

In vitro Callus Induction and Preliminary Phytochemical Studies of *Cissampelos pareira* L

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Abstract- The present investigation was aimed to evaluate the effect of growth regulators on induction of callus from leaf segments of *Cissampelos pareira*. The surface sterilized young leaves were inoculated on Murashige and Skoog (MS) medium fortified with different concentration and combination of growth regulators for induction of callus. The phytochemical constituents were analyzed in the different solvent extracts using standard methods. Maximum callus induction was observed on MS medium supplemented with 2 mg/L of NAA or 2, 4-D alone and in combinations of NAA (2 mg/L) + BAP (1 mg/L) and NAA (2 mg/L) + KIN (0.5 mg/L). The preliminary phytochemical screening of leaf and leaf derived callus revealed the presence of flavonoids, alkaloids, phenols, terpenoids, coumarins etc. The developed protocol is useful for large scale production of callus from leaf explants which may be useful for isolation of important bioactive compounds for the treatment of various diseases.

Key words: *Cissampelos pareira*, callus, growth regulators, phytochemical screening.

I. INTRODUCTION

Medicinal plants are widely used in traditional system of medicine and even in modern medicine [1, 2]. Medicinal plants have enormous potential to synthesize aromatic substances like phenols or their oxygen substituted derivatives as secondary metabolites that serve as plant defense mechanisms against microorganisms, insects and herbivores. Medicinally active constituents of plant tissues are extracted with a broad spectrum of organic solvents of choice through standard procedures [3]. The extracts obtained in liquid, semi-solid, powder form are complex mixtures of metabolites like alkaloids, glycosides, terpenoids, flavonoids, lignin and others [4].

Cissampelos pareira L. (*C. pareira*) is an important medicinal plant, belongs to the family Menispermaceae. It is commonly called as Patha or Laghupatha in Indian traditional medicine [5]. There are 37 plant species which are distributed worldwide come under this genus, but only one species occurs in India [6]. The plant is commonly found in orchards, hedges, parks and gardens mainly on moist soil. It is distributed throughout the tropical and subtropical India. The plant has various medicinal properties and is used for dyspepsia, indigestion, flatulence, abdominal pain, diarrhea, dysentery, blood disorders, cough, asthma, bronchitis, skin disorders, leprosy, migraine, leucorrhoea, gonorrhoea, burns, wounds, astringent, antispasmodic, piles, urogenital affections and scabies [7].

Traditionally the roots are prescribed in combination with other drugs for the treatment of snake bite and scorpion sting [8-10]. The plant contains number of alkaloids like hayatine, hayatinine, hayatidine, bisbenzylisoquinoline, d-4''omethyl berberine, isochandrodendrine, dicentric, dehydrodicentrine, insularine, cycloanine and berberine [11, 12]. The plant has various activities like anti-inflammatory, antioxidant, analgesic, antitumor, anticarcinomic, gastro protective, cardioprotective, antileukemic, antispasmodic, antiseptic, diuretic and stimulant [8].

This medicinal plant is over exploited for its wider range of phytoconstituents and diverse applications. Micropropagation of plants through tissue culture is an alternate method to prevent the loss of important medicinal plants from the natural habitat. Therefore, the present study aims to develop the efficient protocol for callus induction from leaf explants and to screen the phytoconstituents present in different solvent extracts of leaf and leaf derived callus of *C. pareira*.

II. MATERIAL AND METHODS

Collection of plant material and sterilization

Plants were collected in and around Jnanabharathi campus and maintained at the Department of Botany in the greenhouse condition. The leaves segments (0.5 to 1 cm²) were surface sterilized with 0.1% bavastin fungicide for 20

mins, then washed thoroughly with tap water followed by liquid detergent (1-2 drops of teepol) and kept under running tap water to remove the traces of detergent followed by three to four rinses with distilled water. Finally, the explants were sterilized with 0.1% mercuric chloride for 3 min and rinsed 3-4 times with sterile distilled water.

