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Hyperoside or Isoquercitrin in Ethanolic Extracts from the Petals of *Talipariti elatum* S.W

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A B S T R A C T

Liquid chromatography (LC) coupled with UV detection and electrospray ionization (ESI) tandem mass spectrometry (MS/MS) was used for the generation of chemical fingerprints and the identification of phenolic compounds in four solid samples extracted and isolated from the red petals of *Talipariti elatum* utilizing Soxhlet extraction with ethanol at 95 % during 20 hours. Three compounds were detected and two of them were identified as flavonoid glycosides while another one, is supposed to be and alkaloid with impair molecular weight. The compound with m/z of 464 after MS/MS analyses in both positive and negative ion mode is suggested to be Isoquercitrin. The mentioned compound has been identified in *T. elatum* for the first time in Cuban and Martinican flowers.

Introduction

There is an ever-increasing interest in the biological effects of the bioflavonoids, members of the large group of plant polyphenols. Because of the aromatic character of these compounds, they have been analysed by several chromatographic methods. In the case of high-performance liquid chromatography, they are readily detected by their ultraviolet absorbance or electrochemical properties. More evidence that the bioflavonoids undergo extensive metabolism during uptake from the gut and distribution around the body and in specific tissues is accumulating. In addition, free radicals products at sites of inflammatory processes react with bioflavonoids and their metabolites, generating important new compounds of as yet unknown properties. For these reasons, careful examination of the chemical nature of bioflavonoids and their products in biological systems is absolutely

required. Combination of mass spectrometry with the various chromatographic methods has proved to be highly successful in this regard (Prasain *et al*., 2004). A number of analytical techniques have been used to evaluate the metabolism and bioavailability of flavonoids in vitro and in vivo (Kulling *et al*., 2002; Heinonen *et al*., 1999). These methods include gas chromatography (GC), reverse-phase high-pressure liquid chromatography (HPLC), and capillary electrophoresis (CE) in combination with UV absorbance, fluorescence, electrochemical detection, and mass spectrometry.

In the last years, several biological samples such as plant and fruit extracts containing mixtures of phenolic compounds have been analyzed with the use of hyphenated techniques such as liquid chromatography (HPLC, UPLC) coupled to DAD or PDA, (photodiode array detectors), and time of flight (ToF) or electrospray ionization-ion trap (ESI) mass spectrometers (He, 2000; Zhou *et al*., 2009). In this context we have analyzed using these precise toolsseveralsolid samples from Caribbean flowers of *Taliparitielatum*S.w.

Taliparitielatum (S.w) Fryxell (Malvaceae) is a tree with a wide distribution in Cuba and Jamaica that grows in any type of soil, particularly in swampy ones, with a reported wide range of healing properties such as appetitive, emollient, sudorific, antasthmatic and excellent expectorant. It can get about 25 m of height. Its leaves are heart shaped at the basis and peciolated (Roig, 1974; Acosta and Rodríguez, 2006). In Cuba, this specie is known as Majagua. In Jamaica it is known as Majó, Blue Mahoe, Cuba Bark and Mountain Mahoe. The mixture is used in traditional medicine as expectorant and antasthmatic. The antioxidant and antasthmatic activities of gossypitrin are enhanced by complexation with transition metals (González and Cuéllar, 2010; Cuéllar& González, 2010).The aim of the present study was to evaluate the amount of chemical components of several extractselaborated from the petals of the flowers of *Taliparitielatum*from Cuba and Martinica.

Experimental

Plant material

Flowers were collected in January 2015 in the gardens of the Faculty of Pharmacy and Foods at Havana University, and identified at the herbarium of National Botany Garden of Havana, where the voucher specimen no. HAJB 82587 has been deposited. The collection of the flowers in Martinic was realizing at the same time. A voucher specimen is deposited and registered in French Pharmacopeia as Fournet 1752 (4232 Guad). Both, Cuban and Martinican specimens are registered as *Hibiscus elatus* S.w.

Solvents

LCMS grade water (Merck), LCMS grade acetonitrile (Merck), analytical grade ethanol (Merck), analytical grade acetic acid (Merck), analytical grade n-butanol (Merck) and LCMS grade methanol (Merck) were used in the analysis work. All solvents were degassing previously before used in an ultrasonic bath without filtration.

Extract and samples preparation

Dark red flowering types were collected daily. The isolated petals used were dried by three different methods: in an oven with controlled temperature, at 40 ˚C, during 5 days (Cuban sample #1); in an oven with controlled temperature, at 45 ˚C, during 5

days (Martinican sample #2); at shadow at room temperature during a week (Martinican sample #3). The extracts were prepared with the ground material (60 g) without screen extracted in a Soxhlet apparatus with 675 mL of ethanol at 95 % during 20 hours. The ethanolic extracts were concentrated and evaporated under vacuum to 200 mL at 120 rpm, a temperature of 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) (10x20 cm) using n-butanol: acetic acid: water (4:1:5) as eluent (v/v/v).

