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IN VIVO AND IN VITRO PRODUCTION OF TRITERPENOIDS OF CENTELLA ASIATICAL URBAN

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ABSTRACT

Ayurveda had classified selected plants under "Madhya Rasayana". These are used both in herbal andconventional medicine and offer benefits that pharmaceutical drugs lack, helping to combat illness and support the body's efforts to regain good health and intellect. In the present study production of phytoactive substances of such well known memory enhancing medicinal plant of Indian origin Centellaasiatica L. Urban was attempted through tissue culture and protocol for in vitro regeneration of plantlets.

Leaf and Node explant of C. asiatica cultured on MS basal medium containing 3 mg/l IBA and 3 mg/l kinetin gave maximum proliferation of callus. Triterpenoidsare the important phytoactive substances of the C. asiatica used to increase mental ability.Attempt was made to produce triterpenoids of C. asiatica in vitro. In vivo and in vitro produced triterpenoids were compared using HPTLC. Substances with Rf. 0.09, 0.11, 0.19, 0.24, 0.29, 0.32, 0.43, 0.54, 0.66 and 0.78 were common in both in vivo and in vitro whichwas later confirmed by absorption spectra. Substances withRf. 0.05 and 0.70 were only restricted to in vitro.

Key words: *Centellaasiatica, callus, triterpenoids*

INTRODUCTION

Centellaasiatica (L.) Urban of Apiaceae family commonly known as 'Indian Pennywort' or 'Mandookaparni' (Nadkarni, 1954, Chopra et al. 1956) is an important medicinal plants used for centuries in the ayurvedic system of medicine. In the traditional system of Indian medicine, *C. asiatica* is a reputed nervine tonic (Kakkar, 1988).The leaves of the plant are considered beneficial in improving memory (Nalini et al. 1992; Bakhru, 2003). Asiaticoside, a trisaccharidetriterpenoid, has been identified as the most active compound in the plant (Plohmann et al. 1994). The estimated annual requirement of *C. asiatica*is around 12,700 tons of dry biomass valued at Rs. 1.5 billion (Ahmad, 1993) and is met solely from the natural populations leading to their gradual depletion. Plant tissue culture technology offers alternativeto fulfill the demand of raw material with continues supply and also conserve the endangered medicinal plants (Erdeiet al. 1981, Shoyamaet al. 1983, Huang et al. 2000). Callus culture is used as a tool to obtain the pharmaceutically important triterpenoidcompounds without destroying the whole plant. The interest in *C. asiatica*also led to their production *in vitro*. The present study reports *in vitro* plantlet regeneration as well asproduction of triterpenoids.

MATERIALS AND METHODS

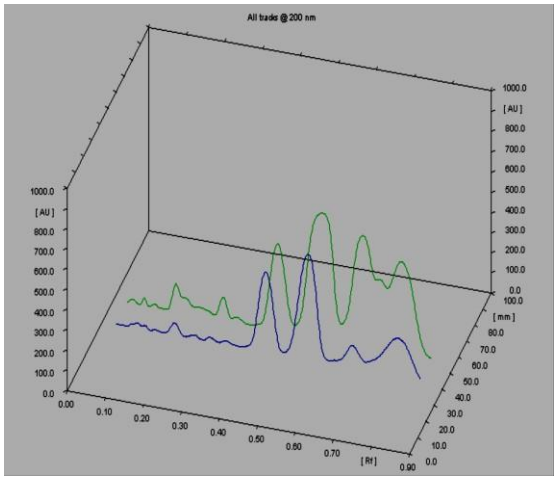
Source material of *C. asiatica* was collected from the Gujarat University botanical garden. The collected material was thoroughly washed with running tap water for 10 min followed by double distilled water. Leaves and nodes were taken as explant. They were surface sterilized with 0.1% (w/v) systemic fungicide - Bavestine followed by 0.1 % (w/v) $HgCl_2$, 5% (v/v) sodium hypochlorite (NaOCl) and 5% (v/v) Tween-20 solution respectively. Finally they were rinsed 5 to 6 time with sterile double distilled water to remove the traces of chemicals and were used for inoculation. The explants were cultured on MS basal medium (Murashige

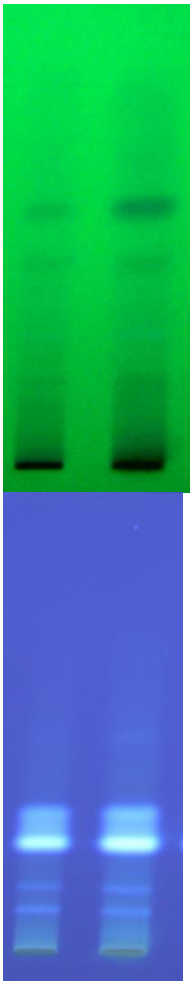
andSkoog1962) supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar-agar and with different levels of auxin(2, 4–D, IBA) and cytokinin (kinetin) to produce callus. The callus so obtained was harvested at the end of 8 week and was used for phytochemical analysis. Triterpenoids were extracted fromleaf and callus using standard method of Daniel, 1991 and were analysed using HPTLC.

