase
2	2,4 D -2.0 NAA-1.0	10	60	Very slow growth, browning occur at the the starting phase
3	NAA – 0.5 BAP – 2.0	10	90	Friable, bulky callus

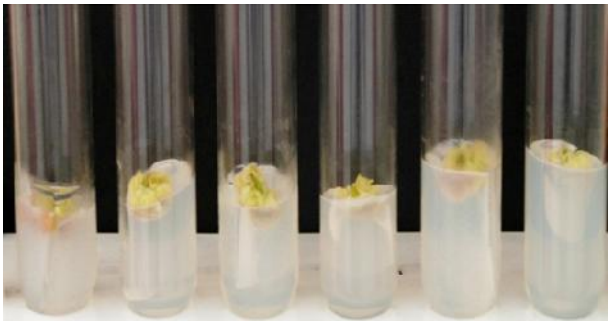

Fig. 2. Callus induction at various concentration Of plant growth regulator

Callus Induction

The leaf explants were sterilized and inoculated on MS media fortified with NAA and BAP. The percentage of response and the type of calli were observed and tabulated (Table 4). Then the callus was transferred on media enriched in cytokinin for

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shoot production. The results were observed (Fig.2).

Table 5. Comparison of Preliminary Phytochemical Screening for leaf, stem and root extracts of *Justicia gendarussa*

	Leaf extract			Stem extract			Root extract		
	Ethanol	**Methanol**	**Water**	**Ethanol**	**Methanol**	**Water**	**Ethanol**	**Methanol**	**Water**
Tannins	+	+	+	+	+	+	+	+	+
aponins	-	-	-	-	-	-	-	-	-
Terpenoids	+	+	+	-	-	+	-	-	+
Glycosides	+	+	+	+	+	+	+	+	+
Steroids	-	+	-	-	+	-	-	+	-
Phenolics	+	+	-	+	+	-	-	-	-
Flavanoids	-	-	+	-	+	+	+	+	+
tarch	-	-	-	-	-	-	-	-	-

Comparison of phytochemical study of the leaf stem and root extracts of *Justicia gendarussa*

Preliminary phytochemical screening of leaf, stem, and root extracts of Justicia gendarussa was performed, and the results were compared in the Table 5. The leaf showed better result for preliminary phytochemical screening than the stem and root extract.

In the present study, efficient plant regeneration protocol was developed for *Justicia gendarussa* Burm.f. (Acanthaceae), an important medicinal shrub. The nodes, shoot tips of *Justicia gendarussa* were selected as an explants for shoot initiation. The explants were surface sterilized with freshly prepared 0.1% w/v aqueous solution of mercuric chloride for 5 minutes. After mercuric chloride, treatment explants were thoroughly washed for 4 to 5 minutes with sterile water to remove the traces of mercuric chloride. These surface sterilized explants were inoculated in MS medium containing different combination of NAA and BAP. Nodal segments grown on Murashige and Skoog (MS) medium containing BAP (2 mg/l) showed better growth response and produced 3.2±3 shoots per explant with an average length of 7.8±1.2 cm after 35 days. Similarly Janarthanam *et al.* (2010) developed an efficient plant regeneration protocol for *Justicia gendarussa* Burm.f., (Acanthaceae). Nodal segments are grown on Murashige and Skoog (MS) medium containing 1 mg LG1 6-Benzyl adenine (BA) with 10 % coconut milk showed better growth response and produced 10.5 ± 0.6 shoots per explant with an average length of 4.4 ± 0.3 cm after 35 days.

In the present study, both BAP (2 mg/l) and NAA (0.5mg/l) induced friable callus and browning in different concentration of 2, 4-D and NAA. Similar results have been reported for legumes (Vijayakumari *et al.,* 2001) and Foxtail millet.

Preliminary phytochemical screening of plant extract has been reported in several medicinal plants (Amerjothy *et al.,* 2007). In the present study the different extracts of *justicia gendarussa* contain tannin, terpenoids, flavanoides, phenolic groups, glycosides were analysed.

Conclusion

In the present study, micropropagation of *Justicia gendarussa*, a medicinal plant has been done using explants like node and shoot tip. BAP 2.0mg/l promoted shoot proliferation. Callus was produced on media supplemented with BAP 2.0mg/l and NAA 0.5mg/l. Preliminary phytochemical screening of different plant extract was also performed and the results were compared. The different extracts of *justicia gendarussa* contain tannin, terpenoids, flavanoides, phenolic groups, glycosides was analysed. In conclusion, the present investigation has resulted in a protocol that could be used for *ex-situ* conservation and true to type mass propagation of this herb of immense pharmaceutical relevance.

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