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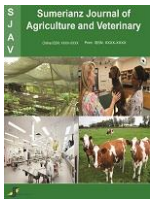

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UHPLC-DAD-ESI-MS/MS Analysis of Gossypitrin Sample from *Talipariti Elatum* (Sw.) With Antioxidant and Neuroprotective Effects

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Abstract

A sample of gossypitrin isolated from the ethanolic extracts of the petals of *Talipariti elatum* (Sw.) were scrutinized using an UHPLC/DAD/MS Thermo scientific Dionex Ultimate 3000 RS coupled to a Thermo scientific LTQ XL mass spectrometer. In addition, about six different chemical compounds were tentatively identified in this specie based on chromatography retention time (R_t), UV and MS/MS spectra and compared with those of isolated authentic compound and literature data. The flavonoids identified after UV and MS analyses were gossypitrin, isoquercitrin, hyperoside, quercetin-*O*-sambubioside, and possibly for the first time, two isomeric forms of kaempferol: 8-methoxy-kaempferol-3-*O*-glucose and 8-methoxy-kaempferol-3-*O*-galactose.

Keywords: UHPLC; MS; Flavonoids; Gossypitrin, petals; Ethanolic extracts.

1. Introduction

Gossypitrin (gossypetin-7-*O*-β-D-glucoside) is a natural flavonoid that was isolated from the petals of the flowers of *Talipariti elatum* (Sw.), also known as Blue Mahoe. The petals of the flowers of this medicinal tree are used in Cuba as anti-asthmatic, expectorant and against flu. Up today, four different kinds of biological activities have been demonstrated by our research group: antimicrobial, antioxidant, transition metal quelating and neuroprotective against chemical hypoxia-induced PC_{12} cell death [1-4].

The results of the test to determine the oral acute toxicity of gossypitrin demonstrated that the glucoside flavonoid classified as non-toxic according to the classification system of the Commission of the European Communities. The flavonoid belongs to class CTA_0 in which the Mortality > 2000 [5].

Related with the importance of protect our nervous system a sample of gossypitrin was used to evaluate the antioxidant and cytoprotective effects against cyanide-induced oxidative stress and cell death in PC_{12} cells. The flavonoid 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 [4].

Taking into account the difficulty to isolate and purify this glucoside, and considering the previous result with the same sample of this natural product done by GC-MS where were found several chemical components in the sample like d-turanose, thymol-β-d-glucopyranoside, ribitol, 2-deoxy ribose and hydroquinone-β-d-glucopyranoside and another 13 compounds, more of them were organic acids [6], the aim of this investigation was to analyze the purity of a "sample of gossypitrin" using UHPLC coupled to MS used as protector of hypoxia induced injury to get information about the chemical components present in the sample.

2. Material and Methods

2.1. Plant Material

Flowers were collected in January 2016 in the gardens of the Faculty of Pharmacy and Foods at Havana University after their mature. They were identified at the herbarium of National Botany Garden of Havana, where the voucher specimen no. HAJB 82587 was deposited and registered as *Talipariti elatum* S.w. Petals were separated manually from the rest of components of the flowers and packaged in a nylon bag strictly closely.

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2.2 Extract and Samples Preparation

Dark red flowering types were collected daily. The petals used were dried in an oven with controlled temperature at 40°C, during 5 days. The extracts were prepared with the ground material (60 g), using a Soxhlet apparatus and 95% ethanol (675 mL) for 20 hours. The ethanolic extracts were concentrated and roto-evaporated under vacuum to 200 mL at 120 rpm, 70°C, and 500 mbar. For to the purification, 1g of solid was dissolved in 25 mL of diethyl ether and the volume was completed to 100 mL with ethanol. The sample was refrigerated until an abundant solid appear and it was recuperated to filtration. This process was done twice, to obtain only a yellowish-green solid monitoring by TLC on silica gel with fluorescent indicator 254 nm on aluminum cards (layer thickness 0.2 mm) (10 × 20 cm) using n-butanol: acetic acid: water (4:1:5) as eluent (v/v/v) [7].

