HPTLC FINGERPRINT PROFILE AND CHARACTERIZATION OF DOPAMINE FROM CALLUS CULTURE OF *MIRABILIS JALAPA* L.

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ABSTRACT

Mirabilis jalapa L., the Four-o'clock plant is a multibranched perennial herb of family Nyctaginaceae. The plant is rich in many biomolecules of pharmacological importance and has been used in traditional medicine. One of the secondary metabolites in this plant is Dopamine, a Catecholamine. In the present study, attempts were made for a reliable chromatographic fingerprint profile for dopamine and to quantify the same from callus culture obtained from hypocotyl, epicotyl, cotyledon of invitro seedling of Mirabilis jalapa.L and leaf (ex vitro) by a simple, precise, accurate and rapid high-performance thin-layer chromatographic method. The callus obtained from leaf (ex vitro) of M.jalapa showed a high content of dopamine compared to other callus cultures

Key words: *Mirabilis jalapa,* Dopamine, Callus, Catecholamine, HPTLC, Nyctaginaceae.

INTRODUCTION

Mirabilis jalapa L., the Four-o'clock plant is a multibranched perennial herb of family Nyctaginaceae. The plant is rich in many biomolecules of pharmacological importance and has been used in traditional medicine. One of the secondary metabolites in this plant is Dopamine, a Catecholamine. In the present study, attempts were made for a reliable chromatographic fingerprint profile for dopamine and to quantify the same from callus cultures of *M.jalapa* (white cultivar) by a simple, precise, accurate and rapid high-performance thin-layer chromatographic method. The callus obtained from leaf of Mirabilis jalapa showed a high content of dopamine compared to other callus cultures (0.058%).

MATERIAL & METHOD

Mirabilis jalapa L., a plant of horticultural and medicinal importance was selected for the study. Seeds were collected from greenhouse grown plants of a White flower cultivar (WFC). They were germinated *in vitro* and various parts of seven day old axenic seedlings were used, to study the effect of various phytohormones for initiation and regeneration of callus. Leaf explants from garden grown cultivar was also used.

The leaves of *M.jalapa* were cut into required sizes, surface sterilized with 70% alcohol and 0.1% $HgCl_2$ and inoculated onto MS basal medium (Murashige and Skoog, 1962) supplemented with 2,4-dichlorophenoxyacetic acid(2,4 –D) and Adenine Sulphate (AdS) (M1) and Picloram and AdS (M2) at lower concentrations for callus initiation and proliferation. The cultures were incubated under a photoperiod of 12 hr at 25 ± 2ºC.

EXTRACT PREPARATION FOR PHYTOCHEMICAL STUDIES : 10 gm dried powder of callus obtained from hypocotyls, epicotyls, cotyledon and leaf of *M.jalapa* were accurately weighed and extracted with 50ml of methanol. The mixture was vortexed for 1 minute and kept standing for 1.0 hour. Further it was filtered through Whatmann filter paper no. 41 and the filtrate obtained was subjected to HPTLC analysis.

Standard stock solution of dopamine of concentration 1000.0 µg/ml was prepared in methanol and stored at 4±10ºC.

INSTRUMENTATION AND OPTIMIZED CHROMATOGRAPHIC CONDITIONS: The chromatographic analysis was performed using CAMAG TLC Scanner 4 supported by winCATS planar chromatography manager software version 1.4.7. Samples were spotted using CAMAG Linomat 5 automatic sample spotter equipped with Hamilton syringe (100.0 µL) and CAMAG Reprostar 3 system for photo-documentation. Chromatographic separation was achieved on HPTLC plates (Merck) pre-coated with silica gel 60 F254 (0.2 mm thickness) on aluminum sheet support. Plates were developed in CAMAG twin trough glass chamber pre-saturated with mobile phase of n-butanol: glacial acetic acid: distilled water (8: 2: 2, v/v/v) for 30 minutes. The plates were derivatized in ninhydrin reagent and scanned at 548 nm to detect dopamine in the samples. All measurements were performed at

22 ±1°C

STATISTICAL ANALYSIS: The data was subjected to Analysis of Variance (ANOVA) using IRRISTAT software (IRRI,2003). Treatment means were compared using Least Significance Difference (LSD) values at $p \leq 0.05$. Differences among treatments were tested by Ducan's New Multiple Range Test (DMRT). In the table given in results, mean values followed by same alphabets in superscript (a,b,c,d...) within a column or alphabets above the bars in graphs are not significantly different at ≤ 0.05 level and error bar indicates standard deviation. Percentage values were transformed into arcsine value and were used for comparing treatments by ANOVA.

RESULT

HPTLC analysis using the selected mobile phase showed good resolution. Presence of dopamine in calli obtained from various explants and *in vitro* plantlets was indicated by R_f value 0.5-0.52 and compared with the standard R_f value of dopamine (0.5), (Fig.1).

