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Short Report

Diosgenin Quantification by HPLC in a *Dioscorea polygonoides* Tuber Collection from Colombian Flora

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As concentrações de diosgenina em uma coleção de tubérculo de *Dioscorea polygonoides* do Eje Cafetero (EC, Colombia) foram determinadas por cromatografia líquida de alta eficiência. Os teores obtidos variaram de 0,02 a 2,64%. A maior concentração de diosgenina (2,64%) foi obtida de um tubérculo coletado perto de Salento (Quindio). A média de recuperação desta foi de 97%. A identificação foi feita por cromatografia gasosa-espectrometria de massas (CG-EM) e por coeluição com o padrão autentico. Os resultados destes estudos foram importantes uma vez que os teores de diosgenina obtidos se mostraram superiores quando comparados aqueles relatados para outras espécies medicinais de *Dioscorea*, tornando *Dioscorea polygonoides* uma fonte potencial de diosgenina.

The diosgenin concentrations in a *Dioscorea polygonoides* tuber collection from the Eje Cafetero (EC, Colombia) were determined by HPLC and their percentages ranged from 0.02 to 2.64%. The highest diosgenin concentration (2.64%) was obtained in a tuber collected near Salento (Quindío). The average of diosgenin recovery was 97%. Diosgenin was identified by gas chromatography-mass spectrometry (GC-MS) and by coelution with authentic diosgenin standard in both HPLC and GC-MS techniques. The results of this study are important since comparatively they are higher than most of those reported on other medicinal *Dioscorea* species, making *Dioscorea polygonoides* a potential new source of diosgenin.

Keywords: bioprospection, Colombian biodiversity, *Dioscorea*, diosgenin screening, steroidal sapogenin

Introduction

The most important families that biosynthesize steroidal saponins are Agavaceae (genus *Agave*), Dioscoreaceae (genus *Dioscorea*) and Liliaceae (genera *Allium*, *Asparagus*, *Lilium*).[1] Yams belong to genus *Dioscorea* (Dioscoreaceae family) with near 400 species growing in the tropical and subtropical wet areas around the world. This family is characterized by the production of subterranean or aerials tubers. Usually its species are climbing vines and many of them are dioicous.[2]

Several *Dioscorea* species are economically important as staple food,[3,4] and others are used for the production of steroidal saponins, which on hydrolysis render sapogenins, such as diosgenin (Figure 1), which is important as starting material for the production of corticosteroids, sexual hormones, oral contraceptives as well as other steroids via hemisynthesis.[5-7]

Dioscorea polygonoides Humb. et. Bonpl. ex Willd. belongs to the Dioscoreaceae family, genus *Dioscorea*, subgenus *Eudioscorea* and to the section *Lynchnostemon*, which is well distributed from Mexico to Brazil, crossing by Colombia and the Antilles.[2,8] From *D. polygonoides* two steroidal saponins, diospolysaponin A and prosapogenin A of dioscin have been isolated and characterized. The last compound showed cytotoxic activity against the HSC-2 (IC_{50} 3.4 μg mL^{-1}) cell line which was as potent as the positive control, doxorubicin (IC_{50} 2.5 μg mL^{-1}).[9] In addition, three

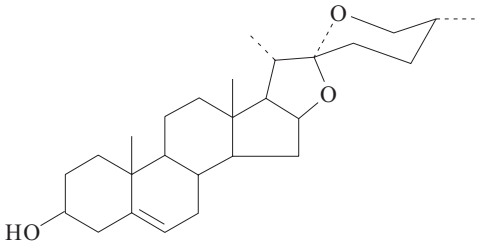

Figure 1. Diosgenin.

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new polyhydroxylated spirostanol saponins were isolated from *D. polygonoides* tubers and their structure determined on the basis of extensive spectroscopic analysis.[10]

To date, diosgenin and related sapogenins are commercially available from tubers of different *Dioscorea* species; however, recent experimental evidence has showed that diosgenin has proved human health benefit.[11] These discoveries have increased diosgenin demand world wide and motivated the search for new and renewable sources for this important raw material for the pharmaceutical industry. The aim of this study was to quantify by high pressure liquid chromatography (HPLC) the levels of diosgenin in a collection of seventy four tubers of *D. polygonoides* from different localities of the Eje Cafetero (EC, Colombia).

