

Original Article

PHENYLPROPANOIDS AND FATTY ACIDS LEVELS IN ROOTS AND LEAVES OF *DATURA STRAMONIUM* AND *DATURA INNOXIA*

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ABSTRACT

Objective: The aim of this research was to determine and compare phenylpropanoids and fatty acids composition in two plant species, *Datura innoxia* and *Datura stramonium*.

Methods: Phenylpropanoids and fatty acids composition in leaves and roots extracted from *Datura innoxia* and *Datura stramonium*, grown under greenhouse conditions, was analyzed by gas chromatography–electron impact/time of flight-mass spectrometry (GC-EI/TOF-MS) chromatography techniques. Analyses were carried out at the Max Planck Institute for Molecular Plant Physiology of Golm (Germany).

Results: We revealed that *Datura stramonium* (DS) contains hydroxy-hexanedioic acid while hexanoic acid was found in *Datura innoxia* (DI). Also, two fatty acids are common to both *Datura* species, hexadecanoic acid and octadecanoic acid, with an almost equal rate between leaves and roots. However, phenylpropanoids composition revealed eight compounds; luteolin, quercetin, trans-caffeic acid, trans-ferulic acid, cis-caffeic acid, cis-4-hydroxy-cinnamic acid, trans-4-hydroxy-cinnamic acid and trans-sinapic acid in DI. However, in DS, five compounds were detected: luteolin, quercetin, trans-caffeic acid, trans-ferulic acid and dihydroferulic acid. Also in both *Datura* species, phenylpropanoids concentration in leaves was significantly higher than in the roots.

Conclusion: Our results showed a difference in phenylpropanoids and fatty acids compositions between the two *Datura* species, with a significantly higher concentration of phenylpropanoids in *Datura innoxia* than in *Datura stramonium*

Keywords: Phenylpropanoids, Fatty acids, *Datura stramonium*, *Datura innoxia*, GC-EI/TOF-MS.

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INTRODUCTION

Datura, a member of Solanaceae, is distributed in warm regions of the world [1]. Various species of *Datura* are known and widely employed for their medicinal and toxic properties based on the presence of tropane alkaloids that are used as parasympatholytics. These compounds, highly used in pharmaceutical industry, are only present in plants [2]. Tropane alkaloids can have critical pharmaceutical applications in small doses; for instance, they are used in making muscle relaxants, painkillers, tranquilizers and psychotropic drugs [3]. In addition to alkaloids, *Datura* also produces significant metabolites such as phenylpropanoids and fatty acids. Phenylpropanoids are secondary metabolites, particular to plant kingdom, derived from phenylalanine pathway: they are involved in several functions such as fertility, pigmentation, woodiness, protection against biotic and abiotic agents [4, 5]. Flavonoids are the main phenolic compounds of phenylpropanoids class and consist of several subgroups including flavones (apigenin, luteolin), flavonols (kaempferol, quercetin), flavanones (naringenin, eriodictyol), flavanonols (dihydroquercetin, dihydromyricetin), and isoflavones (daidzein, genistein). Flavonoids are used to enhance renal excretion; they are also used as a treatment of senile cerebral insufficiency, dementia and problem of memory loss [6]. In chemistry, a fatty acid is a carboxylic acid with a long aliphatic chain. All fatty acids are primary metabolites present in plants. These include palmitic acid, stearic acid, oleic acid, linoleic and linolenic acid [7]. Most fatty acids present in nature take the form of glycerol esters. They are found in most living organisms where they play a key role in energy storage. Also, some lipid molecules affect the biogenesis and the function of various cell membranes among many other biological functions [8].

The application of gas chromatography–mass spectrometry (GC–MS) to the analysis of metabolites in complex samples has now become routine. It is a useful method for separation and identification of complex mixtures of tropane alkaloids [9, 10]. Time-of-flight (TOF)-GC–MS metabolite profiling is based on highly reproducible electron

impact ionization and can be provided by single chromatograms, 200–1000 mass spectral components [11]. It represents, until now, an unknown magnitude of metabolite detection due to a unique combination of high-resolution gas chromatography with a rapid and sensitive time-of-flight mass analyzer [12, 13].

