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Research Article

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HPTLC fingerprint profile and characterization of dopamine from different parts of four cultivars 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 flower, leaves, root and seed of four different cultivars of M.jalapa (white, pink, yellow and multicolour) by a simple, precise, accurate and rapid high-performance thin-layer chromatographic method. The seed of white flower cultivar showed a high content of dopamine compared to other parts of four cultivars (0.059%).

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

INTRODUCTION

Plants are very important commercial source of chemical compounds with various pharmacological activities. Standardization of the plant material is the need of the day. Modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbs and its formulations [1].

Mirabilis jalapa L. of Nyctaginaceae is a popular ornamental plant grown worldwide for the beauty of its flowers. Available literature on the phytochemical constituents of *M. jalapa* showed that the plant is rich in many active compounds including proteins, alkaloids, terpenes, flavonoids and steroids and has been used in traditional medicine in many parts of the world for the treatment of various diseases [2]. One of the secondary metabolites in this plant is Dopamine [3]. Dopamine is a Catecholamine. Catecholeamines have been found in 44 plant families, but no essential metabolic functions have been established so far [3]. The role of dopamine in plants is poorly documented. It has been proposed to be a precursor for various alkaloids. Dopamine is associated with defense against herbivores, processes such as nitrogen fixation, flowering and prevention of IAA oxidation, intercellular regulation of ion permeability and photophosphorylation of chloroplasts[4] [5]. Dopamine is a neurotransmitter occurring in a wide variety of animals.

EXPERIMENTAL SECTION

1.1. Chemicals: n-butanol GR grade: Loba Chem (Batch No.1120), Glacial acetic acid GR grade: Loba Chem (Batch No.2789), Methanol GR grade: Loba Chem (Batch No.1230), HPLC grade water:Obtained using a Milli-Q purification system (Millipore,USA), Dopamine: Fluka Chemicals (Batch No. 607176) and Ninhydrin: Loba Chem (Batch No. G414808).

1.2. Plant materials: Leaf, flower, root and seed of four different cultivars (WFC- white flower cultivar, PFC-pink flower cultivar, YFC- yellow flower cultivar and MFC- multicolour flower cultivar) of *M.jalapa* were collected

from residential garden at Thane, Mumbai. Authentication of the plant was carried out from Blatter Herbarium, St. Xavier's College, Mumbai (Accession No.2398) and was deposited in the same herbarium. The plant materials were cleaned, air dried and homogenized to fine powder and stored in air tight bottles with proper labelling.

1.3. Sample Preparation: 10 gm dried powder of flower, leaf, root and seed from four cultivars 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±10^{o}C.

1.4. 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^{o}C

1.5. 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.

RESULTS AND DISCUSSION

HPTLC analysis using the selected mobile phase showed good resolution. Chromatograms shown in Fig.2 indicate that all sample constituents were clearly separated without any tailing and diffuseness. After derivatiazation and scanning the plates, R_f value of standard dopamine was found to be 0.51. Presence of dopamine in parts of cultivars obtained was indicated by R_f value 0.5-0.52 and compared with the standard R_f value of dopamine (0.5) (Fig.2). Relative response and R_f values of the characteristic band in *M.jalapa* samples related to the band from standard dopamine were obtained as per the chromatographic conditions. The values were then used to estimate the content of dopamine using regression equation in each sample.

Table 1 depicts a comparative analysis of dopamine content from various parts of four cultivars of *M.jalapa.* Among the plant parts, irrespective of cultivars, seed extract showed high content of dopamine (0.052%) followed by flower (0.040%), leaf (0.035%) and root extract (0.030%) (Table 1).Within the cultivars irrespective of plant parts dopamine content was found to be high in white flower cultivar followed by multicolour flower cultivar, yellow flower cultivar and pink flower cultivar.

