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Catecholamines are active plant-based drug compounds in *Pisum sativum*, *Phaseolus vulgaris* and *Vicia faba* Species

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A R T I C L E   I N F O

A B S T R A C T

Introduction: Catecholamines (L-DOPA and dopamine) are the key metabolites found in nervous system and their endogenous deficiency is associated with different patho-physiological disorders. Therefore, it is important to screen the new herbal sources of catecholamines for drug preparation. In this study, the amount of L-DOPA and dopamine were investigated in the leaves and roots of three species from legume family such as *Pisum sativum* (garden pea), *Phaseolus vulgaris* (haricot bean) and *Vicia faba* (broad bean); using TLC and HPLC.
Methods: The seeds of *P. sativum*, *P. Vulgaris* and *V. faba* were treated and cultured under the glasshouse conditions. The extraction from 1 gram of each plant sample was obtained and assayed for L-DOPA and dopamine using thin layer chromatography (TLC) and reversed-phase HPLC.
Results: The results indicated that all cultivars accumulated different levels of L-DOPA and dopamine in leaves and roots. The quantitative results showed that the metabolites concentrations were high in the leaves of *P. Sativum* and *V. faba* compared to that in roots.
Conclusion: The present study may provide a new avenue for preparation and estimation of L-DOPA and dopamine from plant sources and may be used for further analysis and therapeutic studies.

Implication for health policy/practice/research/medical education:
The results of the study may provide a new avenue for preparation and estimation of L-DOPA and dopamine from plant sources and may be used for further analysis and therapeutic studies.

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Introduction

High-tech herbal medicine is a growing field of biotechnology for screening and development of plant components, both for the quality evaluation of crude therapeutic compounds found in plants and understanding of their mechanism of action (1,2). There are different types of herbal drugs to treat pathophysiological disorders. Among them, a number of important herbal-based drugs such as Menoherb, Diacimi and Diablack cohosh tablets have been introduced to treat nervous system. Moreover, it is important to find other valuable herbal sources for drug preparation, each of which may have new opportunities for pharmaceutical industry.

L-DOPA and dopamine are the crucial metabolites found in the brain as well as the rest of the nervous system. These metabolites play the critical role in the control of movement; and different physiological impacts such as stimulating effect on the heart, the circulation and the rate of metabolism (3). L-DOPA and dopamine, moreover, help to modulate brain activity, control coordination and movement, and regulate the flow of information to different areas of the brain. Biochemically, dopamine is a derivative of the tyrosine. Tyrosine is modified by tyrosine hydroxylase to form L-DOPA. L-DOPA decarboxylase then removes carbon dioxide from L-DOPA to for dopamine family (4,5) (Figure 1).

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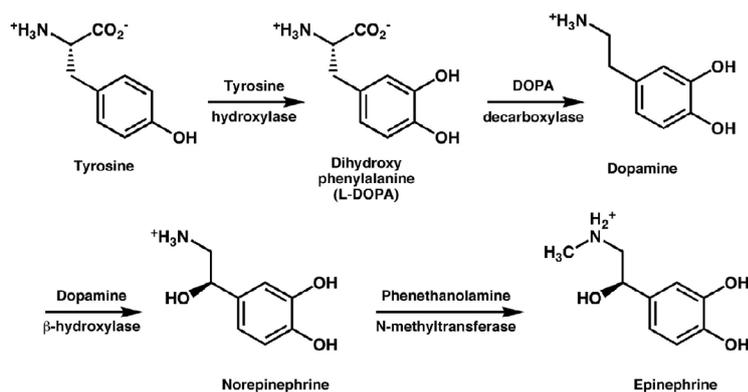
Figure 1. The L-DOPA and dopamine metabolic pathways

Although a number of crops such as beets and bananas contain amino acids, phenylalanine and tyrosine the precursor for synthesis of L-DOPA and dopamine but the occurrence of these compounds have been directly reported in a number of plants (6,7). It was shown that *Prurient mucuna* (the Indian pea) has large amount of dopamine (8). Therefore, the presence of catecholamines in other species of legume including *Pisum sativum* (garden pea), *Phaseolus vulgaris* (haricot bean) and *Vicia faba* (broad bean) was questioned. In Iran, *Pisum sativum* and *Phaseolus vulgaris* are used as herbal drugs due to their medicinal properties, to control the nervous irritability and restlessness in traditional medicine. *V. Faba*, moreover, is recommended for Parkinson's disease (9).