To the best of our knowledge, no information is available on phenylpropanoids and fatty acids composition in roots and leaves of *Datura stramonium* and *Datura innoxia*, which is the focal point of the present work.

MATERIALS AND METHODS

Plant materials

Datura stramonium (DS) and *Datura innoxia* (DI) seeds were harvested in the experimental area of the National School of Agronomy (ENSA-Algiers) (36 ° 43' 15" north, 3 ° 08' 59" East). Before sowing (in loam as substrate), seeds were scarified with nail clipper and glass paper (80 points) for DI and DS respectively [14]. Four weeks later, DI seedlings (10 seedlings) were transferred to pots with loam as substrate and kept for three weeks, then the roots and leaves were collected and dried for 48 h at 50 °C. On the other hand, *Datura stramonium* seedlings (10 seedlings) were transferred to a hydroponics system, containing nutritional MS [15] mineral solution without sugar and vitamins (macro-elements, micro-elements, iron, pH 5.8). After three weeks, roots and leaves were collected and dried for 48 h at 60 °C.

Chemicals and reagents

Chemicals and reagents comprise: MS medium (Duchefa Biochimie), Methanol gradient grade for liquid chromatography (Merck), Chloroform for liquid chromatography (Merck), Ribitol (Sigma), DL-Alanine, 2,3,3,3-d4 (Sigma), nonadecanoic acid methyl ester (Sigma),

1.5-mL Safe-lock, tapered-bottom microvial (Eppendorf, Hamburg, Germany) and 2.0-mL Safe-lock, round-bottom microvial (Eppendorf).

Phenylpropanoids and fatty acids extraction

Dried roots and leaves samples of *Datura stramonium* and *Datura innoxia* were pulverized by liquid nitrogen. Phenylpropanoids and fatty acids were extracted from 20 mg powder of roots or leaves in 360 µl of mixture made of 300 µl pre-cooled methanol, 30 µl internal standard solution (2 mg/ml ¹³C₆-sorbitol), and 30 µl nonadecanoic acid methyl ester (2 mg ml⁻¹ stock in chloroform) followed by vortexing and incubation at 70 °C for 15 min under shaking. Afterwards, 200 µl of chloroform were added, followed by incubation at 37 °C for 5 min under shaking. After adding 400 µl water, the extract was vortexed and the polar phase was separated by centrifugation. Aliquots of 160 µl from the polar metabolite fraction were dried by a speed Vac and stored dried under inert gas at -20 °C (Protocol given by the group of Applied Metabolome Analysis, Max Planck Institute for Molecular Plant Physiology of Golm (Germany)).

GC-IE-TOF-MS chromatography

Phenylpropanoids and fatty acids profiling was performed as detailed previously [16, 11] by gas chromatography coupled with electron impact ionization/time-of-flight mass spectrometry (GC-EI/TOF-MS) using an Agilent 6890N24 gas chromatograph (Agilent

Technologies, Böblingen, Germany; <http://www.agilent.com>) with 1/30 split and splitless injection of 1 µl onto a Factor Four VF-5 ms capillary column, 30 m length, 0.25 mm inner diameter, 0.25 µm film thickness (Varian-Agilent Technologies, Waldbronn, Germany) which was connected to a Pegasus III time-of-flight mass spectrometer (LECO Instrumente GmbH, Mönchengladbach, Germany; <http://www.leco.de>).

Statistical analysis

Each compound was analyzed with three replicates and data were statistically assessed by the standard deviation (SD) of the mean values.

