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Isolation and Characterization of Flavonols by HPLC-UV-ESI-MS/MS from *Talipariti elatum* S.w

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Abstract

The red petals of the flowers of *Talipariti elatum*, commonly named majagua is used as antitussive, expectorant and antasthmatic in phytotherapy, although the plants' composition has not been determined in detail so far. Hence, in this study, we present a validated, sensitive, reliable, and cheap narrow-bore LC-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, the structures of two compounds have been elucidated by LC-MS experiments after isolation. Structure analyses allow proposing the presence of gossypitrin (gossypetin-7-*O*-glucoside) or gossypetin-3'-*O*-glucoside, a quercetin derivative, possibly quercetin-3-*O*-glucoside and an unidentified compound with an impair number of *m/z*, probably an alkaloid.

Keywords

Flavonoids, HPLC, MS, Petals, Chemical Composition

1. Introduction

Blue Mahoe or Cuba Bark (*Taliparitielatum*) is an edible plant used in various applications including foods. The tree grows in Cuba, Jamaica, and others countries in the Caribbean area such as Puerto Rico, Panama and Martinic Island. The flower of the plant is an important source of bioactive compounds, such as organic acids,

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phytosterols, and polyphenols, some of them with antioxidant properties. The phenolic content in the flowers mainly consists of flavonoids like gossypitrin, rutin and quercetin [1] and besides the known flavonoid gossypitrin, the presence of more than 40 different kinds of chemical compounds such as *β*-sitosterol, γ-sitosterol, red anthocyanin, phenolic acids such as propionic acid, pentatonic acid, hydroxypropionic acid, hydroxyacetic acid, 2-hydroxypropionic acid and hexanoic acid was reported [2] [3]. Gossypetin -3'-*O*-glucoside was isolated for the first time from the flowers of the plant in Martinic Island by maceration with methanol (24 h), and Soxhlet extraction with methanol, ethyl acetate and 1,2-dimethoxyethane as solvents [4] [5].

A solution of gossypitrin at 5% in distilled water has been found to exhibit antibacterial activities against *Salmonella typhi*, *Klebsiella pneumoniae*, *Providencia* sp., *Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri*, *Citrobacter freundi*, *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus vulgaris*, *Enterococcus faecium*, *Citrobacter freundii*, *Staphylococcus epidermidis* and antifungal activities against *Candida subtilis* and *Candida albicans*, this last one, an opportunity fungi present in inmunodepression conditions in human health [6] [7]. Antibacterial effects of this plant compound against *Escherichia coli*, *P. aeruginosa* and *S. aureus* and *S. epidermidis* suggest that it may possess remarkable therapeutic action in the treatment of gastrointestinal infection and diarrhea in man and skin diseases [8]. The antibacterial effect against *K. pneumoniae* suggests that the flavonoid may possess remarkable therapeutic action in the treatment of lungs infection and bronchial asthma. The antioxidant and antasthmatic activities of gossypitrin are enhanced by complexation with transition metals [7].

The aim of the present work was to determine the presence of the major components from the petal of the flowers of *Taliparitiy elatum* S.w in order to find out if there exist any differences among samples that could help to give a special place in the national market in Cuba and Martinic.

2. Experimental

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. 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.

2.2. 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.

2.3. 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) (10 × 20 cm) using n-butanol: acetic acid: water (4:1:5) as eluent (v/v/v).

2.4. 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, 150 mm × 2.1 mm × 3 µm. 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 ThermoFinnigan (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.

3. Results and Discussion

After extraction from the three different samples of flowers collected in Cuba and Martinic, four solid samples were isolated from them. One from both Cuban and Martinican sample # 3, and two from Martinican sample # 2, after first and second crystallization.

Identification of main flavonoids and their derivatives by LC-UV-ESI-MS/MS

Figure 1 shows the total ionic current of the three natural compounds (1, 2 and 3) investigated 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 derivatives in 50 min. The four solid samples are comparable, and they exhibits two majoritarian peaks at 14.68 and 16.70 min, respectively.

Table 1 lists the retention times (Rt), MS data spectra and maximal ultraviolet wavelength (λ_{max}) for the chemical constituents found in the samples. Compound 1 was found only in the Martinican sample # 3, showed a supplementary signal with a molecular mass of 480 in positive mode ion and a retention time of 11, 38 min. This result revealed that we are in presence of a chemical compound with impairing number of mass (*m/z* 479).

