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LCMS/MS QUANTITATION OF CATECHOLAMINES AND THEIR PREDECESSORS FROM *PORTULACA OLERACEA* AND GOMPHRENA GLOBOSA

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ABSTRACT: The plants *Portulaca oleracea* and *Gomphrena globosa* belong to the order Caryophyllales, and their flowers are known to produce betalains. The biosynthesis of betalains involves various intermediate molecules such as catecholamines or biogenic amines. Catecholamines include dopamine, adrenaline (epinephrine), and noradrenaline (norepinephrine) and are normally believed to be released in response to stress. They are derived from the amino acid tyrosine, with L-DOPA being formed as an intermediate. Catecholamines and their predecessors (tyrosine and L-DOPA) have many medicinal applications and are used in the treatment of Parkinson's disease, hypotension, low cardiac output, glaucoma, and allergic conditions like asthma, to name a few. These biogenic amines and their predecessors have been found in many plant families. Their presence has been reported in *Portulaca oleracea* and *Gomphrena globosa*. The current study focuses at the identification and quantification, by LCMS/MS, of the different catecholamines or their predecessors that are present in *Portulaca oleracea* and *Gomphrena globosa*.

INTRODUCTION: Aromatic biogenic amines are basic nitrogenous compounds formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones. In human and animal cells, they play an important role as neurotransmitters, sources of nitrogen and predecessor for the synthesis of molecules like hormones, alkaloids, nucleic acids and proteins. Biogenic amines have been reported to occur in 44 plant families [1]. Though biogenic amines and their predecessors, as well as their derivatives, have been reported to occur in plant families, no important metabolic role has been assigned to them [2].

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Their role and action are partly understood. Many plant species in the human diet contain biogenic amines and are reported to be active principle ingredients of many medicinal plants [1]. Plants belonging to the order Caryophyllales are known to produce tyrosine-derived pigment betalains, an alternative to anthocyanin [3-5]. Most families under the order are reported to produce betalains [6, 7]. The metabolic pathway of betalains found in plants suggests that they are converted to L-Dopa and tyrosine [8].

L-Dopa and tyrosine have multiple metabolic fates, one of which is their conversion to medicinally important molecules, dopamine, and epinephrine. L-DOP Aquantitation in different *Mucuna* sp., *Vicia faba*, and *Abrus precatorius* has been reported [9-12]. A method for detection of L-DOPA from different parts/tissues from varieties of *Vicia faba* by LCMS has been reported [13, 14]. Dopamine and other alkaloids from *Papaver* rhoeas, callus of

Celosia cristata and *Celosia argentea* var. *plumosa* have also been reported [15- 17]. Screening of plants belonging to the order Caryophyllales has reported the presence of catecholamines, dopamine and epinephrine, in flowers of *P. oleracea* and inflorescence of *G. globosa* [18]. Catecholamines are reported in callus tissue of *P. grandiflora.* Dopamine and norepinephrine has also been quantitated from *P. oleracea* plant by capillary electrophoresis. The current work focuses on quantitation of catecholamines, from flowers of *P. oleracea* and inflorescence of *G. globosa,* by LCMS/MS.

MATERIALS AND METHOD:

Sample Preparation: Inflorescence of *Gomphrena globosa*, the flower of *Portulaca oleracea* were used for preparing extracts for the study. The plant material was collected from the garden of Ramnarain Ruia College, Matunga. Fresh samples were used for preparing the extract. Two types of extracts were made: Acidic and Methanolic. The acidic extract was made by macerating 2 gm of plant material in 1 ml of 0.1N HCl, and the volume made upto10ml with methanol. The methanolic extract was made by crushing 2 gm of plant material in a clean mortar and pestle in 10 ml of methanol. Both the samples were kept for overnight extraction and filtered through a Whatman filter paper no. 41 the next day. The samples were then stored at 4 °C until further use.

Standard Preparation: 10 ml of the1000 ppm stock solution of the standards; L-tyrosine, L-DOPA, Dopamine, and Epinephrine; were prepared. The standards were dissolved in a minimum quantity of 0.1 N HCl, and the volume was made up to 10 ml with methanol.

LC Parameters: HPLC (Shimadzu Prominence Binary Gradient System, Shimadzu Corporation, Japan) equipped with a binary pump (20AD), degasser, an autosampler (SIL-20AC), a temperature-controlled column compartment CTO-20 AC and photodiode array detector (SPD-M20A) was used. Chromatographic data were acquired using Lab solutions software. The analysis was done using Shim-pack MAqC-ODS I (150mm x 4.6 mml. D., 5µm) column. The mobile phase comprised of-(A) 0.1% formic in water (B) 0.1% Formic acid in acetonitrile in a gradient mode.