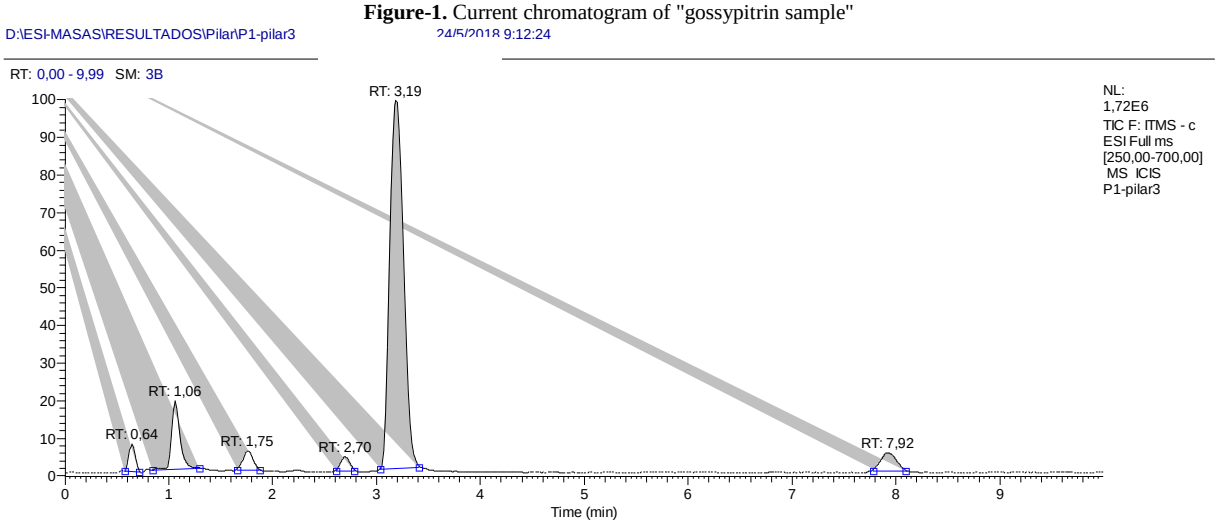
2.3 UHPLC-DAD-ESI-MS/MS Procedures, Instrumentation, and Parameters

The LC system consisted of an UPLC/DAD/MS Thermo scientific Dionex Ultimate 3000 RS with quaternary pump, autosampler, DAD (diode array detector) Dionex with a UV-VIS at 250 (UV1), 280 (UV2), 330 (UV3), and 380 (UV4) nm, coupled to a mass spectrometer Thermo scientific LTQ XL with ESI (trap ion analyzer) in negative mode. Conditions of detection were optimized with a Tune archive based on the behavior of quercetin. Temperature: 225 °C, Voltage 5 KV, Capillar voltage 50 V. Column: Accucore RP-MS (100 x 2.1 mm x 2.6 µm). Temperature: 35 °C. Chromatographic system: eluent ACN: HCOOH (0.1%) (15 % of ACN). Isocratic. 15 min. Flow: 0.4 mL/min. Nitrogen gas flow: 34, auxiliary gas: 16, barrier gas: 3. Induced fragmentation gas: Helium. Was realized an experiment Full Scan in dependent mode to identify the principal ions (TIC) and get the MS and MS^2. The sample was dissolved in methanol HPLC grade at 1 mg/mL filtered by a nylon filter of 0.20 µm. Injection volume: 2 µL. Mass scan between 200-700 u.m.a.