RESULTS AND DISCUSSION

In the present study, we identified phenylpropanoids and fatty acids composition of roots and leaves in *Datura stramonium* and *Datura innoxia*

The results showed that four phenylpropanoids are common to DS and DI: luteolin (1), quercetin (2), trans-caffeic acid (3) and trans-ferulic acid (5). Moreover, DS contains dihydroferulic acid (4), whereas DI contains cis-caffeic acid (6), cis-4-hydroxy-cinnamic acid (7), trans-4-hydroxy-cinnamic acid (8) and trans-sinapic acid (9) (table 1). In a study conducted in 2014 by Pant [17], two phenylpropanoids (cis-sinapic acid and trans-ferulic acid), and one fatty acid (octadecanoic acid) were found in *Arabidopsis thaliana*.

Table 1: Phenylpropanoids composition in *Datura stramonium* and *Datura innoxia*

<i>Datura stramonium</i>	Molecular formula	Molecular weight (g/mol)	<i>Datura innoxia</i>	Molecular formula	Molecular weight (g/mol)
Luteolin (1)	C ₁₅ H ₁₀ O ₆	286.24	Luteolin (1)	C ₁₅ H ₁₀ O ₆	286.24
Quercetin (2)	C ₁₅ H ₁₀ O ₇	302.236	Quercetin (2)	C ₁₅ H ₁₀ O ₇	302.236
Trans-Caffeic acid (3)	C ₉ H ₈ O ₄	180.157	Cis-Caffeic acid (6)	C ₉ H ₈ O ₄	180.157
Dihydroferulic acid (4)	C ₁₀ H ₁₂ O ₄	196.2	Trans-Caffeic acid (3)	C ₉ H ₈ O ₄	180.157
Trans-Ferulic acid (5)	C ₁₀ H ₁₀ O ₄	194.18	Cis-4-hydroxy-Cinnamic acid (7)	C ₉ H ₈ O ₃	164.158
			Trans-4-hydroxy-Cinnamic acid (8)	C ₉ H ₈ O ₃	164.158
			Trans-Ferulic acid (5)	C ₁₀ H ₁₀ O ₄	194.18
			Trans-Sinapic acid (9)	C ₁₁ H ₁₂ O ₅	224.21

The concentration of phenylpropanoids in leaves was higher than that in roots in both species. Compounds 1 and 2, identified in DS and DI, belong to the flavonoids family.

Compound 1 was characterized as luteolin (3', 4', 5, 7-tetrahydroxyflavone) (fig. 1), which is a common flavonoid that exists in many species of plants and organs, including fruits and medicinal herbs [18]. In the leaves of both species, the same amount of luteolin was found, while DS leaves the amount of luteolin was almost three times more than in DS roots. However, no luteolin was found in DI roots (table 2, table 3).

Compound 2 was identified as quercetin (sophoretin; meletin; xanthaurine) (fig. 1). The molecular formula of quercetin is C₁₅H₁₀O₇ with a molecular weight and density of 302,236 g/mol and 1, 8 g/cm³, respectively. Quercetin concentration in DS leaves was 0.549 mg/g, which is the double of quantified amount in roots (0.224 mg/g). Similar to luteolin, quercetin was not detected in DI roots (table 2, table 3).

Compound 3, trans-caffeic acid (3,4-dihydroxyphenyl, acrylic acid) (fig. 1) has the molecular formula C₉H₈O₄ and the molecular weight of 180.157 g/mol. Levels of trans-caffeic acid in leaves were almost

the same in both species; however, in roots, only traces were found (table 2, table 3).

Compound 4, dihydro ferulic acid (4-hydroxy-3-methoxy-benzenepropanoic acid; 4-hydroxy-3-methoxy-hydrocinnamic acid; β-3-methoxy-4-hydroxyphenylpropionic acid; 3-methoxyphloretic acid; dihydroconiferyllic acid; hydro ferulic acid) (fig. 1), has almost the same amount in both leaves and roots of DS (table 2).

Compound 5 was identified as trans-ferulic acid (4-hydroxy-3-methoxycinnamic acid; trans-4-hydroxy-3-methoxycinnamic acid) (fig. 1). This compound is a ubiquitous plant constituent that arises from the metabolism of phenylalanine and tyrosine by shikimate pathway in plants [19]. Trans-ferulic acid concentrations in the leaves and roots of DS were 0.767 mg/g and 0.521 mg/g, respectively. In DI leaves, the concentration was 0.67 mg/g and only 0.02 mg/g in the roots (table 2, table 3).