TABLE 1: GRADIENT PROGRAM USED FOR LIQUID CHROMATOGRAPHY ANALYSIS

Gradient phase HPLC			
Mobile Phase A	0.1% formic in water		
Mobile Phase B	0.1% Formic acid in Acetonitrile		
LC time program	Time(mins)	Mobile Phase A (%)	Mobile Phase B (%)
	3.00	90	10
	5.00	50	50
	6.00	50	50
	6.10	90	10
	12.0	Stop	
Column	Shim-pack MAqC-ODS I (150 mmI. x 4.6mml.D.,5µm)		
Flow rate	0.5 ml/min		
Column Temperature	30°C		
MS Parameters			
Source Type	ESI		
Scan Type	Multiple Reaction Monitoring (MRM)		
Nebulization Gas	Nitrogen,3.0 L/min		
DL Temperature	250°C		
Heat block temperature	400°C		
Drying Gas Flow	15 L/min		

The gradient program is given in **Table 1**. The flow rate was maintained at 0.5 ml/min; injection volume was 20 µl, and the column temperature was maintained at 30 °C. Run time for the analysis was kept 12 min. The quantitation was carried out using LCMS triple quadrupole (LCMS-8040).

LCMS-8040, equipped with the electrospray ionization (ESI) source, was operated in positive ionization mode. Nitrogen gas was used as nebulizing and drying gas at 3.0 L/min and 15 L/min, respectively. The detailed source parameters are given in **Table 1**.

Argon was used as collision gas maintained at 230 kPa. The Multiple Reaction Monitoring (MRM) transitions were optimized using auto MRM optimization function of Lab Solutions software for individual analytes. The software automatically optimizes the CE to form a specific product to get a stable and maximum response. Such 3 products were optimized with different CE for each analyte. The other MS voltages were optimized to get a maximum response of all analytes. The optimized voltages and MRM parameters are given in **Table 2**. The calibration levels of standards were prepared from 10 ppb to 1000 ppb. A batch of calibration levels was acquired, followed by the samples for quantitation. The linearity coefficient (r^2) was achieved more than 0.99. The quantification was carried out through Lab Solution software with appropriate dilution factors of sample preparation. The calibration curves are represented in **Fig. 1.**

Statistical Analysis: Statistical analysis was performed using SPSS 16.0 software. Mean values were compared using ANOVA. A probability P-value of ≤0.05 (P≤0.05) was considered statistically significant. In **Table 3,** given in results, mean values followed by the same alphabet in superscript (a, b, c, d....) within a column are not significantly different [19, 20].

RESULTS AND DISCUSSION: A gradient LC method was developed and optimized for the separation and quantitation of plant catecholamines and their predecessors from plant extracts. Two catecholamine predecessors and two catecholamines were detected from the samples. The catecholamine predecessor L-tyrosine was detected at a retention time (RT) of 3.25 min and the predecessor L-DOPA was detected at 2.32 min. The catecholamine dopamine was detected at 2.10 min while epinephrine was found at 1.88 mins. The representative chromatograms are given in **Fig. 2.**

The concentration of catecholamines as determined from the samples is represented in **Table 3**. Since two different extraction methods have used, a comparison was made across the two methods for each analyte or component from the two plants separately and then statistically validated using SPSS 16.0 software. The highest content of catecholamines and predecessors was reported in methanolic extracts of *G. globosa*, whereas for *P. oleracea* acidic extracts were found to be better. The highest content of L-tyrosine (2673.24 ppb) was reported in *G. globosa,* whereas L-DOPA (68.18 ppb), dopamine (3101.15 ppb), and epinephrine (8801.99 ppb) were present in higher concentrations in *P. oleracea.*

Since, the predecessors were low while catecholamines were in comparatively higher concentration in *P. oleracea* it indicated that the predecessors were most likely directed maximally towards the synthesis of catecholamines. The fate of L-DOPA, wherein it can be converted to a variety of metabolites like epinephrine, betalamic acid, berberine, morphine, codeine, and many others, has been reported [3]. The presence of dopamine from leaves and stem of *P. oleracea* is already reported [21].

