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-cafeic acid, trans-feluric acid, cis-cafeic 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-cafeic acid, trans-feluric acid and dihydrofeluric 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.

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), flavonoids (kaempferol, quercetin), flavonones (naringenin, eriodictyol), flavonoloids (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 Biochemie), Methanol gradient grade for liquid chromatography (Merck), Chloroform for liquid chromatography (Merck), Ribitol (Sigma), DL-Alanine, 2,3,3-d4 (Sigma), nonadecanoic acid methyl ester (Sigma),...
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 1-C-sorbitol), and 30 µl nonadecanoic acid methyl ester (2 mg/ml 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-IE-TOF-MS) using an Agilent 6890N/24 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).

**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-cafeic acid (3) and trans-ferulic acid (5). Moreover, DS contains dihydroferulic acid (4), whereas DI contains cis-cafeic 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***

| Phenylpropanoids         | Molecular formula | Molecular weight (g/mol) | Leaves (mg/g) | Roots (mg/g) |
|--------------------------|-------------------|--------------------------|---------------|--------------|
| Luteolin (1)             | C₁₀H₁₂O₅          | 296.24                   | 0.7676±0.02829| 0.5213±0.0767|
| Quercetin (2)            | C₁₀H₁₄O₅          | 302.236                  | 0.5494±0.2025 | 0.2246±0.0987|
| Trans-Caffeic acid (3)   | C₉H₁₀O₄           | 180.157                  | 0.5803±0.08991| 0.1759±0.0492|
| Dihydroferulic acid (4)  | C₉H₁₂O₄           | 194.18                   | 0.2246±0.0987| 0.1759±0.0492|
| Trans-Ferulic acid (5)   | C₁₀H₁₀O₄          | 194.18                   | 0.5803±0.08991| 0.1759±0.0492|

**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-tetraydroxylavone) (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; melethon; xanthaurine) (fig. 1). The molecular formula of quercetin is C₁₅H₁₀O₅ with a molecular weight and density of 302.236 g/mol, 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-cafeic acid (3,4-dihydroxyphenyl, acrylic acid) (fig. 1) has the molecular formula C₉H₁₀O₄ and the molecular weight of 180.157 g/mol. Levels of trans-cafeic 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-hydrocinamic acid; β-3-methoxy-4-hydroxyphenylpropanionic acid; 3-methoxysphloric acid; dihydroconiferyl 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-sinapic 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 (1)             | 0.6677±0.2829 | 0.2782±0.1373|
| Quercetin (2)            | 0.5494±0.2025 | 0.2246±0.0987|
| Trans-Caffeic acid (3)   | 0.5803±0.08991| 0.1759±0.0492|
| Dihydroferulic acid (4)  | 0.2246±0.0987 | 0.1759±0.0492|
| Trans-Ferulic acid (5)   | 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-enolic acid; 3-[3,4-Dihydroxyphenyl] prop-2-enolic 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 primary components of plant biomass and its residues [20]. (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 (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 (fig. 2), the most common fatty acid saturated found in animals, stea ric acid is used in the production of detergents, soaps, and cosmetics. This compound concentration in leaves and roots of DI were 0.34 mg/g and 0.19 mg/g respectively (table 6).

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

| Phenylpropanoids         | Leaves (mg/g) | Roots (mg/g) |
|--------------------------|---------------|--------------|
| Luteolin                 | 0.632±0.2887  | 0±0          |
| Quercetin                | 0.604±0.2714  | 0±0          |
| Cis-Caffeic acid         | 0.371±0.0755  | 0±0          |
| Trans-Caffeic acid       | 0.504±0.0070  | 0.0106±0.0033|
| Cis-4-hydroxy-Cinnamic acid | 0.529±0.0111 | 0.294±0.0419 |
| Trans-4-hydroxy-Cinnamic acid | 0.412±0.0225 | 0.139±0.0153 |
| Trans-Ferulic acid       | 0.670±0.0385  | 0.029±0.0035 |
| Trans-Sinapic acid       | 0.915±0.1833  | 0±0          |

Data represented as mean±SD (n=3).

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

| Fatty acid                                | Molecular formula | Molecular weight (g/mol) | Leaves (mg/g) | Roots (mg/g) |
|------------------------------------------|-------------------|--------------------------|---------------|--------------|
| Hexadecanoic acid (10)                   | C16H32O2          | 256.424                  | 0.3428±0.0886 | 0.1994±0.0699|
| Hydroxy Hexadecanoic acid (12)           | C17H36O5          | 262.440                  | 0.8186±0.0375 | 0.3719±0.0755|
| Octadecanoic acid (11)                   | C18H34O2          | 284.477                  | 0.3428±0.0886 | 0.1994±0.0699|

Data represented as mean±SD (n=3).

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

| Fatty acids                                | Leaves (mg/g) | Roots (mg/g) |
|-------------------------------------------|---------------|--------------|
| Hexadecanoic acid                         | 0.3428±0.0886 | 0.1994±0.0699|
| Hexanoic acid                             | 0.3428±0.0886 | 0.1994±0.0699|
| Octadecanoic acid                         | 0.3428±0.0886 | 0.1994±0.0699|

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                         | 0.3428±0.0886 | 0.1994±0.0699|
| Hexanoic acid                             | 0.3428±0.0886 | 0.1994±0.0699|
| Octadecanoic acid                         | 0.3428±0.0886 | 0.1994±0.0699|

Data are represented 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-coumaroyllooctopamine, 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].

**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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