Culture condition

The leaf explants were transferred into culture bottles on MS medium supplemented with 3% sucrose and was solidified with 0.8% agar (Plant tissue culture grade, Himedia) with different concentrations (0.5-4 mg/L) of auxins (2, 4-D, NAA, IAA and IBA) and in combination with cytokinin (BAP, KIN) for callus induction. The pH of the media was adjusted to 5.6-5.8, then autoclaved at 121°C for 20 mins. The cultures were incubated at 25±2°C under 16 hrs photoperiod with light intensity (3000-4000 Lux) provided by white florescent bulbs.

Preparation for plant extracts

Leaf and leaf derived callus were collected and finely powdered. 10 gms of the powdered material was extracted with different solvents (methanol, ethanol, chloroform, aqueous, hexane and petroleum ether) by soaking the material for 72 hrs. The extracts were filtered through Whatman No. 1 filter paper. The procedure was repeated for another two cycles to ensure complete extraction of phytochemical compounds. The filtrates were lyophilized and stored at 4°C until further analysis.

Preliminary phytochemical analysis

The preliminary phytochemical analysis was carried out by standard methods [13-17]. Briefly, the lyophilized extracts were dissolved in respective solvents and screened

for the qualitative analysis for the presence of alkaloids, flavonoids, phenols, tannins, saponins, steroids, terpenoids, glycosides, gums and mucilage, carbohydrates, reducing sugars and volatile oils.

Statistical analysis

The data were expressed as Mean ± SE. All the experiments were repeated thrice and 15 replicates for each experiment. The data were analyzed statistically by one-way analysis of variance followed by Turkey's HSD test using SPSS software. Probability values $P < 0.05$ were considered significant.

III. RESULTS AND DISCUSSION

Effect of auxins

Auxins like 2, 4-Dichlorophenoxyacetic acid (2, 4-D), Indole-3-acetic acid (IAA), Indole 3-butyric acid (IBA), α -Naphthalene acetic acid (NAA) were used for the induction of callus from leaf explants. The initiation of callus was found to be more profuse in the leaf explants on MS medium supplemented with 0.5-4 mg/L of 2, 4-D and NAA. Whereas, less profused callus was observed on MS medium supplemented with IBA (0.5-4 mg/L). The initiation of callus was observed after 4-6 weeks of cultures. The callus was profuse and friable in the initial stages and later turned to brown. The significant amount of callus induction was observed at 2 mg/L of NAA (93.33±06.66), IBA (71.66±11.66) and 4 mg/L of 2, 4-D (100.00±0.00), IAA (66.66±08.33) respectively (Table 1; Figure 1 & 2 A-C). The results are in concordance with the reports of Gokul & Tejavathi and Shad & Deepa wherein, maximum callus induction was observed from leaf explants of *C. pareira* on MS medium fortified with different concentrations of 2, 4-D, NAA and IBA [18, 19].

Table 1. Effect of auxins on callus induction from leaf explants of *C. pareira*

Conc. of GRs in mg/L	% of response in Mean ± SE			
	2, 4-D	NAA	IAA	IBA
0.5	38.33±6.66 ^a	28.33±8.33 ^a	33.33±8.33 ^a	40.00±7.63 ^{ab}
1.0	55.00±5.70 ^{ab}	46.60±1.66 ^{ab}	41.66±6.00 ^a	48.30±3.33 ^{ab}
2.0	75.00±5.00 ^{bc}	93.33±3.33 ^{bc}	36.66±8.33 ^a	71.66±11.66 ^b
3.0	95.00±2.88 ^{cd}	66.66±6.66 ^c	41.66±8.33 ^a	61.66±7.26 ^{ab}
4.0	100.00±0.00 ^d	93.33±6.66 ^c	66.66±8.33 ^a	30.00±2.88 ^a

