Journal of Pharmacy and Pharmacology 5 (2017) 489-496
doi: 10.17265/2328-2150/2017.08.001

New Phytochemical Profile of Ethanolic Extract from *Taliparitielatum* (Sw.)

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Abstract: Genus Taliparitihas long history of use in various traditional medicine therapeutic applications in Cuba, especially in treatment of bronchial asthma and flu. Petals of genus Taliparitiare rich source of secondary metabolites and most of these metabolites are reported to possess expectorant, antasthmatic, appetitive, antioxidant, and antibacterial properties. Hence, in our study, we present a validated, sensitive, reliable, and cheap narrow-bore 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. In addition, about twelve compounds were identified in this specie based on chromatography retention time (t_R), UV and MS/MS spectra and compared with those of isolated authentic compound and literature data. About eighth constituents were reported for the first time from *Taliparitielatum*. Our results demonstrated the developed method could be employed as a rapid and versatile analytical technique for identification of chemical constituents and quality control of *Taliparitielatum*.

Key words: UHPLC-DAD-ESI-MS/MS, flavonoids, petals, chemical-compounds, taliparititi.

1. Introduction

Genus Taliparititi is a Pantropical genus belonging to family Malvaceae and is represented by 22 species, but only two of them are present in Cuba, *Taliparitielatum* var. *macrocarpum* and *Taliparititiliaceum* var. *tiliaceum*, and from this last one, a sub specie var. *pernambucense* [1]. Taliparititi elatum is native to the islands of Cuba, Jamaica, US, Virgin Islands, Puerto Rico and Martinica. In wetter areas it will grow in a wide range of elevations, up to 1,200 meters (3,900 Ft.) and is often used in reforestation. It is the national tree of Jamaica. Taliparititi elatum 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 (Fig. 1). It grows quite rapidly, often attaining 20 meters (66 Ft.) or more in height. Commum names are *Majagua*, *Majagua azul*, *Majagua común*, etc. The English name is *Blue Mahoe*. The name mahoe is derived from a Caribe word. The "blue" refers to blue green streaks in the polished wood, giving it a distinctive appearance [2].

A very few data on flavonoids and other constituents in *T. elatum* are available. Until now, only gossypitrin (gossypetin-7-*O*-β-D-glucoside), gossypetin-3'-*O*-glucoside and quercetin-3-*O*-glucoside (Isoquercitrin) have been reported from ethanolic extracts of the petals of the flowers in this plant [3-5].

Hence, we investigated the composition of an ethanolic extract from *T. elatum* in-depth and presented hereby the first detailed and comprehensive report on the phenolic compound of *T. elatum*, being highly interesting because of the medical use of this plant. The structures of 12 chemical compounds were elucidated by UHPLC-DAD-ESI-MS/MS experiments after isolation.

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Fig. 1 Flower of *Taliparitiy elatum* (Sw.) Fryxell (Malvaceae).

2. Material and Methods

2.1 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.

2.2 Solvents

LCMS grade water (Merck), LCMS grade acetonitrile (Merck), LCMS grade isopropilic alcohol, 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.

2.3 Extract and Samples Preparation

Dark red flowering types were collected daily. The isolated 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) without screen extracted in a Soxhlet apparatus with 675 mL of ethanol at 95% during 20 h. 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) (10 × 20 cm) using n-butanol: acetic acid: water (4:1:5) as eluent (v/v/v) [6].

2.4 HPLC-DAD-ESI-MS/MS Procedures, Instrumentation, and Parameters

The LC system consisted of an UHPLC Ultimate 3,000 Dionex with a DAD (diode array detector) Dionex with a UV-VIS at 254 nm (Wavelength) coupled to a Mass Spectrometer Ion Trap 500 MS Varian (USA) fitted with an Electrospray source with the scan range from *m/z* 0 to 700. Configuration Turbo DDS Method (Automatically Fragmentation) in Negative mode (-), Needle voltage -5 kV and Spray shield Voltage -600 V, Column Waters Atlantis C18 3 μm (i.d) 120 Å, 100 mm × 4.6 mm. UV data were recorded from 190 to 400 nm (PDA). A gradient of A (H_2O) = 80.0%, B (ACN) = 20.0% g during 2 min, was followed by holding the gradient during 12 min, then changing the gradient of A = 20.0%, B = 80.0% and C = 0.00% during 6 min and reversing to A = 0.0%, B = 100.0% and C = 0.00% during 6 min, changing to A = 80.0%, B = 20.00% and C = 80.00% during 8 min and finally reversing to A = 80.00%, B = 20.00% and C = 0.00% during 2 min. The total time of running was 34 min with a flow of 0.8 mL/min and split 350 μL/min for MS and the rest for waste.