Experimental

General experimental procedures

The solvents *n*-hexane, chloroform, methanol, ethanol, *n*-butanol and acetonitrile used for the steroidal sapogenin extraction and analysis were purchased from Mallinckrodt (Phillipsburg, NJ, USA); diosgenin [CAS: 512-04-9] standard and the enzyme naringinase (E.C.: 232-962-4) were purchased from Sigma (St. Louis, MO, USA).

For diosgenin quantification a HPLC Hewlett Packard instrument model HP-1100 (Palo Alto, CA, USA) equipped with the software version ChemStation A.06.01, a diode array detector (DAD-UV) was used; in addition, a Hypersil ODS C18 column (250 × 4.0 mm, 5 μm) and a 20 μL Rheodyne manual injector were used.

Plant material

Tubers of *D. polygonoides* were collected during the period between November 2002 and November 2003 at different zones in the EC. This area is constituted by the departments of Caldas, Quindío and Risaralda (Colombia), and they are located on the Central Andean mountain chain, with an extension area of 13.893 km^2, with different altitudinal zones (900–5400 m) and high annual precipitation. Plant samples were authenticated by the authors, see Table 1, and a voucher for a specimen was deposited at the Universidad de Antioquia Herbarium (Medellín, Colombia), under the code number HUA 132745.

Diosgenin extraction

Each collected tuber was washed, chopped to in fine slices and oven-dried at 50 °C to constant weight and ground to a fine powder, as described by Mahato *et al*.[12] Next, for each colleted tuber 10.00 g aliquot were taken and defatted with *n*-hexane in a Soxhlet extractor. The degreased plant materials were extracted three times with methanol in Soxhlet, and the combined methanol extracts were concentrated to dryness at reduced pressure. After that, each methanol extract was resuspended in 10 mL of water HPLC grade and partitioned three times with portions of 6 mL of *n*-butanol saturated with water, to obtain the crude saponins.[13] Finally, 50 mg of each butanol extract were hydrolyzed by treatment with the enzyme naringinase to obtain the crude sapogenins, following the procedure described by Niño *et al*.[9]

Diosgenin quantification

Diosgenin quantification was performed on a HPLC instrument applying the external standard method, through a diosgenin calibration curve with six points in a concentration range between 50-300 mg L^{-1}. The experimental conditions were an isocratic binary system of acetonitrile/water (90:10), a flow rate of 1 mL min^{-1} and a temperature of 35 °C. Detection was performed at 194 nm, according to the procedure described by Oncina *et al*.[14]

The diosgenin concentrations in the different samples were calculated through a regression analysis from the peak area and the known concentrations of authentic diosgenin samples and are the average of three consecutive readings for each tuber sample.

Results and Discussion

Tuber collection

Seventy four *D. polygonoides* tubers were collected at 10 different localities from the Eje Cafetero (EC) as showed in Table 1.

Diosgenin quantification by HPLC

The hydrolysis of the *n*-butanol extracts with the enzyme naringinase produced diosgenin as well as other sapogenins. The use of the enzyme hydrolysis reduced significantly diosgenin degradation as well as the production of artifacts and this might increase the diosgenin yields in this study.[1,15] The diosgenin recovery using the method reported here was 97%, which is higher than that obtained by the acid hydrolysis of *n*-butanol extracts from *Trigonella foenum-graecum* L.[16]

The experimental conditions used for diosgenin quantification by HPLC in the different *D. polygonoides*

Table 1. Places and number of *D. polygonoides* tubers collected in the Eje Cafetero

Recolection Places	Geographic Coordinates	Numer of Sample
Salamina (Caldas)[1]	N 6° 16´ 48" W 73° 18´ 05"	1 to 7
Jardín Botánico UTP[2] (Risaralda)	N 4° 42´ 89" W 70° 35´ 00"	8 to 10
Circasia (Quindío)	N 6° 5´ 37" W 74° 5´ 46"	11 to 16
Parque Ucumarí (Risaralda)	N 4° 42´ 89" W 73° 35´ 00"	17 to 27
Ecoparque LosYarumos (Caldas)	N 6° 11´ 40" W 73° 35´ 55"	28 to 38
La Nona (Risaralda)	N 5° 58´ 41" W 73° 48´ 06"	39 to 48
Salento (Quindío)	N 6° 12´ 48" W 73° 46´ 69"	49 to 52, and 56
Valle del Cocora (Quindío)	N 6° 12´ 48" W 75° 40´ 00"	53 to 55
Filandia (Quindío)	N 6° 0´ 10" W 173° 59´ 40"	57 to 63
Alto del Nudo (Risaralda)	N 5° 57´ 93" W 73° 44´ 66"	64 to 74