Table 2: Composition and levels of phenylpropanoids in *Datura stramonium* roots and leaves

Phenylpropanoids	Leaves (mg/g)	Roots (mg/g)
Luteolin	0.6677±0.2829	0.2782±0.1373
Quercetin	0.5494±0.2025	0.2246±0.0987
Trans-Caffeic acid	0.5803±0.08991	0.1759±0.0492
Dihydroferulic acid	1.1961±0.3370	1.0064±0.3017
Trans-Ferulic acid	0.7676±0.1158	0.5213±0.0767

Data represented as mean±SD (n=3).

Compound 6, cis-caffeic acid (caffeic acid pure, caffeic acid; 3-(3,4-dihydroxyphenyl)prop-2-enoic acid; 3-(3,4-Dihydroxyphenyl) acrylic acid) (fig. 1), is an organic compound classified as a hydroxycinnamic acid. It is found in all plants because it is a key intermediate in the biosynthesis of lignin, one of the principal components of plant biomass and its residues [20]. Cis-caffeic acid was present only in the DI leaves with a concentration of 0,371 mg/g (table 3).

Compound 7 was identified as cis-4-hydroxy-cinnamic acid ((2Z)-3-(4-hydroxyphenyl) acrylic acid, (2Z)-3-(4-hydroxyphenyl) prop-2-enoic acid) (fig. 1). The molecular formula is C₉H₈O₃; with the molecular weight of 164.158 g/mol. In our study, we recorded the roots content of cis-4-hydroxy-cinnamic acid twice higher than in the leaves of DI (table 3).

Compound 8 was identified as trans-4-hydroxy-cinnamic acid ((2E)-3-(4-hydroxyphenyl) acrylic acid; (4-Hydroxycinnamic acid; (E)-p-

coumaric acid; 2-Propenoic acid, 3-[4-(trimethylsiloxy)-phenyl]) (fig. 1). DI contents were 0.41 mg/g and 0.13 mg/g in leaves and roots, respectively (table 3).

Compound 9, trans-sinapic acid (trans-4-Hydroxy-3,5-dimethoxy-cinnamic acid) (fig. 1) a small naturally occurring hydroxycinnamic acid and a member of the phenylpropanoids family with a molecular formula C₁₁H₁₂O₅; and a molecular weight 224.21 g/mol, was the most abundant phenylpropanoid in the DI leaves. However, it was null in the roots (table 3).

In the second part of this work, the composition and contents of fatty acids in DS and DI were also determined. Hexadecanoic acid and octadecanoic acid were found in DI and DS with approximately equal rates between the leaves and the roots of each species. Additionally, the third fatty acids found in DS and DI were hydroxyl-hexanedioic acid and hexanoic acid respectively (table 4).

Table 3: Composition and levels of phenylpropanoids in *Datura innoxia* roots and leaves

Phenylpropanoids	Leaves (mg/g)	Roots (mg/g)
Luteolin	0.6322±0.2887	0±0
Quercetin	0.6044±0.2714	0±0
Cis-Caffeic acid	0.3719±0.0755	0±0
Trans-Caffeic acid	0.5045±0.0070	0.0106±0.0033
Cis-4-hydroxy-Cinnamic acid	0.5293±0.0111	0.2944±0.0419
Trans-4-hydroxy-Cinnamic acid	0.4129±0.0225	0.1391±0.0153
Trans-Ferulic acid	0.6702±0.0385	0.0297±0.0035
Trans-Sinapic acid	0.9157±0.1833	0±0

Data represented as mean±SD (n=3).