3. Results and Discussion

Fig. 2 shows the total chromatographic profile of the investigated extract by LC-MS. 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

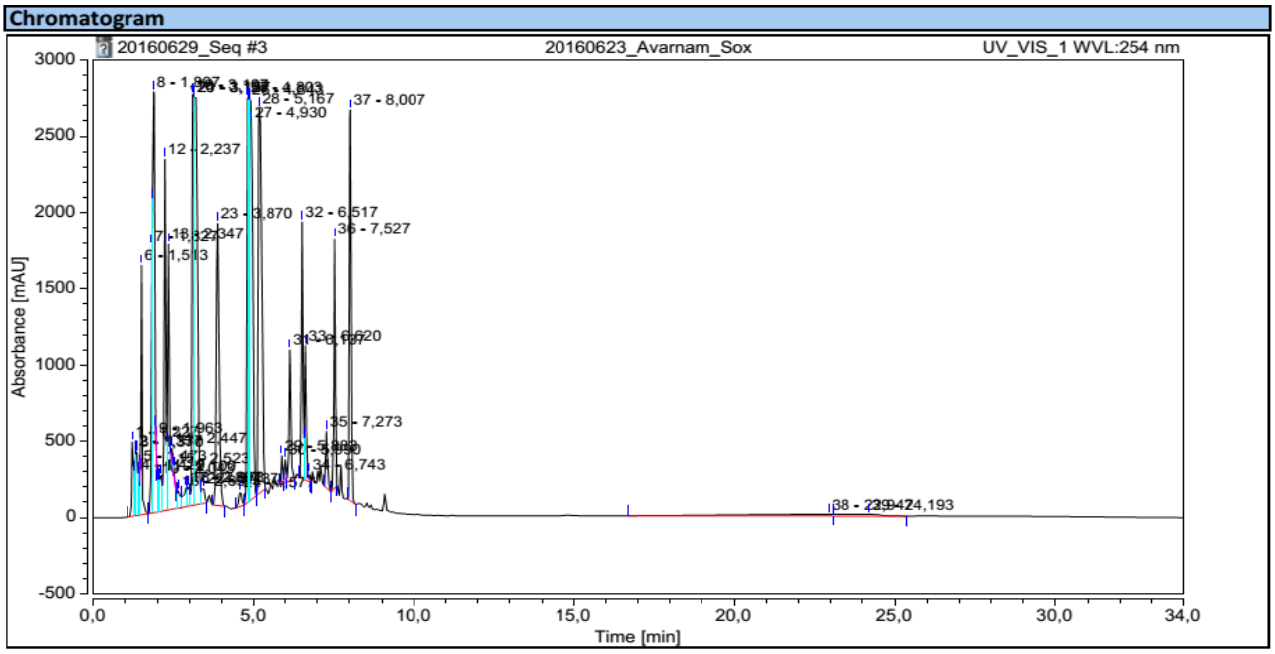

Fig. 2 Chromatogram of ethanolic extract from the petals of *T. elatum* recorded from 190 to 400 nm (PDA).