HPLC-UV-ESI-MS/MS procedures, instrumentation and parameters

The LC system consisted of an Agilent 1100 HPLC system (Agilent, Palo Alto, CA) including Degasser (G1322A), Quaternary pump (G1311A), Autosampler (G1313A), Column heater (G1316A) and DAD (G1315B). The HPLC column was a Waters Atlantis C18, 150mm x 2.1 mm x 3µm. Elution was performed at a flow rate of 3 mL/min., using as eluent (A) H_2O 0.1% and eluent (B) ACN 0.1%.

All solvents were degassing previously before used in an ultrasonic bath without filtration. A gradient of A = 90.0% and B = 10.0% during 3 min, was followed by holding the gradient during 37 min, then changing the gradient of A = 0,0% and B = 100.0% during 5 min and reversing to A = 90.0% and B = 10.0% during 5 min.

LC–MS analyses were performed on a Thermo Finnigan (Thermo Electron, San Jose, CA) 3D ion trap mass spectrometer fitted with an Electrospray source.LC–MS analysis was performed with the above described HPLC method, except that UV data were recorded from 190 to 400 nm (PDA). For MS analysis both positive and negative ion mode of ESI were examined with the scan range from m/z 50 to 1500. Capillary Temp (C): 275, 00, Sheath Gas Flow (ua): 50, 00, Aux/Sweep Gas Flow (): 10, 00, Source Type: ESI. POSITIVE POLARITY: Source Voltage (kV): 4,50, Capillary Voltage (V): 37,00, Tube Lens Offset (V): 30,00, Multipole RF Amplifier (Vp-p): 400,00, Multipole 1 Offset (V): -4,00, Multipole 2 Offset (V): -6,00, InterMultipole Lens Voltage (V): -30, 00. NEGATIVE POLARITY: Source Voltage (kV): 4,50, Capillary Voltage (V): -10,00, Tube Lens Offset (V): -50,00, Multipole RF Amplifier (Vp-p): 400,00, Multipole 1 Offset (V): 3,00, Multipole 2 Offset (V): 7,00,InterMultipole Lens Voltage (V): 16,00. MS^2 of three compounds were recorded from 130,0 to 650,0 m/z in negative mode.

Results and Discussion

HPLC-DAD and MS Analysis of Phenolic Compounds

Figure 2and 3 show the total ionic current of the three natural compounds (1, 2 and 3) investigated by LCMS. The LC conditions permitted a good separation of these compounds and were optimized for further separations of crude plant extracts containing aglycones or glycosylated flavonoids derivatives in 50 min. The four solid samples are comparable, and they exhibits two majoritarian peaks at 14.68 and 16.70 min, respectively. In figure 2 an additional peak appear at 11.38 min and

corresponding to a natural product with molecular weight of 479 a.m.u.

In mass spectrometry, C-glycosyl flavones experiment cross-ring cleavages of sugar residues yielding main signals (ions produced by losses of 60, 90 and 120 a.m.u) (Cuyckens & Claeys, 2002; Figueirinha *et al*., 2008) that allowed differentiation with O-glycosyl flavones (losses of 162 a.m.u. for hexose, 146 a.m.u. for rhamnose and 132 a.m.u. for pentose moieties, respectively) (Simirgiotis *et al*., 2012).

In this work we report one tentative alkaloid (Peak 1) and two *O*- glycosyl flavones (Peaks 2 and 3).The four samples are comparable. Only the Martinican sample #3 showed a supplementary signal at 480 a.m.u in mass spectrometry in positive ion mode that corresponding to a product with molecular mass of 479 a.m.u (Peak 1). Impair mass implicates the presence of nitrogen in natural products. The corresponding retention times of each compound were 11.38 (1), 14.68 (2) and 16.70 min (3), respectively (Fig. 2).

Fig.1 Structure of the compounds 2 and 3 identified in Taliparitielatum.

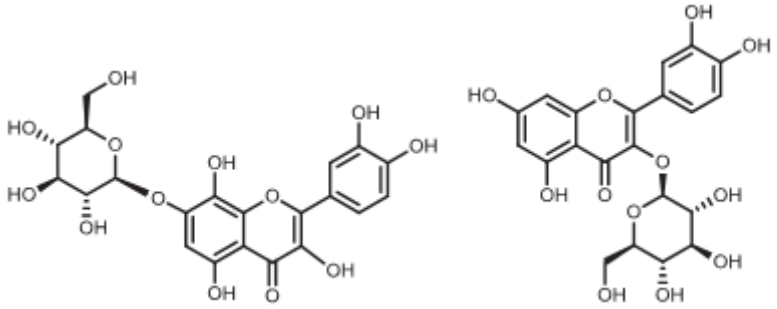


Fig.2 Chromatograms in positive and negative ion mode of the Martinican sample #3.