In addition to dopamine callus extracts also showed other bands with R_f value 0.10, 0.23,0.26,0.29,0.35, 0.41, 0.58.0.68,0.82,0.84 and 0.88 respectively indicating the presence of other components (Fig.1).

Quantitative analysis of dopamine by HPTLC revealed the **presence of dopamine in callus** obtained from *in vitro* as well as *ex vitro* explant and *in vitro* regenerated plantlets of *M.jalapa*. Among the different callus extracts, callus obtained from leaf explant on MS2 showed high amount of dopamine (0.058%) followed by *in vitro* plantlet (0.057%) and epicotyl callus (0.055 %) compared to other callus extracts (Table 2).

Table 1 HPTLC profile of dopamine from callus obtained from *in vitro* explants of axenic plantlet and proliferated callus from leaf explant of *M.jalapa*

Extracts	R_f value
Cotyledon callus on MS1	0.51
Hypocotyl callus on MS1	0.51
Epicotyl callus on MS1	0.52
In vitro plantlet on MS1	0.52
Leaf callus on MS1 medium	0.51
Leaf callus on MS2 medium	0.50
Standard Dopamine	0.50

Table 2 Percentage content of dopamine in callus obtained from cotyledon, hypocotyl, epicotyls, *invitro* plantlet and *ex vitro* leaf explants of *M.jalapa*.

Extracts	Area	Concentration (µg/ml)	Concentration (mg/g)	Mean	SD	% CV	% Content
Cotyledon callus On MS1	11880	89.50	0.447	0.447	0.002	0.553	0.0447 ^{d} (1.20)
	11795	88.86	0.444				
	11925	89.83	0.449				
Hypocotyl callus on MS1	11616	87.52	0.438	0.438	0.004	0.814	0.0438 ^{e} (1.18)
	11554	87.05	0.435				
	11741	88.46	0.442				
Epicotyl callus on MS1	14714	110.72	0.554	0.554	0.004	0.633	0.0554 ^{c} (1.33)
	14625	110.06	0.550				
	14812	111.46	0.557				
***In vitro* plantlet on MS1**	15222	114.53	0.573	0.573	0.002	0.266	0.0573 ^{b} (1.36)
	15212	114.46	0.572				
	15287	115.02	0.575				
Leaf callus on MS1	5784	43.83	0.219	0.219	0.001	0.249	0.0219 ^{f}

medium	5805	43.99	0.220				(1.38)
	5777	43.78	0.219				
Leaf callus on MS2 medium	15478	116.45	0.582	0.587	0.004	0.723	0.0587 [a] (0.84)
	15628	117.57	0.588				
	15700	118.11	0.591				