RESULTS

Table 1: Media for callus initiation

BasalMedia	Plant Growth Regulators			Rate of callus initiation
	2,4-D (mg/l)	IBA (mg/l)	Kinetin (mg/l)	
Callus **3 mg/l IBA + 3 mg/l kin** MS	-	-	-	-
	1	-	1	-
	2	-	2	-
	3	-	3	-
	4	-	4	-
	-	1	1	-
	-	2	2	++
	-	3	3	++++
	-	4	4	+





Densitometric scanning of bands at 200 nm ***in vivo*** ***in vitro*** ***in vivo*** ***in vitro***

200nm **254 nm**

Table 4: Spots Detected through HPTLC

SubstanceNo.	Samples	Rf	Area %	Remarks / 1 Max
1	C1	-		No peak detected
	C2	0.05	0.20%	200
2	C1	0.09	0.84%	198
	C2	0.09	0.25%	198
3	C1	0.11	0.34%	197
	C2	0.11	0.13%	197
4	C1	0.19	2.03%	198
	C2	0.19	3.26%	198
5	C1	0.24	0.56%	190
	C2	0.24	1.14%	190
6	C1	0.29	0.60%	194
	C2	0.29	1.80%	194
7	C1	0.32	0.54%	196
	C2	0.32	0.37%	196

8	C1	0.43	25.17%	199
	C2	0.43	13.62%	199
9	C1	0.54	40.27%	199
	C2	0.54	30.45%	200
10	C1	0.66	7.15%	195
	C2	0.66	22.25%	195
11	C1	-		No peak detected
	C2	0.70	6.40%	197
12	C1	0.78	22.51%	199
	C2	0.78	20.12%	199

In vitro studies of *C. asiatica* culture showed difficulty due to the heavy fungal contamination. Tap water wash followed by wash with solution of systemic fungicide, Bavistin for 20 minutes, reduces the chances of contamination to a great extent. Callus was obtained from the nodal explant of *C. asiatica* on MS medium supplemented with 2 mg/l IBA + 2 mg/l Kinetin and 3 mg/l IBA + 3 mg/l Kinetin, but best proliferation was obtained on the medium with 3 mg/l IBA +3mg/l Kinetin. Out of various combinations of IBA and Kinetin tried, 1mg/l IBA + 3mg/l Kinetin proved to be the best for multiple shoot regeneration from nodes.
Combinations of 2,4,-D with Kinetin did not responded callus initiation. Surprisingly 2, 4-D, which is generally helpful in initiating callus, did not respond at all. Patraet. al. (1998) reported leaf and stem explant placed on MS supplemented with BA (0.0-5.0 mg/l) Kinetin (0.0-5.0 mg/l
), NAA (0.0-5.0 mg/l) and 2,4–D (0.0-5.0 mg/l) resulted in callus formation in *C. asiatica*within 18-22 days. Earlier Singh and Rastogi (1968) examined the chemical constituent of *C. asiatica* for brahmic acid. Singh and Rastogi (1969) reinvestigated the triterpenoid of *Centellaasiatica*

CONCLUSION

Centellaasiatica L. Urban is a well-known memory enhancing medicinal plant of Indian origin isexploited by local people and pharmaceutical industry. MS medium supplemented with 3 mg/l IBA and 3 mg/l kinetinproduces highly proliferated callus.Triterpenoidsare the important phytoactive substance of the *C. asiatica* used to increase mental ability. *In vivo* and *in vitro* produced triterpenoids were compared using HPTLC. Substances with Rf. 0.09, 0.11, 0.19, 0.24,
0.29, 0.32, 0.43, 0.54, 0.66 and 0.78 were common in both *in vivo* and *in vitro* whichwas later confirmed by absorption spectra. Substances with Rf. 0.05 and 0.70 were only restricted to *in vitro*.Tissue culture technologyis useful in obtaining continuous and reliable source of active components of *Centellaasiatica*through callus.

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