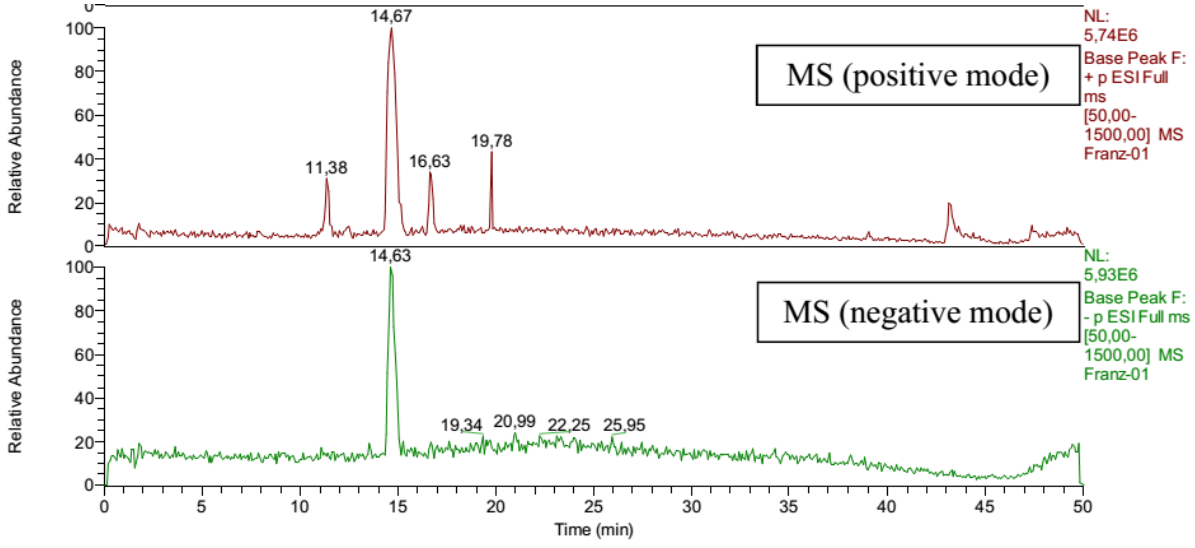


Fig.3 Mass spectrums obtained in LC/MS/MS in negative mode.