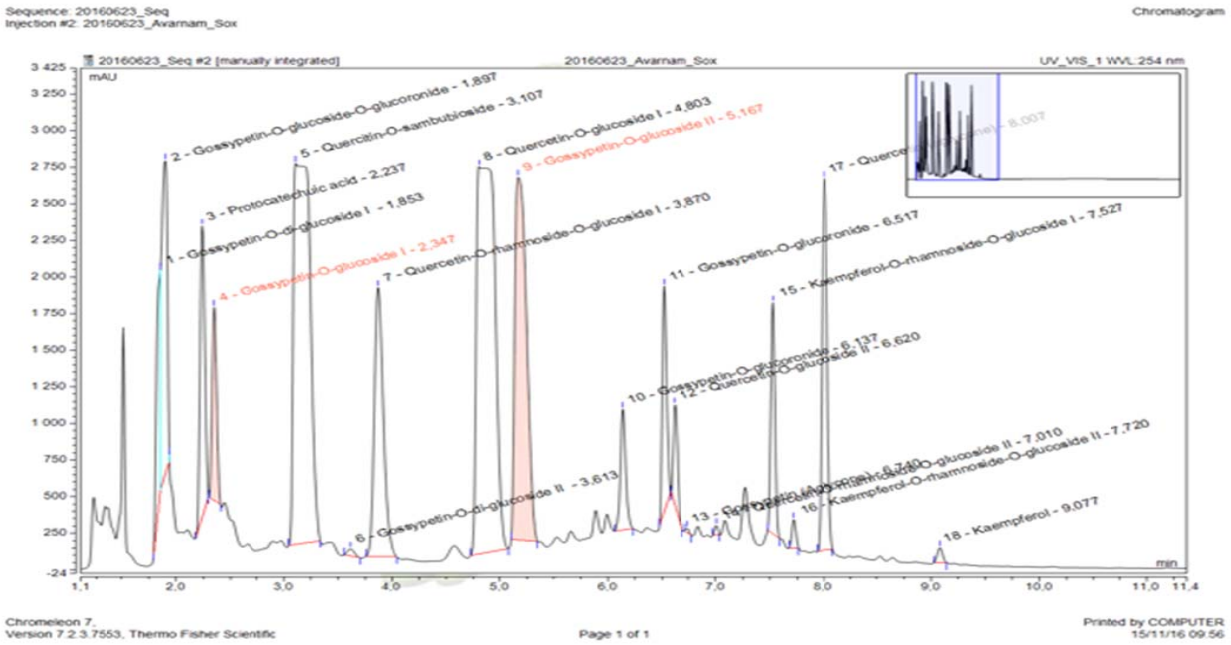

Fig. 3 Chromatogram of ethanolic extract from *T. elatum* with the phenolic profile.

derivatives and other chemical constituents in 34 min. At least 39 different kind of chemical compounds were found in the ethanolic extract using the gradient with A (H_2O), B (ACN), and C (Isoprop).

Fig. 3 shows the chromatogram of ethanolic extract from the plant searching the phenolic profile. In the LC, at least 29 different kinds of chemical constituents are found, giving an idea that the utilized gradient give a good resolution to get the information about how many phenolic compounds were in the ethanolic extract.

Table 1 lists the retention times (t_R), MS data spectra and maximal ultraviolet wavelength (λmax) for the chemical constituents found in the extract. 12 chemical compounds were detected after HPLC-DAD-ESI-MS/MS,

Table 1 Identified compounds in *Taliparititi elatum* by UHPLC-DAD-ESI-MS/MS.

No.	Identified Compound	tR (min)	Peak No. UV Crom.	MS^1 m/z Ion Type $[M-H]^-$	Theorical mass [M-H]	MS^2 m/z Ion Type (Relative Intensity)	UV max
	Organic acid						
1	Protocatechuic acid	2.319	6	153.0 $[M-H]^-$	154.0	153.0 [M-H] (10)-109.0 [M-COOH] (100)	-
	Flavonols						
2	Quercetin (aglycone)	8.118	26	301.0 $[M-H]^-$	302.1	301.0 [M-H] (20)-272.9 [M-H-CO] (14)-257.0 [M-H-CO2] (12)-179.1 [^{1,2}A] (100)-151.2 [^{1,2}A-CO] (85)	250-367
3	Quercetin-*O*-glucoside and Isomer	4.944/ 6.640	12-18	463.1 $[M-H]^-$	464.4	301.1 [M-Hex] (100)-179.1 [^{1,2}A] (2)	225-250-367
4	Quercetin-*O*-rhamnoside-*O*-glucoside	3.998/ 7.110	10-21	609.1 $[M-H]^-$	610.1	463.1 [M-DeoxyHex] (100)-301.1 [M-DeoxyHex-Hex] (100)-179.1 [^{1,2}A] (2)	222-257-356
5	Kaempferol (aglycone)	9.169	27	284.9 $[M-H]^-$	286.2	285.0 [M-H] (100)-241.1 [M-18] (1)-243.1 [M-18] (1)-213.1 [M-18] (1)-151.1 [M] (3)	266-300-373
6	Kaempferol-*O*-rhamnoside-*O*-glucoside and Isomer	7.617 / 7.798	24-25	593.1 $[M-H]^-$	594.1	447.1 [M-Hex] (23)-307.1 [M-2Hex] (8)-285.2 [M-2Hex] (100)	230-267-316
7	Gossypetin (aglycone)	6.718	19	317.0 $[M-H]^-$	318.1	317.1 [M-H] (5)-299.1 [M-18] (100)	261-335-385
8	Gossypitrin (gossypetin-7-O-glucoside) and Isomer	2.407 / 5.287	7-13	479.0 $[M-H]^-$	480.0	317.1 [M-Hex] (100)-299.1 [M-18] (1)	-
9	Gossypetin-*O*-di-glucoside and Isomer	1.998 / 3.730	4-9	641.0 $[M-H]^-$	642.0	479.1 [M-Hex] (100)-317.1 [M-2Hex] (18)	-
10	Gossypetin-*O*-glucoside-*O*-glucuronide	2.034	5	655.1 $[M-H]^-$	656.1	479.2 [M-Glucur] (100)-317.2 [M-Glucur-Hex] (2)	-
11	Gossypetin-*O*-glucuronide	6.209	17	493.1 $[M-H]^-$	494.1	317.2 [M-Glucur] (100)-299.2 [M-Glucur-18] (1)	257-374
12	Quercetin-*O*-sambubioside	3.107	8	595.1 $[M-H]^-$		475.2 (16)-463.6 (22)-445.4 (22)-301.3 (43)-300.4 (100)-[M-H-CO] (14)-271.0 [M-H-CO2] (16)-255.1 [^{1,2}A] (9)-229.3 (1)	248-315-368

