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Research Article

Direct UHPLC-MS/MS Detection of Gossypitrin-7-O-glucoside from *Taliparititi elatum* Sw. (*Malvaceae*)

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Abstract

Flowers petals from *Taliparititi elatum,* a Caribbean tree belonging to *Malvaceae* family, have been extracted respectively with ethanol and 1,2-dimethoxyethane. The crystallization of the resulting extracts led to precipitates, which have been analyzed by UHPLC. Presence of gossypitrin (or gossypetin-7-*O*-glucoside) was revealed as major compound together with four others minor molecules.

Keywords: *Taliparititi elatum*; Ethanolic Extract; 1,2-dimethoxyethane; Flavonoids; Chemical Components

Introduction

The West Indies account for a total of 208 families of species of which only 183 are indigenous to the region. Even if there are no endemic families of seed plants in the West Indies, ten most species rich families are contributing to nearly 60% of the native taxa of the region. Among the countries that have the biggest amount of plant families, number of genera, percent of generic endemism, total taxa, native taxa and percent of endemic taxa, Cuba is the most important contributor [1].

Cuba and Jamaica are the main location that have the most important presence of *Taliparititi elatum* (Sw.) in all Caribbean basin although the plant population is present in different islands of the area like Martinica, Guadalupe, Virgin Islands, Puerto Rico and some other countries like Panama. *T. elatum* is a medicinal plant belonging to *Malvaceae* family. It was renamed in 2007 by Areces and Fryxell [2] when they found out at least five different characteristics that allow them to include the plant in Taliparititi gender: arborescent habit, prominent stipules, coriaceous foliar lamina, margin majoritarian entire, capsule 10-locular and relatively higher chromosomal number (2n = ca. 80, 90, ca. 92, ca. 96 and 120).

T. elatum grows in a wide range of elevations, up to 1200 meters (3900 Ft.) and is often used in reforestation. The tree is quite attractive with its straight trunk, broad green leaves and hibiscus-like flowers. The attractive flower changes color as it matures, going

from bright yellow to orange-red and finally to crimson [3] (Figure 1). Cuban population uses the flowers as cataplasm and infusions mixed with sugar cane or honeybee [4].

Figure 1: Flowers of *Taliparititi elatum* (Sw.).

The main flavonoid glucoside extracted, isolated, and purified from flowers petals named gossypitrin, has shown its "in vitro" scavenging effects on reactive oxygen species (ROS) (O^{-}, $HO^{\cdot}$, HOCl, $ROO^{\cdot}$, and H_2O_2), reactive nitrogen species (RNS) ($ONOO^{-}$ and NO) and $ABTS^{\bullet+}$ (2, 14 mM), DPPH. radicals and Reducing Power assay. Additionally, two enzymatic assays (Inhibition of xanthine oxidase (XO) and Effect on XO activity) were also evaluated [5].

The antibacterial and antifungal activities of gossypitrin were recently demonstrated against a series of microorganisms, and gossypitrin showed a potent intrinsic antioxidant capacity evidenced by low IC_{50} and EC_{50} values for DPPH/ABTS/malondialdehyde and ferric reducing power, respectively. Pre-treatment of PC_{12} cells with gossypitrin, significantly increased their survival against KCN, restored the levels of GSH and the SOD and CAT enzymes activities, as well as reduced the level of lipid peroxidation. Its antioxidant effects were higher than those elicited by rutin [6,7].

In 2016 a research group from Martinica, leading by Frantz François-Haugrin discovered the presence of another flavonoid glucoside named gossypetin-3'-*O*-glucoside which is an isomeric form of gossypitrin using 1,2-dimethoxyetahne as extractive solvent. When the extract was refrigerated at 4 ^{0}C, formation of a precipitate of gossypetin-3'-O-glucoside is observed [8].

Both gossypetin-3'-*O*-glucoside and gossypitrin have the same molecular weight, similar UV and IR spectrometric data, but with differ with their NMR spectrum [9-12]. Until now the components are under controversial explanation due to the possible presence of both chemical substances in the petals of the flowers maybe associated to the extraction methodology and the recovery of the substances.