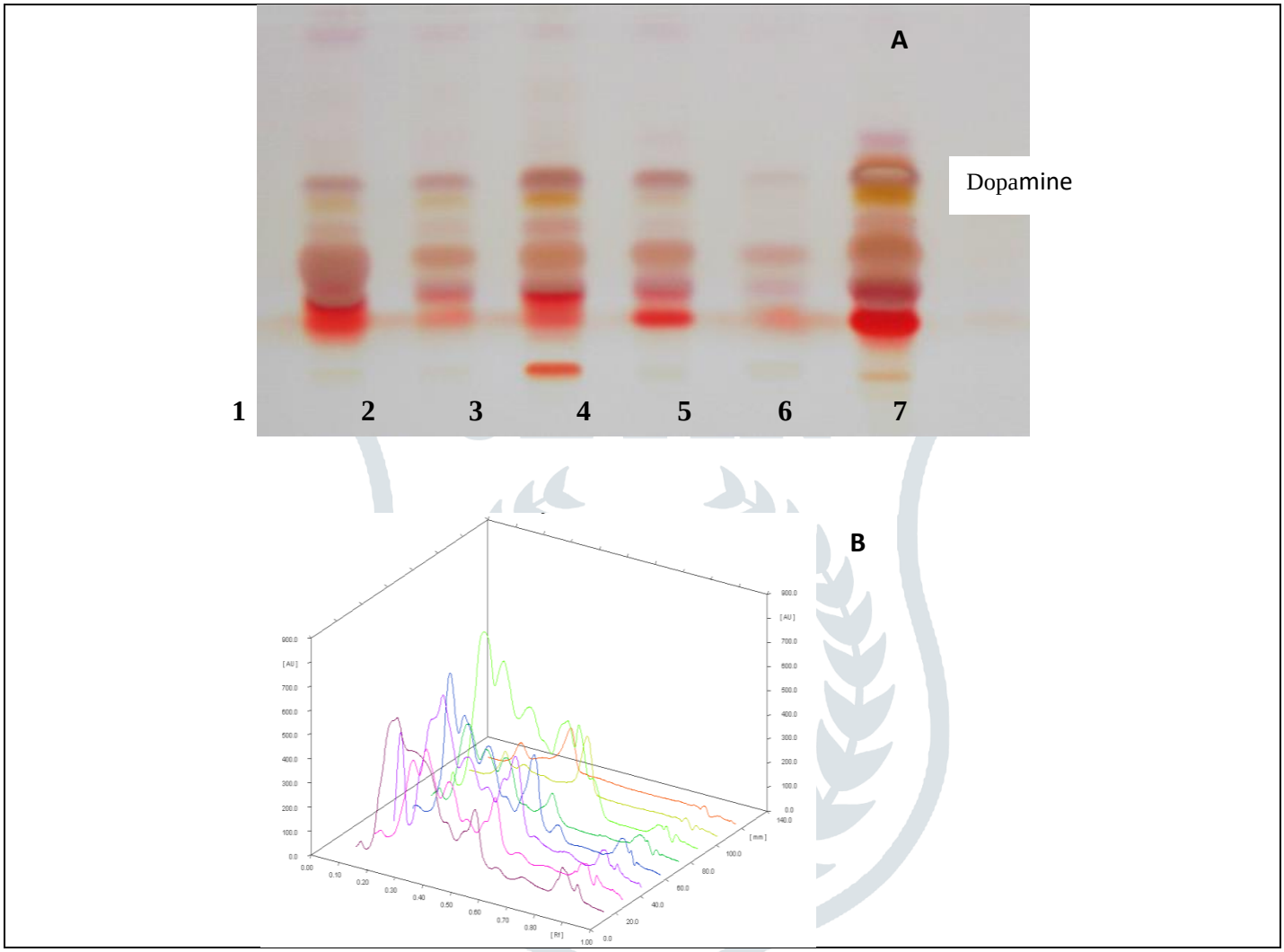

Fig 1 : Visualization and quantification of dopamine in different callus samples of *M. jalapa* by HPTLC method (after derivatization); B : Overlay after derivatization at 548 nm

Track details: 1: Callus from cotyledon, 2: Callus from hypocotyle, 3: Callus from *in vitro* plantlet, 4: Callus from epicotyl , 5: Callus from leaf grown on MS1 medium, 6: Callus from leaf grown on MS2 medium, 7: dopamine (80.0 µg/ml)

DISCUSSION

The study revealed the presence of **dopamine** from callus and *in vitro* regenerated plantlets of *M. jalapa*. Presence of dopamine was reported only in *M. pruriens* and the accumulation of dopamine in callus cultures from leaf explants of *M. pruriens* was reported by Wichers *et al.* (1993). Accumulation of L-dopa in cell cultures of *M. pruriens* is relatively well documented than dopamine (Huizing *et al.*,1985; Chattopadhyay *et al.*, 1994;.Raghavendra *et al.*, 2012) L-Dopa content in callus cultures have also been reported in *Vicia fabia*, *Vicia narbonensis*, *Stizolobium hassjoo*, banana and *Portulaca grandiflora* (Albrecht and Kohlenbach,1990; Sasamoto and Komamine, 1983; Huang *et al.*, 1995; Bapat *et al.*, 2000; Rani *et al*, 2007).

REFERENCES

Albrecht, C. and Kohlenbach, H.W. (1990). L-DOPA content, peroxidase activity and response to H_2O_2 of *Vicia faba* L. and *V. narbonensis* L. *in situ* and *in vitro*. *Protoplasma*, 154 (2-3) : 144 -150.

Bapat, V.A., Suprasanna, P., Ganapati, T.R. and Rao, P.S.(2000) *In vitro* production of L-dopa in tissue cultures of banana. *Pharmaceutical Biology*, 38 : 271-273.

Chattopadhyay, S., Datta, S.K. and Mahato, S.B. (1994) Production of L-DOPA from cell suspension culture of *Mucuna pruriens*. *Plant Cell Reports*, 13 : 519-522.

Huizing, H.J., Wijnsma, R., Batterman, S., Malingre, Th. M.,Wichers, H.J. (1985). Production of L-DOPA by cell suspension cultures of *Mucuna pruriens.* I. Initiation and maintenance of cell suspension cultures of *Mucuna pruriens* and identification of L-DOPA. *Plant Cell Tissue Organ Cult*, 4: 61–73.

Raghavendra, S., Kumar, V., Ramesh, C.K., Khan, M.H. (2012). Enhanced production of L-DOPA in cell cultures of *Mucuna pruriens* L. and *Mucuna prurita* H. *Nat Prod Res*, 26(9) : 792-801.

Wichers, H.J., Wijnsma, J.F., Malingre, T.H. and Huizing, H.J. (1985). Production of L-Dopa by cell-suspension cultures of *Mucuna pruriens*. *Plant Cell Tiss Org*, 4 : 75–82.