[1]Department where the collection took place; [2]UTP = Universidad Tecnológica de Pereira.

extracts gave a highly reproducible retention time (t_R) equal to 11.8 ± 0.05 min. All samples studied gave the same chromatographic pattern. The diosgenin peak in gas chromatography-mass spectrometry (GC-MS) of some tuber extracts displayed similar fragmentation patterns as diosgenin standard (data not shown). In addition, they are in concordance with the information reported on steroidal sapogenins isolation by HPLC.[17]

The results of diosgenin quantification by HPLC are given in Figure 2. The percentages of diosgenin obtained from the seventy four accessions of *D. polygonoides* on this work are in the range from 0.02 to 2.64%, which is significant since there are several literature data where the diosgenin contents are very low, for example: *D. polygonoides* (0.2%);[3] *Dioscorea althaeoides* (0.2-2.3%);[18] *Dioscorea prazeri* (1.92%);[12] *Dioscorea villosa* (1.3%);[19] 2 years old *Dioscorea zingiberenzis* (0.18-0.55%),[20] several *Dioscorea* species (0.04-0.93%),[21] among others.

The tubers with the highest diosgenin contents were collected in Salamina (Caldas) and Salento (Quindío) (see Table 1). These towns are located on the Colombian Central Andes mountain chain with an altitude of 1820 and 1920 meters above the sea level, respectively. Both places have an average temperature of 15 °C and an annual precipitation of 3000 mm *per* year. In both localities there is a predominance of volcanic soils (pH 4.0-7.0). However, these two localities are far away and there is not a connection between them. One possibility that can arise to explain the

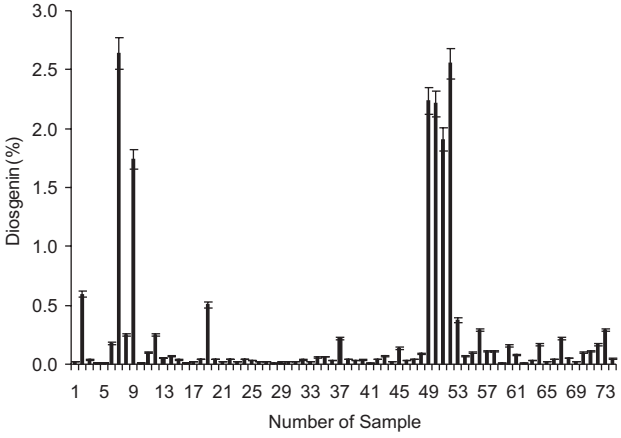

Figure 2. Diosgenin percentage on dry base, for each one of the seventy four *D. polygonoides* tubers collected in the Eje Cafetero.

reason why the tubers from this region gave the highest diosgenin yields is that they would be genetically close to the other ones, due to its anemochorous dispersion at some time, facilitated by the membranous wing around their seeds and by human factors; these aspects could promote the spread and distribution of *D. polygonoides* populations in these two localities.[22] Another possibility is that the screening was done on tubers with different weights, and consequently they had different ages. In the study of Chapagain and Wiesman[11] they found that two years old tubers gave higher diosgenin contents than those one year old, these results are not in concordance with the ones found in this work, since there was not a correlation between the

weight of the tubers collected and their diosgenin production (data not shown).

Furthermore, the heterogeneity found on the steroidal sapogenin contents from the different *D. polygonoides* accessions analyzed in this study might depend on factors such as the genotype, the physiological state, the climatic conditions as well as the geographic localization of plants as pointed out by Dinan *et al.*[1] These findings correlate with the determination of the steroidal sapogenin contents in different *Tribulus terristris* samples by HPLC, where significant differences were found depending on the origin and part of the plant used for extraction.[16, 23]

Conclusions

The *D. polygonoides* tubers collected in the Eje Cafetero (EC, Colombia), showed a wide range of diosgenin contents. As four of the seventy four tuber collected in the EC showed a diosgenin content higher than 2% on dry base, showing that the tubers of *D. polygonoides* could be a new source of diosgenin supply. Further studies looking for high yielding varieties and the effect of environmental factors on diosgenin levels could be required to develop a high diosgenin producing variety.

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