The aim of this study was to determine the structure of the main constituent found contained in precipitates of ethanolic and 1,2-dimethoxyethane extracts from flower petals of *T. elatum* in Martinica using and UHPLC-MS/MS system.

Materials and Methods

Plant material

Flowers were collected in January 2016 along the track road in Balata forest located in Martinica. A voucher specimen is deposited and registered in French Pharmacopeia as Fournet 1752 (4232 Guad). Martinican specimens are registered as *Hibiscus elatus* Sw . (synonym of Taliparititi eratum).

Extracts and sample preparation

Extraction was carried out in a Soxhlet apparatus using the petals of flowers with respectively 95 % ethanol and 1,2-dimethoxyethane 99 % as solvents. In both cases 80 g of crushed petals were extracted from 1/2 L of solvent during 20 h. Then each extract was concentrated in a rotary evaporator (RE Start 300) until a volume of about 50 mL and stored at 4°C for crystallization. After 24 h the precipitate was collected by filtration and dried in an oven at 40°C. The supernatant of the precipitate with 95° ethanol was reset to 4°C and gave again a less abundant precipitate. It was collected, dried and placed in its own vial for future characterizations.

UPLC-MS/MS procedures, instrumentation and parameters

UPLC has been used for the profiling and characterization of the metabolites contained in the extracts. The system used is a Dionex U 3000 equipped with a DAD detector having a C_{18} analytical column (100 x 4.6 mm particles 3 µm). Solvent systems: H_2O-0.1% Formic Acid (H_2O) and Acetonitrile-0.1% Formic Acid (Table 1).

The HPLC is coupled to a Varian 500 MS Mass Spectrometer equipped with an electrospray ionization chamber (ESI) used in negative mode at 5 KV at a capillary temperature of 250 °C. The UV detector and the Mass Spectrometer are used in parallel. A split al-

Time	Solvents	Gradient
0	H_2O:CH_3CN	80:20
5	H_2O:CH_3CN	80:20
10	H_2O:CH_3CN	0:100
25	H_2O:CH_3CN	0:100
30	H_2O:CH_3CN	80:20
34	H_2O:CH_3CN	80:20

Table 1: UPLC Gradient used in the research.

lows the post column eluent flow to be separated into 2 parts when the flow rate used is greater than 500 µL/min. From a flow rate of 1mL/min, 400 µL/min are sent to the mass spectrometer and around 600 µL/min to the trash.

For the comparison of the two powders: Column of 250 mm x 4.6 mm (i.d), particle 5 um. The flow rate is 0.800 µL/min. For the product purification: 150 mm x 10 mm (i.d) column, 5 um particles. Flow rate: 5 mL/min.

Data was acquired in positive or negative mode using the TDDS option "Turbo Data Dependent Scanning" to automatically obtain ion fragmentation spectra that allow the identification of compounds. These mass spectrometry data were compared with free access databases such as “Mass bank", "Spider mass DB", the “in-house” database or data from the literature. If this procedure did not allow identification, an attempt to elucidate the structure was carried out manually.

Results and Discussion

When the flower extract of *T. elatum* (Sw.) is placed at 4 °C, a precipitate is formed. This characteristic is used to obtain a powder containing gossypitrin. To do this, 3 tests were carried out. A first test carried out by recovering the precipitate from a hydroalcoholic extract, a second test obtained from the supernatant of the first precipitation and a third test carried out from an extract with 1,2-dimethoxyethane (100 mm column, 3 µm, 0.450 µL/min).

The study of the chromatograms shows that several chromatographic peaks have been detected, including a largely predominant peak. So, we are not in the presence of a pure compound but of a mixture of products. The majority peak was identified by MS/MS as being gossypitrin or gossypetin-3'-*O*-glucoside (MS/MS data not shown here). Its relative amount varies between 61 and 79% depending on the preparation of the extract. This difference is not significant when using DME or ETOH in the first crystallization (61 and 65%) but more significant between the first (65 %) and the second crystallization (79 %) (Figure 2).