3. Results and Discussion

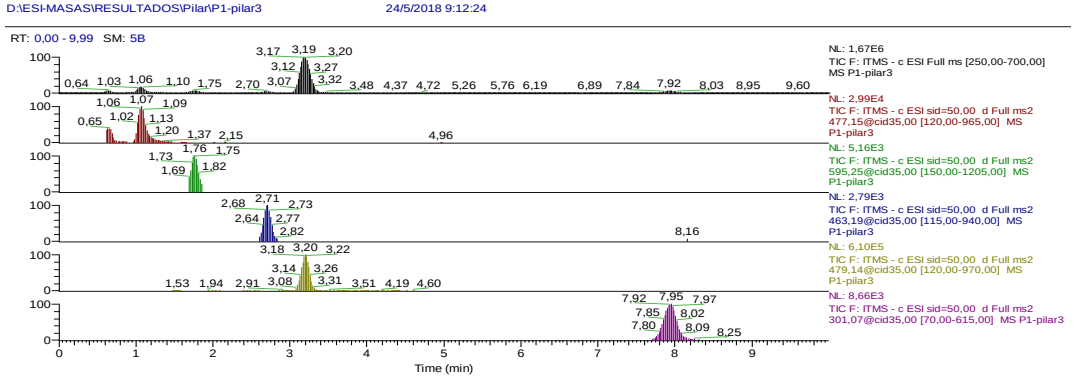
Figure 1 show the TIC chromatogram with the retention times of six different kind of chemical components present in the sample, indicating that the main compound has purity between 75-80 %. Obviously, the "sample of gossypitrin" is not complete pure, for that reason it is necessary to increase the purification of the sample to get only the flavonoid glucoside in future investigations.

Figure-1. Current chromatogram of "gossypitrin sample"



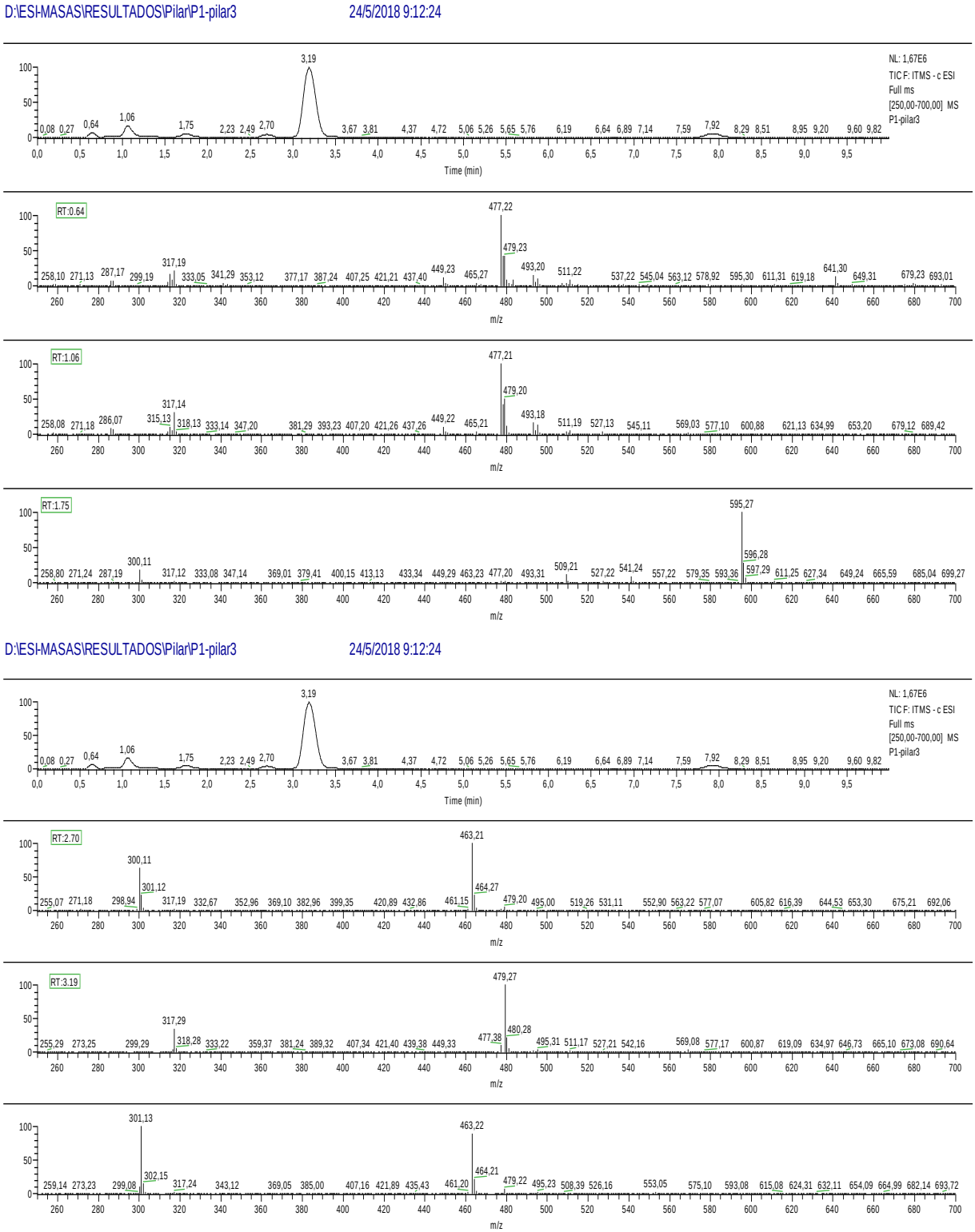
TIC chromatogram and mass spectrum of each product confirm the presence of six different chemical compounds, two of them with a molecular mass of 477 Da, but with different retention times, at 0.65 and 1.07 min, respectively, indicating that both chemical components are closely related (Fig. 2).