Table 4: Fatty acids composition of *Datura stramonium* and *Datura innoxia*

<i>Datura stramonium</i>	Molecular formula	Molecular weight (g/mol)	<i>Datura innoxia</i>	Molecular formula	Molecular weight (g/mol)
Hexadecanoic acid (10)	C ₁₆ H ₃₂ O ₂	256.424	Hexadecanoic acid (10)	C ₁₆ H ₃₂ O ₂	256.424
Hydroxy Hexanedioic acid (12)	C ₆ H ₁₀ O ₅	162.140	Hexanoic acid (13)	C ₆ H ₁₂ O ₂	116.16
Octadecanoic acid (11)	C ₁₈ H ₃₆ O ₂	284.477	Octadecanoic acid (11)	C ₁₈ H ₃₆ O ₂	284.4772

Data represented as mean±SD (n=3).

Compound 10 obtained was hexadecanoic acid (palmitic acid, cetyl acid) (fig. 2), the most common fatty acid saturated found in animals, plants and microorganisms [21]. It is the major component of the palm oil. Palmitic acid is used to produce soaps, cosmetics, and release agents. Hexadecanoic acid concentration in the leaves and the roots of DI and in the leaves of DS was almost equal (table 5, table 6). In DS roots, the amount of hexadecanoic acid was found 10% higher than that in the leaves.

Compound 11, octadecanoic acid (stearic acid) (fig. 2), is one of the most common saturated fatty acids found in nature. Stearic acid is used in the production of detergents, soaps, and cosmetics. This element's rate in DS leaves was 12 % higher than that in roots.

However, its levels in DI roots were higher than those in the leaves: 0.87 mg/g and 0.81 mg/g for roots and leaves, respectively (table 5, table 6).

Compound 12, hydroxy-hexanedioic acid (fig. 2) has a chemical formula C₆H₁₀O₅ with a molecular weight of 162.1406 g/mol. The hydroxy-hexanedioic acid amounts founded in DS were 0.45 mg/g and 0.69 mg/g respectively in leaves and roots (lowest concentration of all identified fatty acids in this study) (table 5).

Compound 13, hexanoic acid (caproic acid) (fig. 2); a hexane-derived carboxylic acid. This compound concentration in leaves and roots of DI were 0.34 mg/g and 0.19 mg/g respectively (table 6).

Table 5: Composition and levels of fatty acids in *Datura stramonium* roots and leaves

Fatty acids	Leaves (mg/g)	Roots (mg/g)
Hexadecanoic acid	0.9036±0.1710	0.9968±0.1175
Hydroxy-Hexanedioic acid	0.4544±0.1025	0.6939±0.0494
Octadecanoic acid	0.8955±0.1802	0.7986±0.0872

Data represented as mean±SD (n=3).

Table 6: Composition and levels of fatty acids in *Datura innoxia* roots and leaves

Fatty acids	Leaves (mg/g)	Roots (mg/g)
Hexadecanoic acid	0.8939±0.1501	0.9016±0.2269
Hexanoic acid	0.3428±0.0886	0.1994±0.0699
Octadecanoic acid	0.8186±0.0375	0.8777±0.1952

Data are presented as mean±SD (n=3).

Datura stramonium and *Datura innoxia* are considered important species in pharmaceutical research because of alkaloids contents with pharmaceutical and medicinal properties. Others chemical compound of this plants, such as phenylpropanoids, flavonoids and fatty acids, have the similar importance of alkaloids, but they are less studied. Analysis of different *Datura* organs and research on other metabolites (besides alkaloids) may offer a new aspect of research in these areas.

To the best of our knowledge, no information is available on phenylpropanoids and fatty acids compounds in roots and leaves of *Datura stramonium* and *Datura innoxia*. However many other plant

species are reported to be the producer of these substances. Pant *et al.* [17] found that *Arabidopsis thaliana* plants contain two phenylpropanoids, cis-sinapic acid and trans-ferulic acid and the fatty acid octadecanoic acid. N-trans-coumaroyloctopamine, N-trans-feruloyl octopamine, guaiacylglycerol- β -ferulic acid ether, guaiacylglycerol- β -caffeic acid ether, trans-coumaric acid and trans-ferulic acid were identified in garlic skin [22]. While hexadecanoic acid and octadecanoic acid are the fatty acids found in *Melissa officinalis* [23]. Phytochemical screening of *Plectranthus hadiensis* revealed the presence of flavonoids (phenylpropanoids) [24].