Not all gossypitrin precipitates from the first precipitation. Further purification by various chromatographic process is needed to obtain pure gossypitrin. If the objective is to have pure gossypitrin, this process is not ideal, but if the objective is to obtain a powder containing mainly gossypitrin in a simple and inexpensive way this precipitation process is adapted. Obviously, the extraction with EtOH get a big amount of recovery than DME extraction and four another chemical compounds are present in the samples.

Gossypetin-7-*O*-glucoside (*G7G*) was detected at 22.287 min of retention time. This result is different comparing with the same evaluation made by our research team in 2017 in which the same product was detect at 2.407/5.287 with a molecular mass of 480 [13]. Up to now, only four flavonoid glycosides have been found in nature derivatives from gossypetin: *G8G* or gossypin (from *H. vitifolius* and *H. sabdariffa*) [14,15]; *G7G* or gossypitrin/gossypetin (*H. sabdariffa*, *T. elatum* and *T. tiliaceum*) [16-18]; *G3G* or gossytrin (*H. sabdariffa* and *T. tiliaceum*) [19,20] and *G3'G* (*A. manihot* and *T. elatum*) [21,22].

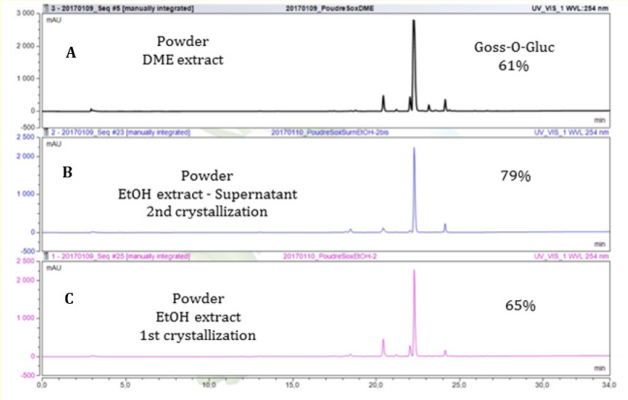

Figure 2: UV chromatograms at 254 nm. (A) RP-UPLC of the precipitate of the Flower extract with dimethoxyethane (B) RP-UPLC of the precipitate of the Flower extract with ethanol in 2nd precipitation (C) RP-UPLC of the precipitate Flower extract with ethanol in 1st precipitation.

Recently, a Martinican research team, after their results of a Computational Study proposed that four gossypetin derivatives: *G8G, G3G*, *G3'G* and finally *G7G,* as good tropical natural compounds candidate that should be further investigated to prevent or treat COVID19. They were able to show that molecules derived from medicinal plants have better physico-chemical interaction properties and potential for inhibition of 3CLpro, as compared to some candidate drugs proposed for the treatment of COVID19 [23].

Flavonoids have a wide range of binding affinity to SARSCoV CLpro due to their hydrophobic aromatic rings and hydrophilic hydroxyl groups. the presence of carbohydrate groups influences severely to the binding affinity and mode of the chromen-4-one moiety. The antiviral activity of some flavonoids against CoVs is presume directly caused by inhibiting 3C-like protease (3CLpro) [24].

Conclusions

A simple and versatile analytical method, the "UPLC-DAD-ESI-MS/MS" was implemented to allow direct identification of the constituents of the hydroalcoholic/1,2-dimethoxyethane extracts of the flower petals from *T. elatum* Sw. (Fryxell). The analysis was carried out by RP HPLC coupled to a DAD detector and to a tandem ion trap mass spectrometer in order to obtain a UV profile and a spectrum of fragmentations in negative mode making it possible to achieve provisional identification. Five different chemical components were isolated using this analytical methodology being the first attempts to purify the majority constituents of the extract. Thus, it has been demonstrated that gossypitrin or gossypetin-3'-*O*-glucoside does not precipitate alone and the use of UPLC-DAD-ESI-MS/MS for preparatory purposes is promising. We were able to isolate 4 major products including the main flavonoid glucoside. This methodology can be used to elucidate the major constituent from the extracts by NMR spectroscopy.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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