TABLE 2: MULTIPLE REACTIONS MONITORING (MRM) DETAILS OF THE CATECHOLAMINESBY LCMS

Compound Name	RT	Transition (m/z)	Q1 PreBias	CE	Q3 PreBias
L-tyrosine	3.25	182.05>91.00	-13	-26	-19
		182.05>136.10	-13	-10	-30
		182.05>165.10	-13	-10	-13
L-DOPA	2.32	198.10>152.10	-10	-13	-30
		198.10>107.05	-10	-13	-22
		198.10>107.05	-14	-24	-21
Dopamine	2.10	154.1>137.10	-11	-13	-29
		154.1>91.00	-11	-24	-19
Epinephrine	1.88	184.1>166.10	-13	-10	-13
		184.1>107.05	-13	-22	-21

The catecholamine content from flowers of *P. oleracea* was not reported. The presence (qualitatively) of catecholamines and/or predecessors from flowers and inflorescence of *P. oleracea* and *G. globosa*re respectively has been reported [18]. However, there are no reports of quantification of the catecholamines and/or predecessors from flowers/inflorescence.

These molecules are medically important and, under normal circumstances, produced as secondary metabolites in low concentrations. Certain kinds of stress in the plant, however, result in an increase in the concentration of these catecholamines. In the future, therefore, these plants can be stressed in the controlled conditions of the laboratory and induced to increase the concentration of catecholamines further. They can then be used as a method of commercially producing catecholamines rather than synthesizing them chemically.