Up to date, there is no more evidence for detection and quantification of dopamine and l-DOPA in legume family particularly, *P. sativum*, *P. Vulgaris* and *V. faba* which are used as cultivars in agriculture. In this study, attempts have been made to discover a new source of L-DOPA and dopamine in legume family. In this study, therefore, the amount of L-DOPA and dopamine were investigated in the leaves and roots of three species from legume family such as *Pisum sativum* (garden pea), *Phaseolus vulgaris* (haricot bean) and *Vicia faba* (broad bean); using TLC and HPLC methods.

Materials and Methods
Materials and chemicals
The RP- TLC plates and analytical grade solvents such as ethyl acetate, n-propanol and acetic acid were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA). L-DOPA and dopamine were obtained from Merck. The HPLC grade water was prepared by a Millipore type water purifier instrument.

Sample preparation
The seeds of *P. sativum*, *P. Vulgaris* and *V. faba* were obtained from the Agricultural Research Center, Isfahan, Iran. Seeds were placed according to the method of Martin *et al.* (10) in a moistened environment at 25 °C in the dark condition. After 24 h, germinated seeds were transferred

into pots and kept under the glasshouse conditions until the four-leaf stages. Then, the plant materials from the roots and leaves in the four-leaf stages were collected and kept away from light, heat and moisture until use.

Extraction of metabolites
Briefly, 1 gram of each plant sample was grinded to powder and was extracted by floating in the extraction solution containing water-ethanol (1:1) and keeping at dark for five days. Then, each sample was subjected to ultrasonication for three minutes, centrifuged at 2000 rpm for 10 min and filtered at 0.22 μm to remove any remaining cell debris.

Preliminary studies by thin layer chromatography
The TLC plates were run as described by Hossaina and Beckerb (11) in ethyl acetate-n-propanol-water-acetic acid (19:2:10:1) at pH 6.2 and spots were visualized by ninhydrin reagent (0.3%). About 10 mg/ml of L-DOPA and dopamine (Merck) were run as standard to detect the place of catecholamines. Moreover, to estimate the concentration of analytes in samples, densitometry–based analysis of each spot was carried out using the GelBandFitter package.

Quantitative studies by high performance liquid chromatography (HPLC)
The spectra from high-resolution reversed-phase HPLC system with a RP C18 column was used to perform the analyses (12) Detection was done using a photodiode array detector and the detector was operated at 280 nm. Chromatographic software Chromoquest 30 was applied for data collection and processing.

Standard stock solutions of L-DOPA and dopamine were prepared with water to a concentration 10 mg ml^{-1} and stored at 4 °C. The working concentration ranges from 0.25-25 mg ml^{-1} were prepared by diluting with water to obtain the calibration curves. Then, the standard graphs were prepared using peak area versus concentrations of working solutions in mobile phase. The concentrations of L-DOPA and dopamine in the plant samples were calculated by comparison with peak intensity obtained

from standard solutions.

Statistical analysis and data mining
The ANOVA analysis was performed using Statgraphics Centurion plus 5. The statistical difference between means of variables were tested using Dancan test (Statgraphics Centurion plus 5).

Results
Sample Preparation and Extraction of Bio-component
The cultured seeds of *P. sativum*, *P. Vulgaris* and *V. faba* were fallowed until the four-leaf stage, under the glasshouse condition. The extracted compounds from 1 g of leaves and roots with the extraction solvent including water-ethanol (1:1) were compared at 280 nm in order to estimate the extraction conditions (data was not shown). The UV-visible spectroscopy analysis showed a reliable increase in OD absorption at 280 nm, when compared with the control. The increase in absorbance at 280 nm was attributed to the increase of the active compounds such as dopamine and L-DOPA. The extracted bio-components were used for further analysis to assay the dopamine and L-DOPA.