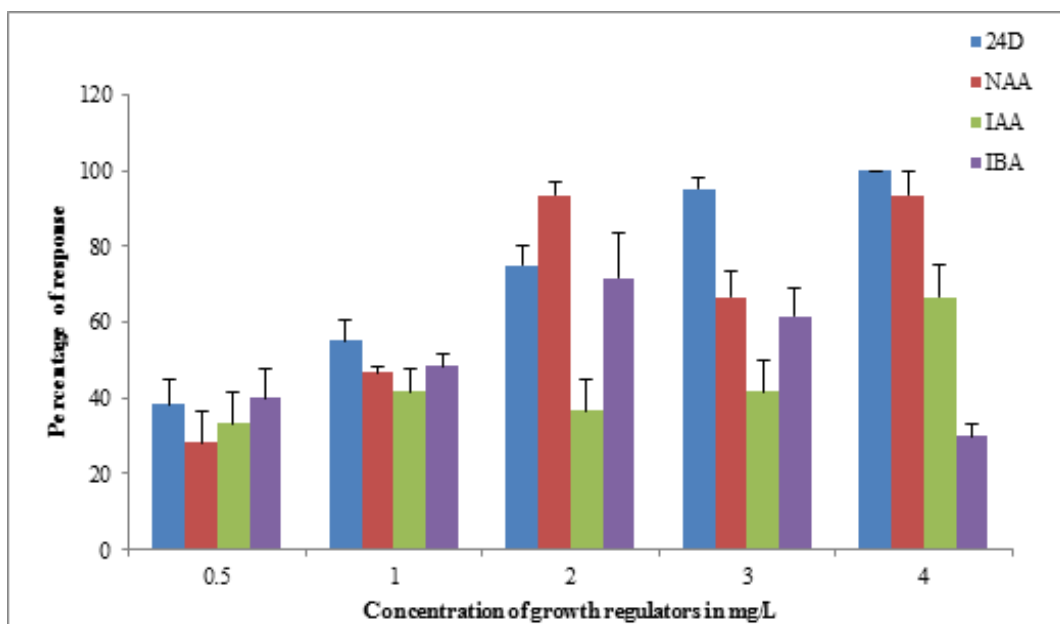


Figure 1. Effect of auxins on callus induction from *C. pareira* leaf explants

Effect of cytokinins

There was no callus induction from leaf explants of *C. pareira* inoculated on MS medium supplemented with different concentrations of cytokinins such as 6-Benzylaminopurine (BAP) and 6-Furfurylaminopurine (KIN) ranging from (0.5-4 mg/L).

Effect of auxins and cytokinins

Synergistic effect of auxins and cytokinins on leaf explants of *C. pareira* on callus induction was studied. The ratio of auxins and cytokinins play a vital role in callus induction. The callogenesis was observed in presence of different concentration and combination of auxins and

cytokinins. The significant callus induction was observed on BAP (0.5 mg/L) + IAA (1.5 mg/L) and BAP (1 mg/L) + NAA (2 mg/L). Combination of NAA (2 mg/L) + KIN (0.5 mg/L) showed 100% callus induction, whereas 95% response was observed on media supplemented with BAP (1 mg/L) + NAA (1 mg/L) (Table 2; figure 2 D-F). Similar observation was reported by Manasa *et al.* in leaf and stem explants of *Cyclea peltata* inoculated on MS medium supplemented with NAA (1 mg/L) + BAP (3 mg/L) and NAA (1 mg/L) + KIN (3 mg/L) [20]. Sugandha also observed the maximum callus induction from leaf explants of *Adansonia digitata* on MS medium fortified with BAP (3 mg/L) + NAA (1 mg/L) [21].

Table 2. Effect of auxins and cytokinins on callus induction from leaf explants of *C. pareira*