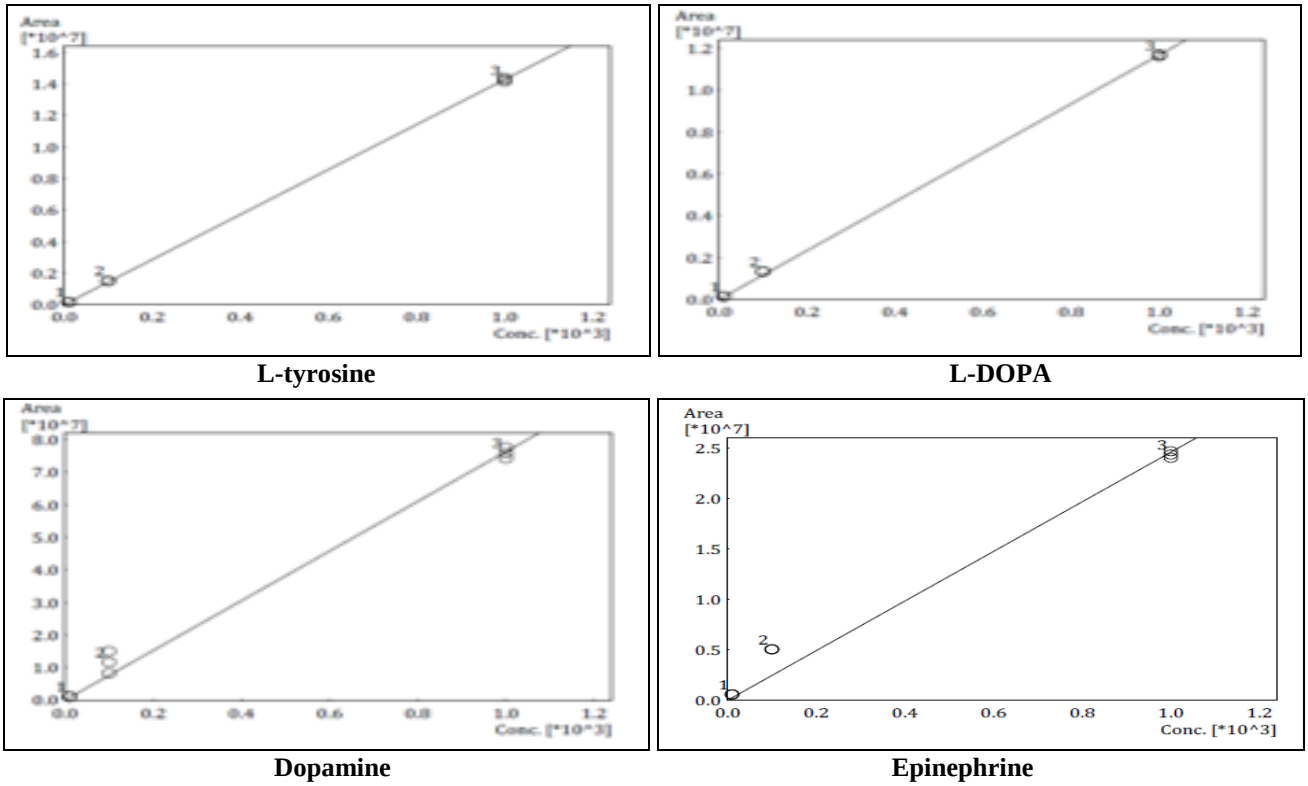
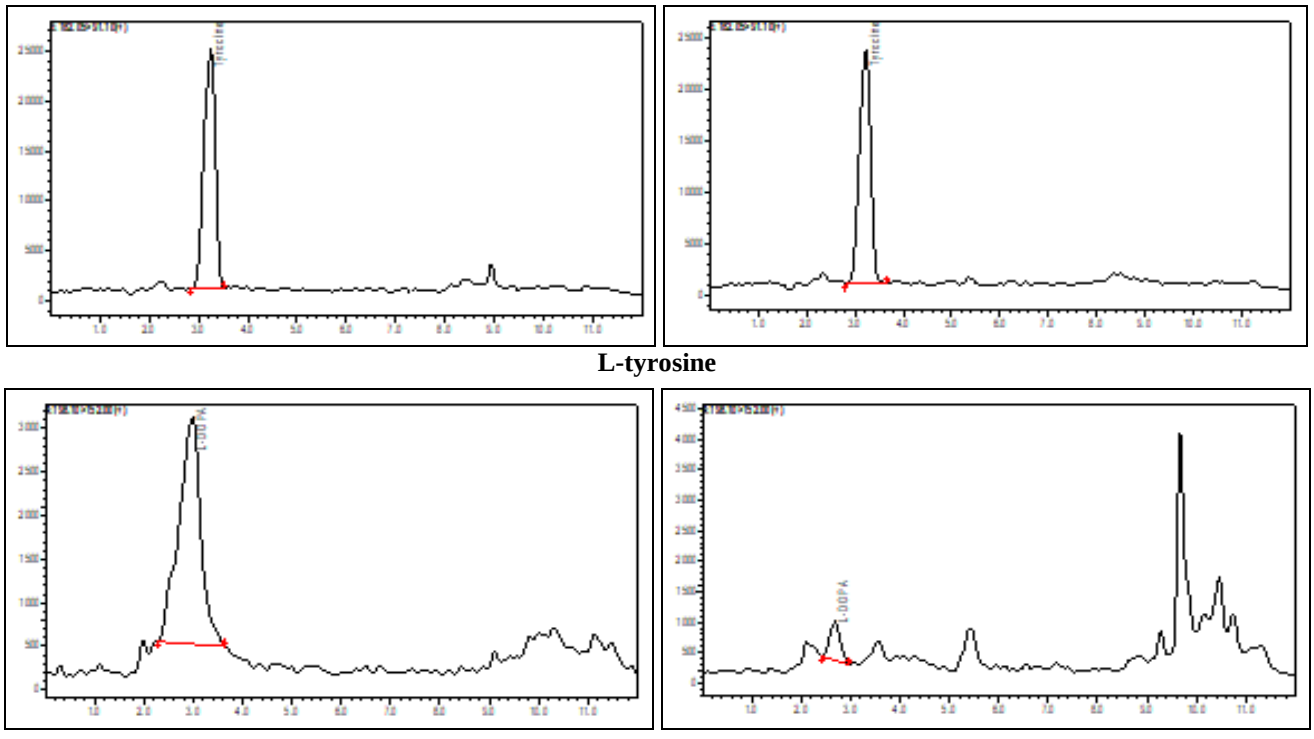
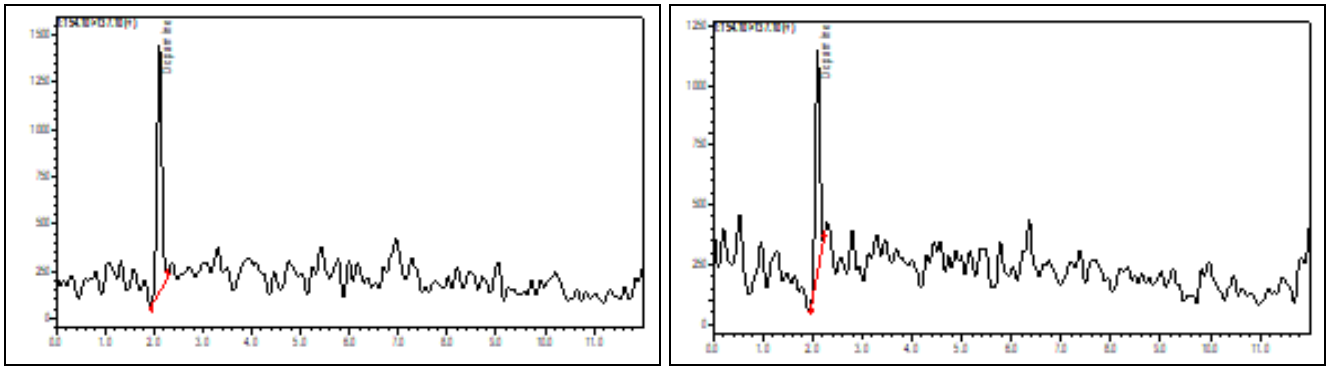
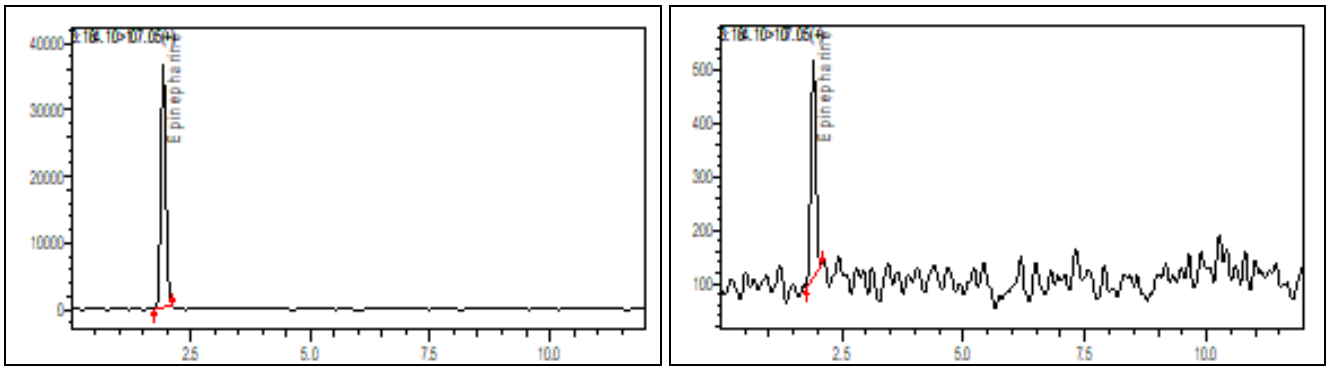