Figure-2. TIC chromatogram and main masses detected



The mass spectrum of each compound detected in the sample confirmed that six chemical compounds were found, as mentioned before, two of them with molecular mass of 477 Da and another two with 463 Da. The first two compounds were tentatively identified as kaempferol derivatives with a methoxy group in position 8 and with a molecule of a hexose attached in position 3. That situation allow to inferred that there are differing only in the sugar moiety attached to the flavonol skeleton, in this case glucose and galactose according to Simirgiotis [8] (Fig. 3).

Figure-3. TIC chromatograms and mass spectrum of each compound



Compounds **4** and **6** yielded an MS^2 spectrum typical for quercetin. The fragments proposed for quercetin reinforces the discussed hypothesis that these successive CO and CO_2 losses involve first the C ring. The most interesting fragments concern the base peaks at m/z 179 ($^{1,2}A^-$) and 151 ($^{1,2}A^-$- CO), respectively. Although in only two cases the peak at m/z 273 was observed, this result allowed us to propose a pathway involving C ring with their corresponding loss of CO (m/z 28 u). For flavonols, shows that this new retrocyclization pathway concerns bonds 1 and 2 leading to $^{1,2}A^-$ and $^{1,2}B^-$ fragments at m/z 179 and 121 for quercetin. This $^{1,2}A^-$ diagnostic ion undergoes further loss of CO giving rise to a $^{1,2}A^-$-CO ion at m/z 151 [9, 10].

According to the data published by Simirgiotis in 2013, this compound was suggested to be Isoquercitrin (quercetin 3-*O*-glucose), which were identified previously in hawthorn, by comparison with authentic compounds showed a molecular anion at m/z 463. The compound has the same mass spectrum behavior detected in negative mode, in particular using the ESI ion trap detector ($[M-H]^-$ (m/z) 463, $[2M-H]^-$ (m/z) 927, fragment ions (m/z) (301, 179, 151) with Hyperoside (quercetin 3-*O*-galactose) and differentiated only in the kind of sugar moiety attached to the aglycone. The same result was found by our team for the first time in Martinica in 2016.

Compound **5** is obviously gossypitrin, the main chemical compound found in the ethanolic extracts of the petals of the flowers of *T. elatum* in Cuba, with a base peak at 317.29 and a molecular mass of 479.27.