Conc. of GRs in mg/L					% of response Mean ± SE
BAP	NAA	IAA	IBA	KIN	
0.5	-	0.5	-	-	68.33±4.40 ^a
0.5	-	1.5	-	-	95.00±2.88 ^b
0.5	-	2.0	-	-	80.00±2.88 ^{a,b}
1.0	-	0.5	-	-	80.00±2.88 ^{ab}
1.5	-	0.5	-	-	73.33±1.66 ^a
1.5	-	1.0	-	-	83.33±3.33 ^{ab}
1.5	-	1.5	-	-	78.33±3.33 ^{ab}
2.0	-	2.0	-	-	71.66±6.00 ^a
0.5	1.0	-	-	-	70.00±2.88 ^a
0.5	2.0	-	-	-	88.33±7.26 ^{ab}
1.0	1.0	-	-	-	95.00±2.88 ^b
1.0	1.5	-	-	-	73.33±1.66 ^a
1.0	2.0	-	-	-	98.33±1.66 ^b
1.5	1.0	-	-	-	78.33±1.66 ^{a,b}
2.0	2.0	-	-	-	73.33±7.26 ^a
0.5	-	-	0.5	-	65.00±2.88 ^a
2.0	-	-	1.0	-	75.00±2.88 ^{ab}
2.0	-	-	2.0	-	65.00±2.88 ^c