Table1.Mean values for percentage content of dopamine analyzed by HPTLC from different parts and cultivars of *M.jalapa*. (Data in parenthesis is arcsine converted value)

Plant parts	Dopamine (%)
Flower	0.040^{b} (1.11)
Leaf	0.035^{bc} (1.04)
Root	0.030^{c} (0.97)
Seed	0.052^{a} (1.31)
LSD	0.12
Cultivars	
WFC	0.043^{a} (1.17)
PFC	0.034^{b} (1.03)
YFC	0.036^{ab} (1.07)
MFC	0.042^{a} (1.16)
LSD	0.12

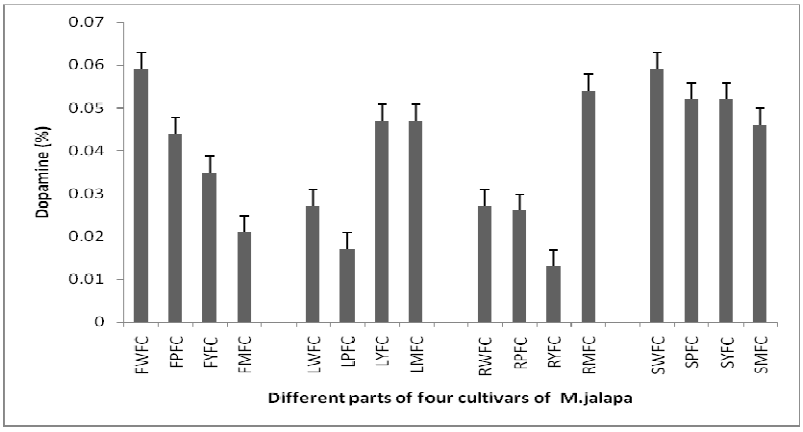
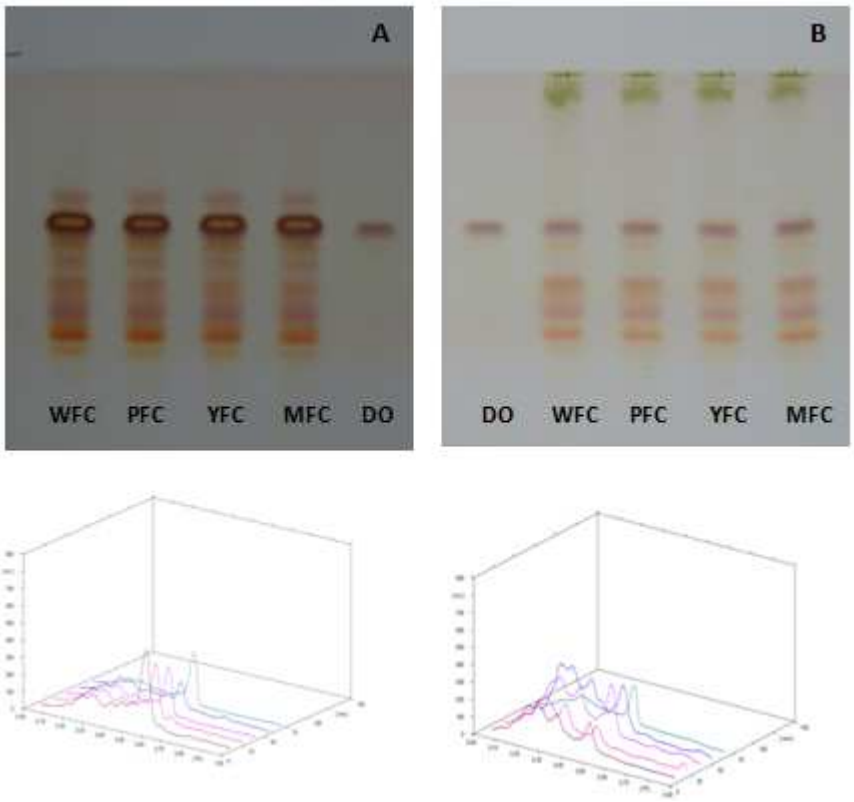


Fig1. The percentage content of dopamine analysed by HPTLC from flower, leaf, root and seed extracts of white, pink, yellow and multicolour flower cultivars of *M.jalapa*

Within the cultivars and plant parts, among flower extracts of all cultivars, dopamine content was found to be high in flower of WFC (0.059%), and in other cultivars it ranged between 0.021- 0.044%. Among leaf extracts, the highest content of dopamine was found in YFC and MFC (0.047%) whereas in other two cultivars it ranged between 0.017 –0.027%. Among root extracts, MFC showed high content of dopamine (0.054%) and in other cultivars it ranged between 0.013- 0.027%. High content of dopamine among seed extracts was found in seed extract of WFC (0.059%) while in PFC and YFC it was found to be similar (0.052%) and in MFC it was 0.046% (Fig1). In this study HPTLC method revealed high percentage of dopamine content in seed extracts of WFC.