TLC of Extracted bio-components
As the preliminary studies, the plants extracted from the roots and leafs were separated on TLC plates (Merck) and process using ninhydrin. The content of L-DOPA and dopamine metabolites were studied by comparing the Rf obtained from both the standard and the sample spots on chromatography (Figure 2) and semi-quantitative study was performed using the GelBandFitter software (data not shown). It has been reported that in *M. purient*, which belongs to the legume family, the
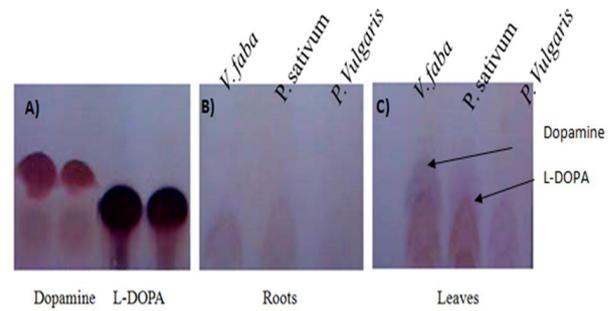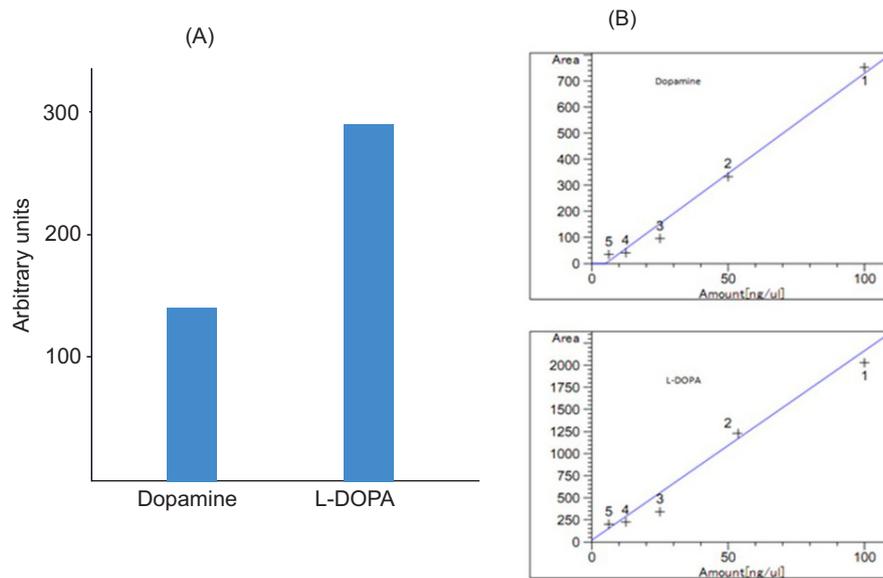
Figure 2. TLC analysis of L-DOPA and dopamine. A. Standard spots of catecholamines. B and C). The amount of L-DOPA and dopamine in roots and leaves of plant samples.

catecholamine compounds synthesis actively. Due to the close phylogenetic relationship between *M. purient* and *P. sativum*, *P. Vulgaris* and *V. faba*, the presence of the catecholamine in these species is interesting. As it can be seen in Figure 2, the amount of L-DOPA and dopamine on the leaves of all plants is greater than the roots.

Quantitative Studies by HPLC
The HPLC-based methods for the determination and evaluation of bioactive compounds from plants have been taken into consideration in recent years. To determine the amount of L-DOPA and dopamine in plant samples, the chromatographic approaches were used. Briefly, the mobile phase was consisting of HPLC grade water and the presence of each analyte was studied at 280 nm. The retention time was 1.8 min and 2.3 min for dopamine and L-DOPA, respectively, at a flow-rate of 1 ml min^{-1}. A good separation (with symmetry peak) from the HPLC was achieved using the RP C18 column for the standard concentrations of L-DOPA and dopamine (Figure 3A).

Figure 3. Figure 3A shows a typical signal obtained from the analysis of a standard and solution of commercial L-DOPA and dopamine using the proposed method. Data are the average results of three independent experiments. Figure 3B shows the regression equation and correlation coefficient (r) for L-DOPA and dopamine. The HPLC-based assays show a good level of linearity.

Furthermore, observed retention times (1.8 min and 2.3 min) allowed a rapid assay of L-DOPA and dopamine.

The linearity of the HPLC method was assayed using the calibration standard solutions of L-DOPA and dopamine. The linear ranges of concentration were found to be 1-100 mg ml^{-1} for both the L-DOPA and dopamine. The regression equation and correlation coefficient (r) were $y=7.7x+38.99$ (y: peak area, x: concentration) and 0.9925 for L-DOPA and $y=21.47x+21.24$ and 0.9926 for dopamine, respectively. The linearity of the HPLC assays was evaluated by the high correlation coefficients (r) for the regression equations, and the curves showed a reliable intensity consistent with the standard concentrations of analytes (Figure 3B). Therefore, the method was supposed as a fast and reliable way for preparation and quantitative assay of L-DOPA and dopamine in both leaves and roots of *P. sativum*, *P. Vulgaris* and *V. faba*.