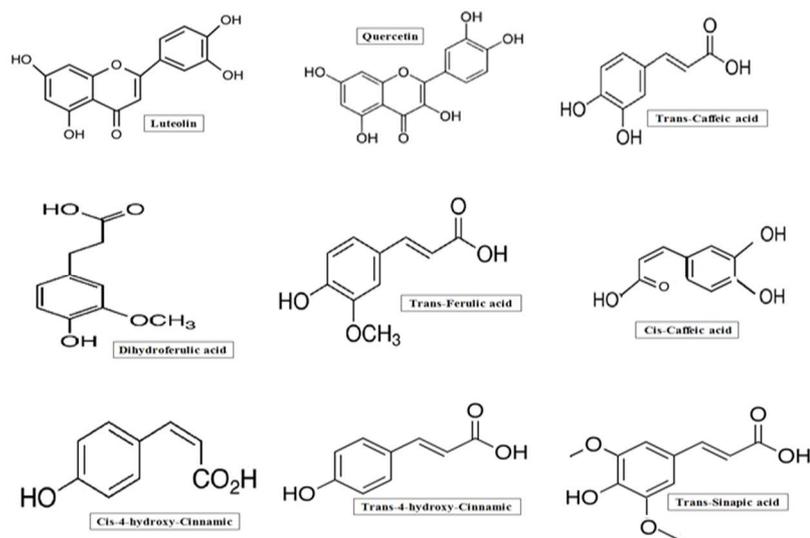


Fig. 1: The chemical structure of phenylpropanoids (1-9) from *Datura stramonium* and *Datura innoxia* (<http://www.chemspider.com>)

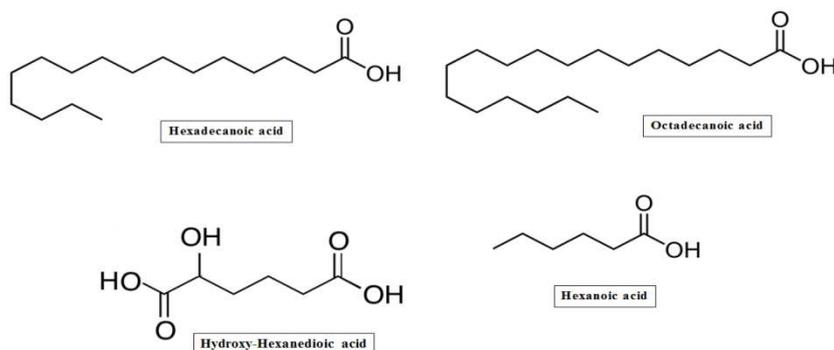


Fig. 2: The chemical structure of fatty acids (10-13) from *Datura stramonium* and *Datura innoxia* (<http://www.chemspider.com>)

CONCLUSION

Phenylpropanoids and fatty acids are very useful substances in foods and pharmaceuticals industry and their demand sudden increases in recent years. Therefore, improving and increasing production of these substances has become a necessity, which requires more studies and research in *Datura* and other medicinal plants.

Analysis carried out by the GC-EI/TOF-MS chromatography techniques allowed to identify different phenylpropanoids and fatty acids metabolites in *Datura sp.* Our studies showed that phenylpropanoids amounts in *Datura innoxia* were higher than in *Datura stramonium* and the concentration in leaves is higher than that in roots, in the two species. Our work revealed that DI and DS contain the same fatty acids rates with equal concentrations in the leaves and the roots, but with different components.

The use of modern culture techniques, such as, *in vitro* or hydroponics systems associated with the application of chemical

elicitors or Plant Growth Promoting Rhizobacteria (PGPR) or genetic engineering can optimize the synthesis of specific molecules that are characterized in previous studies of prospection.

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CONFLICTS OF INTERESTS

Authors declare that they have no conflict of interest

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