As per our knowledge, there is no scientific documentation on quantification of dopamine in *M.jalapa*, though it is reported as a phytoconstituent [6]. Literature survey supports the occurrence of L- Dopa in seeds of many plants [7] with very high concentration in seed of *M. pruriens*[8] [9]. Its presence was also reported in different parts of the plant [10] [11]. Presence of dopamine was reported in the leaves of *M.pruriens* [12].However, in the roots, stems and seeds, no dopamine could be detected at any stage of development. *Mucuna* metabolizes L-Dopa to dopamine in leaves as a protective mechanism against the toxicity of L-Dopa (a potent allelochemical) [13]. Dopamine has also been detected in many other plant families [14] [15].



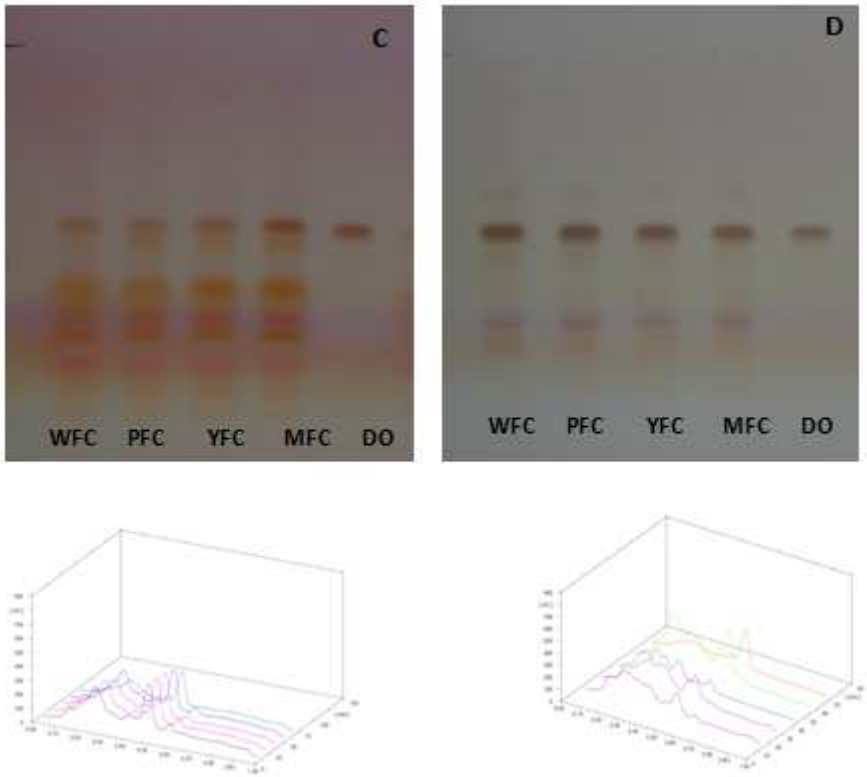


Fig.2 Chromatogram and overlay photo of Flower extracts (A), Leaf extracts (B), Root extracts (C) and Seed extracts (D) of four cultivars of *M.jalapa* after derivatization at 548nm. WFC: White Flower Cultivar, PFC: Pink Flower Cultivar, YFC: Yellow Flower Cultivar, MFC: Multicolour Flower Cultivar and DO: Dopamine standard

CONCLUSION

In the present study we report a comparative analysis of dopamine from different parts (flower, leaf, root and seed) of four cultivars of *M.jalapa* (white, pink, yellow and multicolour flower cultivars). HPTLC proved to be good simple, linear, precise, repeatability, accurate and robust method for dopamine quantification from all parts of four cultivars of *M.jalapa.* This method provides standard fingerprints and can be used as a reference for the identification. In conclusion, the plant can be potentially exploited for dopamine.

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