one organic acid and 11 flavonols, showing that the extraction procedure adopted is efficient for concentration of this class of compounds. The proposed structures were confirmed after fractionation of the extract by HPLC with detection set at 254 nm. They all share the flavonol-skeletons of quercetin, kaempferol and gossypetin and their glucosyl or glucuronide derivatives, glycosylated with common monosaccharides or oligosaccharides.

Among them, 9 chemical constituents are being reported for the ethanolic extracts of the petals for the first time: one carboxylic acid (protocatechuic acid), two aglycones flavonol types (quercetin and kaempferol), three flavonoid-*O*-diglycosides (quercetin-*O*-rhamnoside-*O*-glucoside, kaempferol-*O*-rhamnoside-*O*-glucoside and

gossypetin-*O*-diglucoside), and probably gossypetin-*O*-glucoside-*O*-glucuronide and gossypetin-*O*-glucuronide. Until now, we have not evidence of the presence of quercetin-O-sambubioside in this plant. Quercetin-3-*O*-glucoside, gossypetin aglycone and gossypitrin (gossypetin-7-*O*-glucoside) were previously identified by our research team [3, 4, 6].

MS/MS data obtained was used for structural identification of each compound. Most of these compounds were flavonoids and their MS/MS data revealed some common features such as neutral loss of H, CO, CO_2, H_2O and loss of glucose residue or 2 hexose residue in case of flavonol glycosides/diglycosides. Loss of CO is due contraction of C-ring [7]. Besides RDA (retro-diels alder) fragment ions (pathway I) were also observed, which are important for determination of substitutions on -A and -B rings of flavonoid nucleus. 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. The peak at m/z 273 was observed, and this result allowed us to propose a pathway involving C ring with their corresponding loss of CO (m/z 28 u). For flavonols, having the 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 [8, 9]. According to the data published by Simirgiotis [10], this compound was suggested to be Isoquercitrin (quercetin 3-*O*-glucose), which were identified previously in hawthorn [11, 12], 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.

New information was found with the results from the particular application of neutral loss scans in MS^2 spectra. The presence of a deoxyhexose into the chemical structures of two flavonol glycosides in this medicinal plant, specifically rhamnose. It must be noted that like in some *O*-glycosides an internal sugar loss may take place, in which the aglycone-bound sugar is released first and, simultaneously, the other residue attaches itself to the aglycone [13], which can be used to characterize the distribution of sugar residues. That is the case of quercetin-*O*-rhamnoside-*O*-glucoside (m/z 609.1) and kaempferol-*O*-rhamnoside-*O*-glucoside (m/z 593.1). In both cases, the losses of 134 Da lead to identify radical ions with molecular weights of 463.1 and 447.1, respectively. The loss of the second sugar moiety attached to the aglycone was confirmed by the loss of 162 Da to give arise the radicals ions at m/z 301.1 and 285.2 [14] (Figs. 4 and 5).