The last component identified was quercetin-*O*-sambubioside with a retention time of 1.75 min, that have a molecular mass of 596.494 g/mol and a molecular formula of $C_{26}H_{28}O_{16}$, which was identified too in Martinican's flowers by our research team in 2017 using an UHPLC-UV-ESI-MS/MS coupled to PDA (photodiode array detectors) method for the simultaneous isolation and identification of flavonoids and their glycosidic derivatives in this flower drug [11] and isolated from *Eucommia ulmoides* male flowers in China [12].

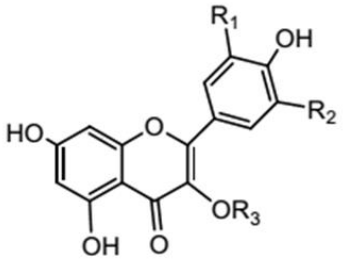
The data of all chemical components and its chemical structures are summarized in Table 1 and their respective proposed chemical structures are represented in Figure 4.

Table-1. Retention times, base peaks, molecular masses and proposed names of compounds detected by UPLC into the sample of gossypitrin isolated from *T. elatum* Sw.

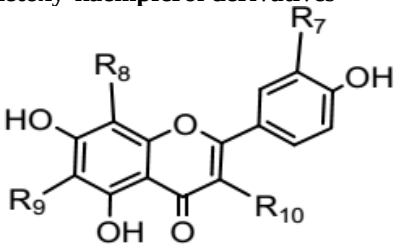
Gossypitrin sample				
Peak	**Rt**	**Compound**	**MS ^{1}**	**MS ^{2}**
1	0.64	8-Methoxy-Kaempferol-3-*O*-glucose	315.09	477.22
2	1.06	8-Methoxy-Kaempferol-3-*O*-galactose	315.04	477.21
3	1.75	Quercetin-*O*-sambubioside	300.03	595.27
4	2.70	Isoquercitrin	301.07	463.21
5	3.19	Gossypitrin	317.29	479.27
6	7.92	Hyperoside	301.05	463.22

Figure -4. Proposed structures of flavonoids derivatives from the petals of *T. elatum* identified by UHPLC-DAD-ESI-MS/MS.

Quercetin-*O*-derivatives



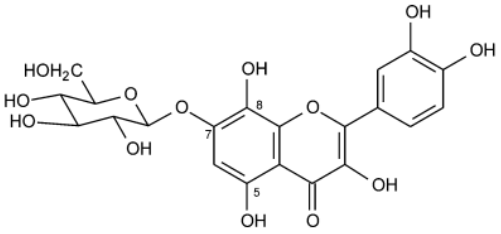
8-metoxy-kaempferol derivatives



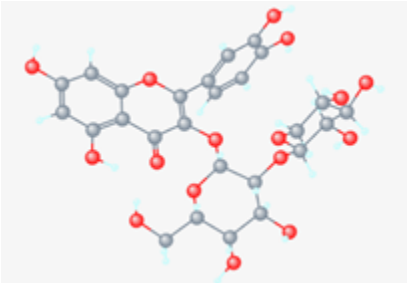
R_1	R_2	R_3
OH	H	Galactose
OH	H	Glucose

R_7	R_8	R_9	R_{10}
H	OCH_3	H	Glucose
H	OCH_3	H	Galactose

Gossypitrin



Quercetin-*O*-sambubioside



4. Conclusion

A simple and versatile analytical method was developed for qualitative identification of major constituents in the petals of the flowers of *Taliparitielatum*, by using UHPLC-DAD-ESI-MS/MS in negative ion mode. Six flavonols derivatives were identified based on retention time (t_R), UV and MS spectra compared with those of authentic compounds and literature data. In our study, flavonol glycosides found major constituents. These flavonol glycosides could be considered as chemotaxonomic markers of these *Taliparitiel* species. This method was successfully applied to identify two new constituents in petals of *T. elatum*, which were not previously reported from this species: 8-methoxy-kaempferol-glucoside and 8-methoxy-kaempferol-galactoside. Due to high sensitivity of this method, some constituents in minor amount were also identified. Furthermore, the results demonstrate that this method could provide full qualitative information of genus Taliparitiel.

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