Table 1. The retention times (R_t), MS data spectra and maximal ultraviolet wavelength (λ_{max}) for the chemical constituents found in the solid samples from *Talipariti elatum* through HPLC-UV-ESI-MS/MS.

Sample	Spectral information	Retention time (min); Mean based on MS^+			
		11.38	14.68	16.70	19.78
Cuba	UV (λ_{max}) MS (+) *m/z* MS (−) *m/z* $[2M-H]^-$		278 481.1 479.2 959.0	252 465.0 463.1	
Martinican 3	UV (λ_{max}) MS (+) *m/z* MS (−) *m/z* $[2M-H]^-$	202 480.0	278 481.1 479.2 959.0	252(370) 465.0 463.2	Artefact
Martinican 2 first cristallisation	UV (λ_{max}) MS (+) *m/z* MS (−) *m/z* $[2M-H]^-$		276 481.1 479.2 959.0	254(368) 465.0 463.2	
Martinican 2 second cristallisation	UV (λ_{max}) MS (+) *m/z* MS (−) *m/z* $[2M-H]^-$		278 481.1 479.2 959.0	252(370) 465.0 463.2	

Note: MS^+ quasimolecular ions are $[M+H]^+$; MS^- quasimolecular ions are $[M-H]^-$.

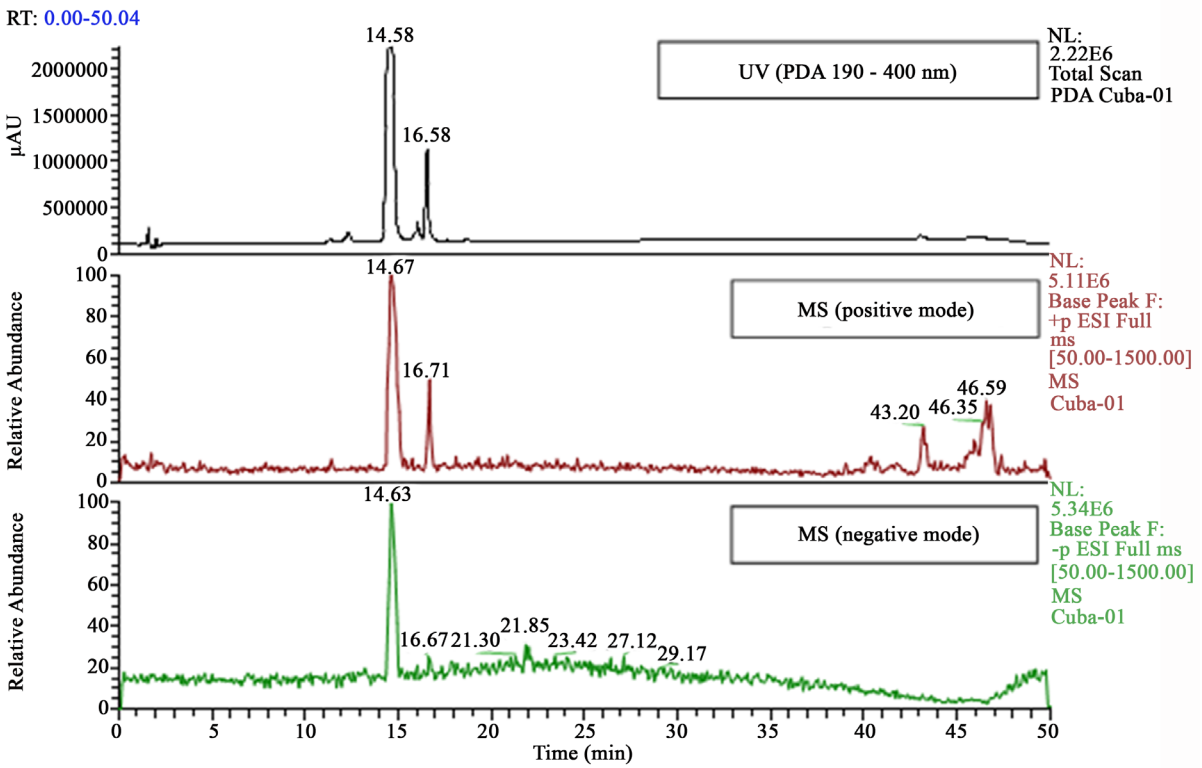


Figure 1. Chromatograms of the three natural compounds (1, 2 and 3) investigated by LC-MS.