Another new three reported compounds are gossypetin-*O*-diglucoside, gossypetin-O-glucoside -*O*-glucoronide, and gossypetin-*O*-glucoronide. In the first two cases, it is obvious that the sugar moiety corresponds to a hexose and in the last two mentioned

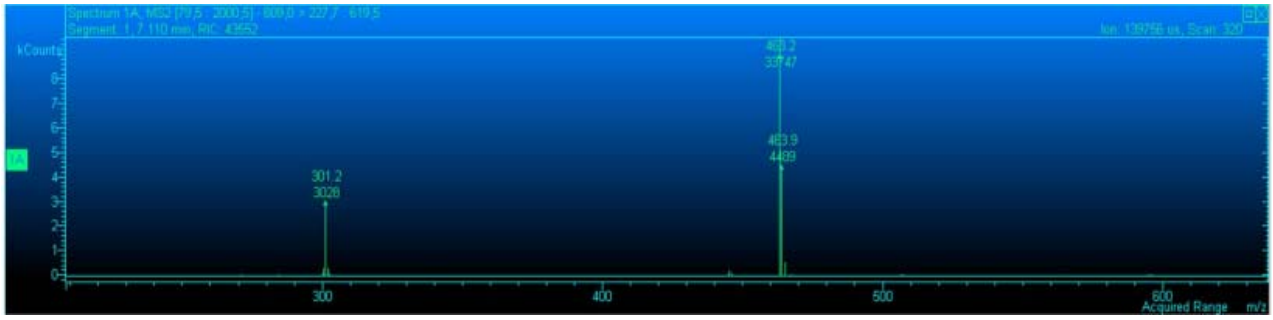

Fig. 4 MS^2 spectrum of quercetin-*O*-rhamnoside-*O*-glucoside.

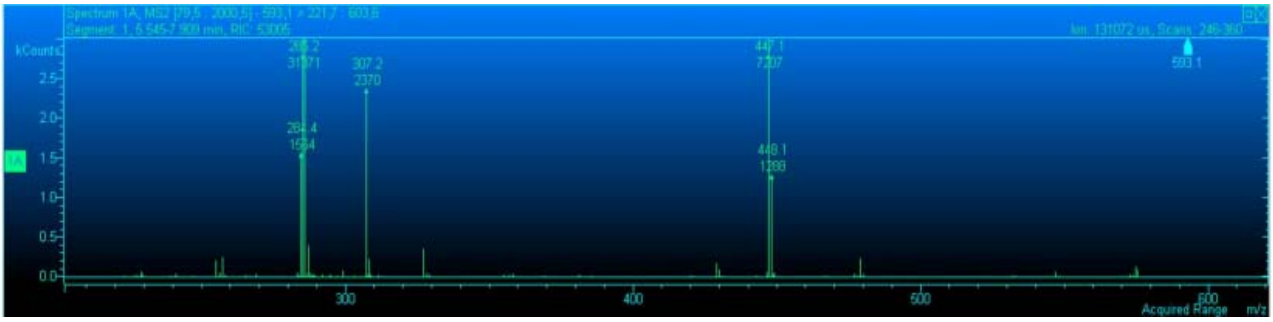

Fig. 5 MS^2 spectrum of kaempferol-*O*-rhamnoside-*O*-glucoside.

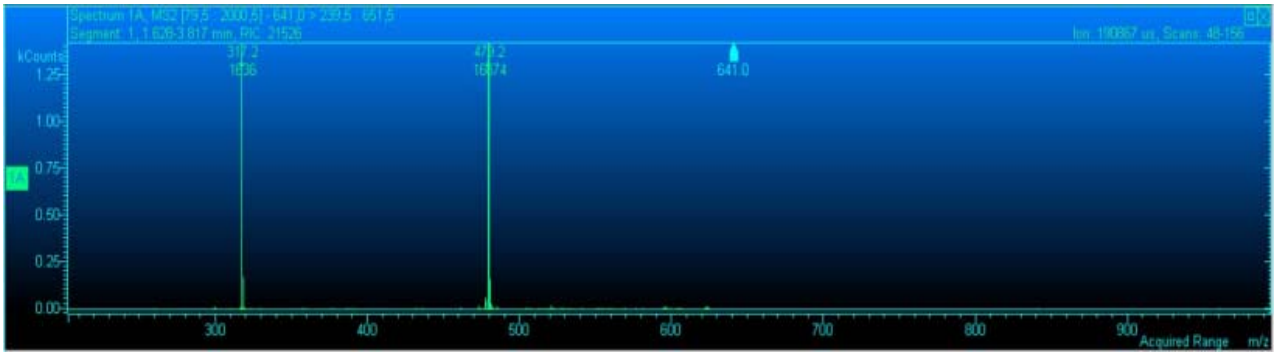

Fig. 6 MS^2 spectrum of gossypetin-*O*-diglucoside.