	2.0	-	-	0.5	100.0±0.00 ^c
	2.0	-	0.5	-	80.00±2.88 ^b

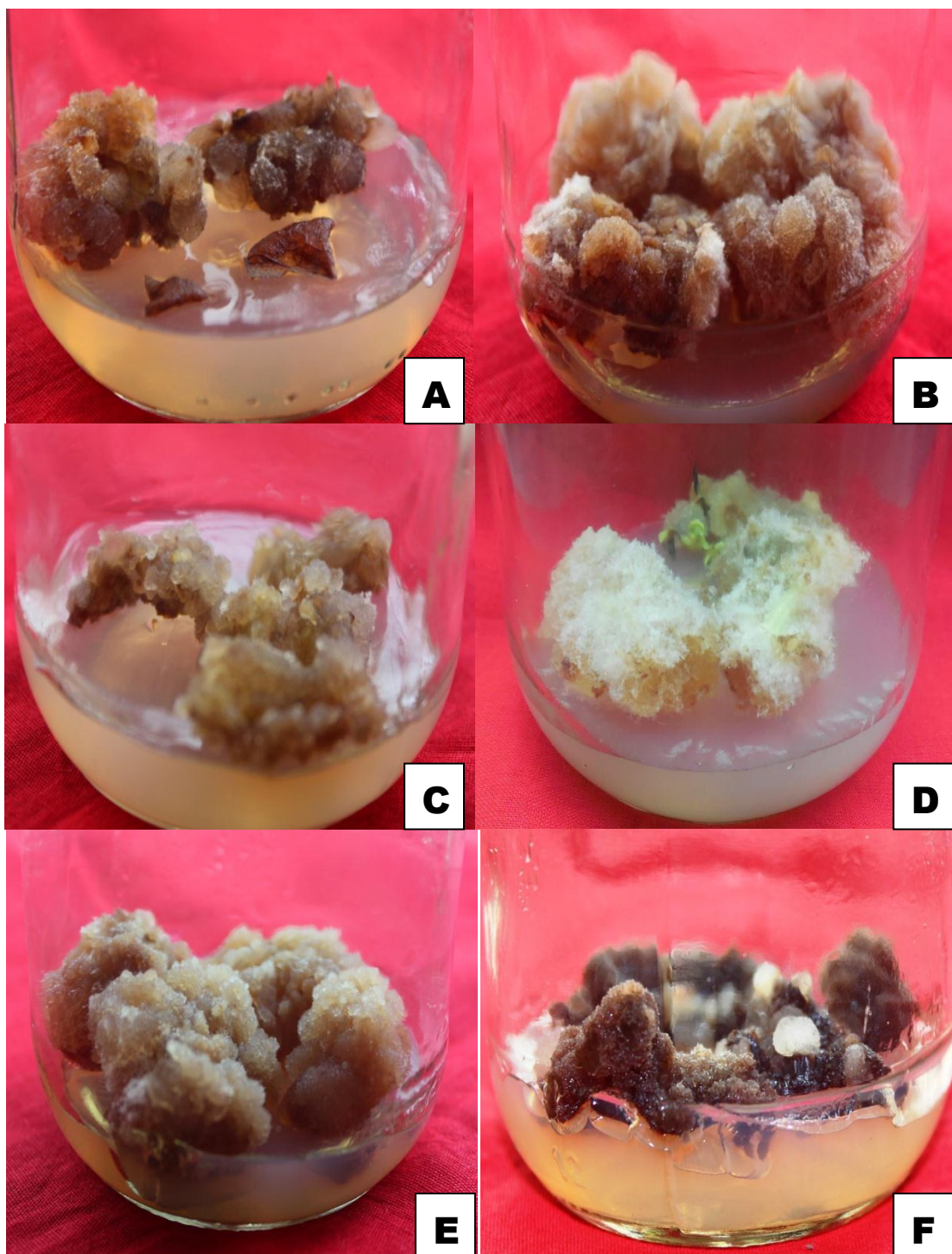


Figure 2 A-F. Shows the stages of callus induction from leaf explants of *C. pareira*

A- MS + 2,4-D (4 mg/L), B- MS + NAA (2 mg/L), C- MS + IAA (2 mg/L), D- MS+ BAP (1 mg/L) + NAA (2 mg/L), E- MS + BAP (0.5 mg/L) + IAA (1.5 mg/L) F- MS + NAA (2 mg/L) + KIN (0.5 mg/L).

Phytochemical screening

Plants have enormous potential to synthesize secondary metabolites and play an important role in plant defense mechanism against prey, microorganisms, insect, herbivores and stress as well as interspecies protection. These secondary metabolites have been used as a drug from the time immemorial, hence screening of phytochemicals serve as the initial steps in predicting the potential active compounds in the plant extracts [22]. The preliminary phytochemical screening of leaf and callus extract of *C. pareira* revealed the presence of major phytochemicals like alkaloids, flavonoids, phenols,

tannins, saponins, steroids, terpenoids, glycosides, carbohydrates, reducing sugars and volatile oils. Gums and resins were found to be absent in leaf and leaf derived callus. Ethanol and methanolic extracts of leaf and callus extracts were proven to be better solvents for the extraction of major phytochemical compared to other solvents (**Table 2**). Arumugam *et al.* reported that maximum phytochemicals were extracted in methanolic extract of leaf and leaf derived callus of *Centella asiatica* [23]. Whereas, Jhonson *et al.* found ethanol to be better solvent for extraction of phytochemicals in *Baliospermum montanum* [24].

Table 3. Preliminary phytochemical screening of leaf and callus extracts

Chemical test	Leaf extract						Callus extract					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Carbohydrate test	+	+	+	-	+	-	-	+	+	-	-	-
Reducing sugars	-	+	+	-	-	-	+	+	+	-	-	-
Alkaloids	-	+	+	+	+	-	+	+	+	+	-	-
Flavonoids	+	-	-	-	-	-	+	+	+	+	-	-
Gums and mucilage	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	+	+	+	-	-	-	-	+	-	+	-	-
Tannins	-	+	+	-	-	-	+	+	+	+	-	-
Phenols	+	+	-	-	-	-	+	+	+	+	-	-
Terpenoids	+	+	+	-	+	-	+	+	+	+	+	+
Saponins	+	-	-	+	+	+	-	-	-	-	-	-
Glycoside	-	-	+	+	-	-	-	-	-	-	-	-
Volatile oil	-	+	+	+	-	-	-	+	+	+	-	+

I-Aqueous, II- Methanol, III- Ethanol, IV-Chloroform, V-Petroleum Ether and VI- Hexane

IV. CONCLUSION

The present study was aimed to develop the effective protocol to induce callus from leaf segments through *in vitro* propagation of medicinally important plant. The preliminary phytochemical screening of leaf and leaf derived callus showed the presence of alkaloids, phenols, flavonoids, steroids, tannins, terpenoids and

volatile oils. This study will provide an efficient protocol for rapid induction of callus for the production of bioactive compounds. There will be possibilities of making plant cell factories for the enhanced production of bioactive compounds, which may prevent the exploitation of natural plant resources.

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