The amount of L-DOPA and dopamine in each extraction solution from *P. sativum*, *P. Vulgaris* and *V. Faba* were analysed using the validated HPLC method. About 10 ml of each extract assayed using HPLC and the concentration of analytes was calculated using the standard regression equation (Table 1 and 2).

The data, which are obtained from HPLC, are consistent with the results obtained with TLC. The concentration of both the L-DOPA and dopamine in leaves are more than the concentrations in roots. The overall content of L-DOPA, the precursor of dopamine was obtained more than dopamine in roots and leaves of *P. sativum*, *V. faba* and *P. vulgaris*, respectively. Therefore, it seems that the L-DOPA is imported not only in the dopamine biosynthesis but also in other metabolic pathways (13,14). Finally, the results showed significant levels for L-DOPA and dopamine in *P. sativum*, *V. Faba* and *P. Vulgaris* which may be used in further studies to examine the

effects of these plants in neurological disorders such as Parkinson's disease.

Discussion

In this paper, the extraction and extermination of dopamine and L-DOPA from *P. sativum*, *P. Vulgaris and V. faba* from the roots and leaves was carried out. The results may be used for further therapeutic studies.

L-DOPA and dopamine are important metabolites found in the nervous system. It was suggested that *Prurient mucuna* (the Indian pea) contains a high content of L-DOPA in the seeds, roots and leaves (14). In this study, the amounts of catecholamines in the roots and leaves of three other species from legume family including *P. sativum* (garden pea), *P. vulgaris* (haricot bean) and *V. faba* (broad bean), were investigated. The fractionation procedure was carried out and samples were subjected to spectroscopy studies. A significant increase in OD absorption was obtained when extracted fractions were assayed at 280 nm. An increase in ultraviolet wavelength was discussed which resulted in increasing of the concentration of bio-active compounds such as dopamine and L-DOPA. Furthermore, it can be assumed that the extraction conditions were effective enough to obtain the aromatic components.

The TLC studies confirmed the presence of L-DOPA and dopamine on the leaves and roots in all samples (Figure 2). Interestingly, the accumulation of L-DOPA and dopamine in leaves was more than roots. Therefore, although the metabolism pathway of catecholamines in legume family is not clear in details, it can be proposed that the metabolic pathway might be active in the production of catecholamine compounds on the leaves (13,14). Furthermore, the results confirmed that the leaves are more appropriate than the roots for the purposes of therapeutic treatment. The quantitative studies to assay the amount of L-DOPA and dopamine in each extraction solution from *P. sativum*, *P. Vulgaris* and *V. Faba* were done using the HPLC method (Table 1 and 2). The results were consistent with the data from TLC and showed that the extraction from *P. sativum*, *P. Vulgaris and V. faba* contained both L-DOPA and dopamine and the concentrations of these metabolites in leaves were more than that was detected in roots. The data provided in this study suggested that TLC was a reliable method for detection of catecholamines and the data obtained from this technique was matched from that gained from HPLC. Furthermore, the results were in consist with the previous reports in embryo of velvet bean and *V. faba* (broad beans) (*Mucuna pruriens*) in which, the catecholamines actively metabolism by plant (8,13,15). In conclusion, the present study may provide a new and reliable protocol for extraction of L-DOPA and dopamine from *P. sativum*, *P. Vulgaris and V. faba* for further analysis and therapeutic studies.

Table 1. The amount of L- DOPA in leaves and roots of *P. sativum*, *P. Vulgaris* and *V. faba*. The Mean values and analysis of variance. Values followed by different capital letters are significantly different per P < 0.01. n = 4

Species	L-dopa in the leaves (ng.g FW^{-1})	L-dopa in the root (ng.g FW^{-1})
P. sativum	67.93A	64.16A
P. Vulgaris	20.33C	14.05C
V. faba	63.41B	20.52B

Table 2. The amount of dopamine in leaves and roots of *P. sativum*, *P. Vulgaris* and *V. faba*. The Mean values and analysis of variance. Values followed by different capital letters are significantly different per P < 0.01. n = 4.

Species	Dopamine in the leaves (ng.g FW^{-1})	Dopamine in the root (ng.g FW^{-1})
P. sativum	19.21A	4.131A
P. Vulgaris	9.67C	3.86B
V. faba	15.21B	3.63C

Author' contributions

MK designed the project; MK, FG, GM performed the experiments; MK provided expertise and access to analysis

of metabolites; MK, FG, GM analysed the data; MK, RE wrote the paper; all authors read it.

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