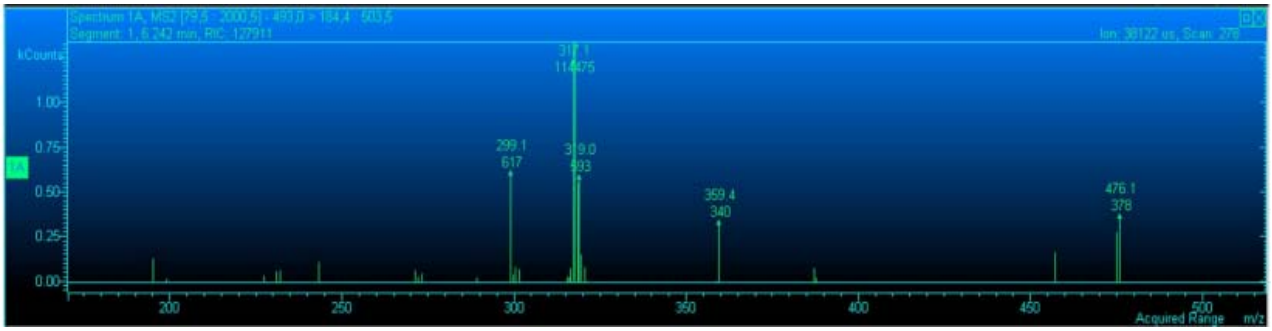

Fig. 7 MS^2 spectrum of gossypetin-*O*-glucuronide.

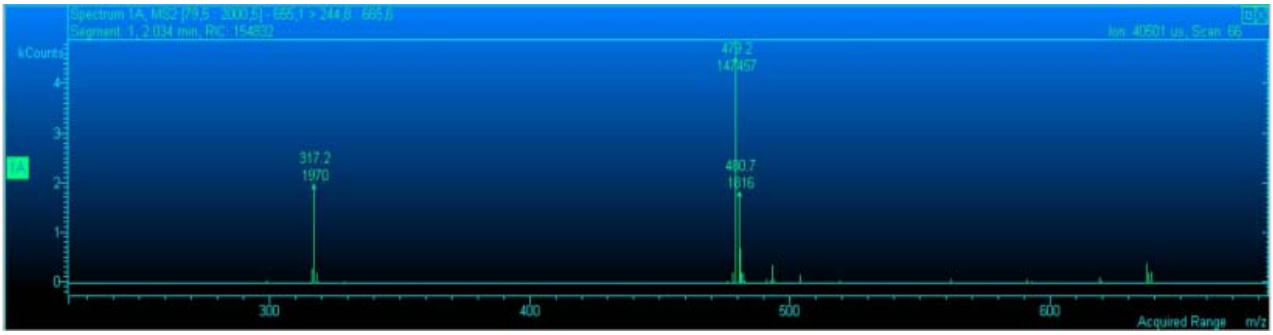

Fig. 8 MS^2 spectrum of gossypetin-*O*-glucoside-*O*-glucuronide.

compound the presence of a glucuronide moiety, respectively (Figs. 6, 7 and 8).

It is possible to confirm the presence of these three chemical components by the losses of 162 Da corresponding to a hexose, when a deglycosylation takes place and becomes the radical ions at m/z 479.1 and 317.1 and the deglucuronidation by losses of 176 Da. The sugar type can be easily determined by the

characteristic m/z values of the A_i, fragments arising from hexoses, which are not directly observable in the mass spectra but can be computed from the m/z differences of the parent ions and corresponding X_i [13, 15, 16].

4. Conclusions

A simple and versatile analytical method was developed for qualitative identification of major constituents in the petals of the flowers of *Taliparitielatum*, by using HPLC-DAD-ESI-MS/MS in negative ion mode. About 11 constituent including one organic acid and 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 Taliparitispecies. This method was successfully applied to identify 8 constituents in petals of *T. elatum*, which were not previously reported from this species. 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 Taliparitii. Further use of innovation studies like MALDI-TOFD-MS and HPLC-DAD-ESI-MS/MS-NMR are necessary for detection and characterization of minor/unidentified compounds.

Acknowledgements

This work is cooperation between the Laboratory BIOSPHERES of FWI University (UA) and ARVARNAM. We extend our sincere thanks to the Regional Council of Martinica for its financial support.

Conflict of Interest

The authors have declared no conflict of interest.

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