FIG. 1: CALIBRATION CURVE FOR STANDARD L-TYROSINE, L-DOPA, DOPAMINE AND EPINEPHRINE





Dopamine



Epinephrine

FIG. 2: CHROMATOGRAMS FOR CATECHOLAMINES AND THEIR PREDECESSOR FROM ACIDIC EXTRACTS OF *P. OLERACEA* FLOWERS AND METHANOLIC EXTRACTS OF *G. GLOBOSA* INFLORESCENCE

TABLE 3: QUANTITATION (PER GRAM PLANT MATERIAL) OF CATECHOLAMINES PRECURSORS AND CATECHOLAMINES FROM *PORTULACA OLERACEA* AND *GOMPHRENA GLOBOSAUSING* LCMS/MS

Sample Name	Sample ID	Extract used	L-tyrosine (ppb) ± S.D.	L-DOPA (ppb) ± S.D.	Dopamine (ppb) ± S.D.	Epinephrine (ppb) ± S.D.
P. oleracea	PF	Methanolic	385.81± 1.34[a]	N.D.	74.05 ± 0.55[b]	60.65 ± 7.60[d]
P. oleracea	PF	Acidic	387.82 ± 1.69[a]	68.18 ± 1.56	3101.15 ± 115.04[c]	8801.99 ± 211.99[e]
G.globosa	GI	Methanolic	2673.24 ± 6.92[f]	59.14 ± 2.54[h]	69.51 ± 2.68[j]	55.92 ± 6.09
G.globosa	GI	Acidic	144.45 ± 6.21[g]	0.36 ± 0.04[i]	0.21 ± 0.01[k]	N.D.

N.D: Not detectable. The results were statistically validated using SPSS 16.0 software at a significance level of 95%.

CONCLUSION: From the LCMS/MS analysis carried out, it was found that the highest concentration of catecholamines is present in flowers of P.oleraceaand catecholamine predecessors were found in highest quantity in the inflorescence of *G. globosa.* A LC-MS/MS method was successfully developed and optimized for the separation and quantitation of catecholamines. Being a fast method, it saves a lot of time and resources. The study was helpful in identifying the catecholamines dopamine and epinephrine as well as catecholamine predecessor tyrosine and L-DOPA from *P. oleracea* and *G. globosa*.

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CONFLICTS OF INTEREST: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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