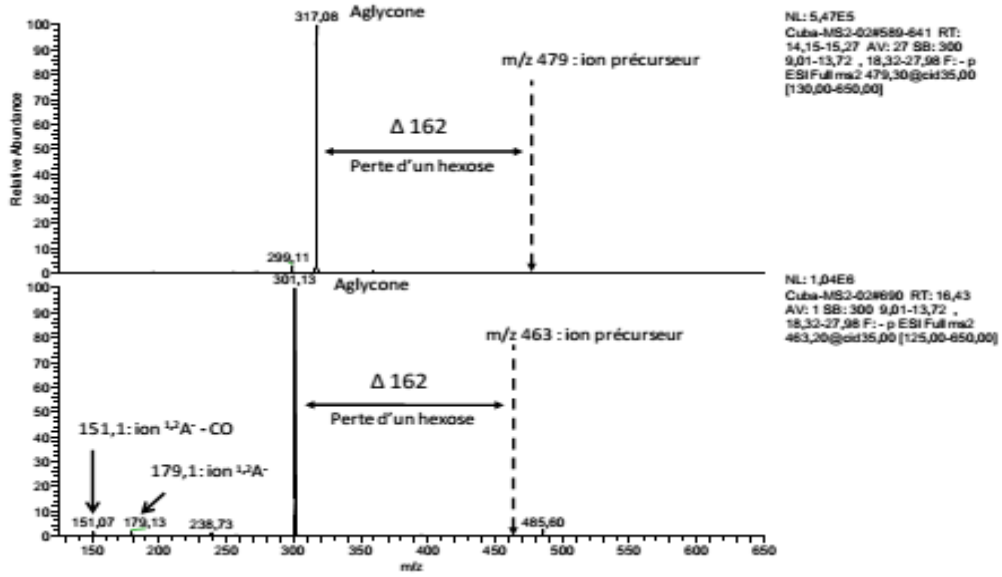
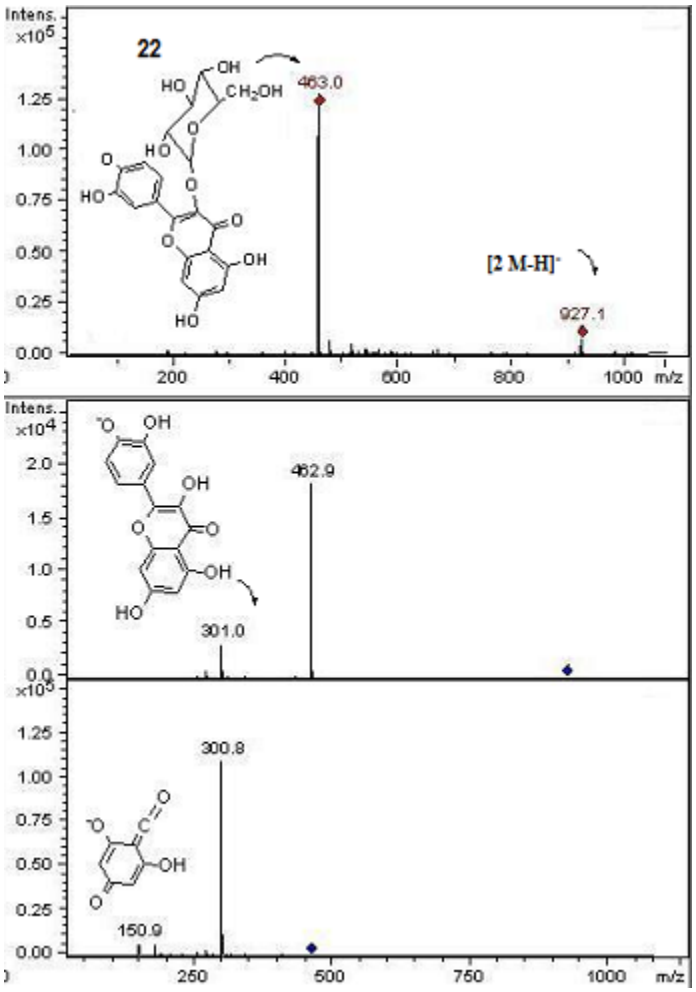


Fig.4 Structures, fragmentation, full ESI-MS and MS-MS spectra of peak 3



Peak 2 with a molecular weight of 481 a.m.u in positive ion mode and 479 a.m.u in negative ion mode is a glucoside flavonoid that was previously reported in *T.elatum* by our research group as gossypitrin (Cuéllar& González, 2010). In negative ion mode the compound showed a parent ion at m/z 479 which produced MS ions at m/z 317 (loss of glucose: 162 a.m.u).

Peak 3 with UV data corresponding to a quercetin derivative and a $[M-H]^-$ion at m/z 463 which produced MS ions at m/z 301 (loss of glucose: 162 a.m.u)was identified as isoquercitrin (quercetin 3-*O*-glucose), which were identified previously in hawthorn (Ding*et al*., 2010; Prinza *et al*., 2007) by comparison with authentic compounds. Two additional ions are present in the fragmentation of 3, the ions at m/z 151.1 and 179.1 corresponding to the fragment ion $[^{1,2}A - CO]$ and $[^{1,2}A^-]$ of quercetin, respectively (Fig. 3). Both ions are reported by literature and they are diagnostic ions to describe the flavonoid fragmentations.

Peak 3 (Figure 4) with UV data 252 and 370 nm, pseudomolecular ion at m/z 463 and MS-MS ions at 301, 179 and 151 a.m.u. was identified asIsoquercitrin. We suggest a tentatively identification of this flavonoid glucoside instead hyperosil (quercetin 3-*O*-galactose), because up to now, in this medicinal plant the reported glycosylated flavonoids showed the presence of glucose in their molecular structures.

Conclusions

The HPLC fingerprints showed in this work can be used to authenticate and differentiate the edible flowers of the two species called*majagua* or *blue mahoe* from Cuba and Martinica which are similar in appearance and are grown in different locations and used for similar medicinal purposes. Furthermore, based on our LC/DAD and LC/MS experiments, the distribution of different phenolics in the two species has been analyzed and a total of two phenolic compounds weredetected and characterized, or tentatively identified for the first time for both speciesfrom both countries. The compounds identified can be also used as biomarkers especially for *T. elatum*since little research has been published for this species. The phenolic profiles of the plant part revealed high predominance offlavonoids, which are antioxidant compounds that modulate a variety of beneficialbiological events. Therefore, *T. elatum* edible flowersmay be considered a source of important phytochemicals (mainly flavonoids and phenolic acids) with bioactive properties to be explored for pharmaceutical applications.

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