Compounds 2 and 3 are all flavonoid-*O*-hexosides that were detected as [M−H] ions at *m/z* 481.1 (positive mode ion) and *m/z* 479.2 (negative mode ion).The second one showed two signals at *m/z* 465.0 and *m/z* 463.2, respectively (**Figure 2(a)** and **Figure 2(b)**).

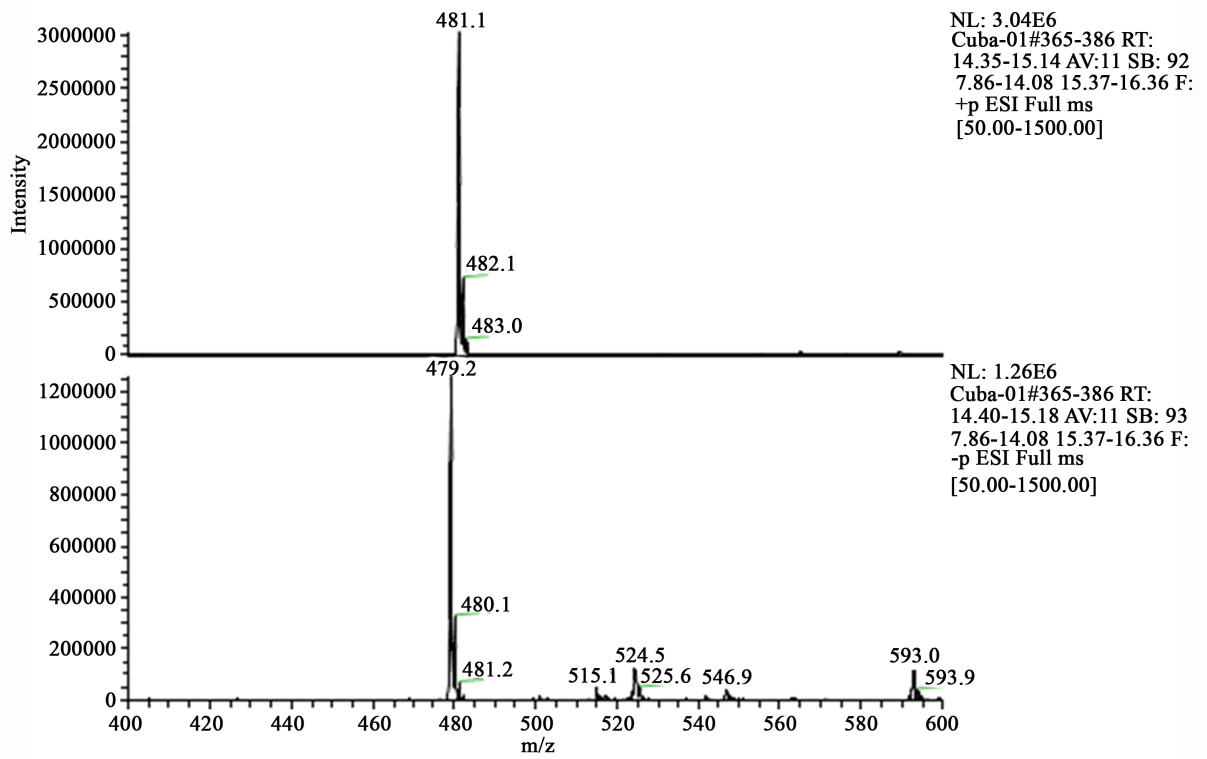
The sample was analyzed in negative mode ion to define the real molecular weight of both compounds. **Figure 3** shows the chromatograms of both compounds in negative mode ion (MS^2).

The MS^2 spectra of the two compounds are dominated by the neutral loss of the O-linked hexosyl-group (−162 u), but differ in the ratio of the resulting Y_0^- and $[Y_0-H]^{\bullet-}$ ions at *m/z* 318.1 and 301.1, respectively, and the MS^3 spectra obtained from these two fragment ions (**Figure 4**). Compound 2 showed one important peak in all analyzed solid samples at *m/z* 299, that involve the loss of H_2O (−18 u). No further fragmentation was observed from 2.

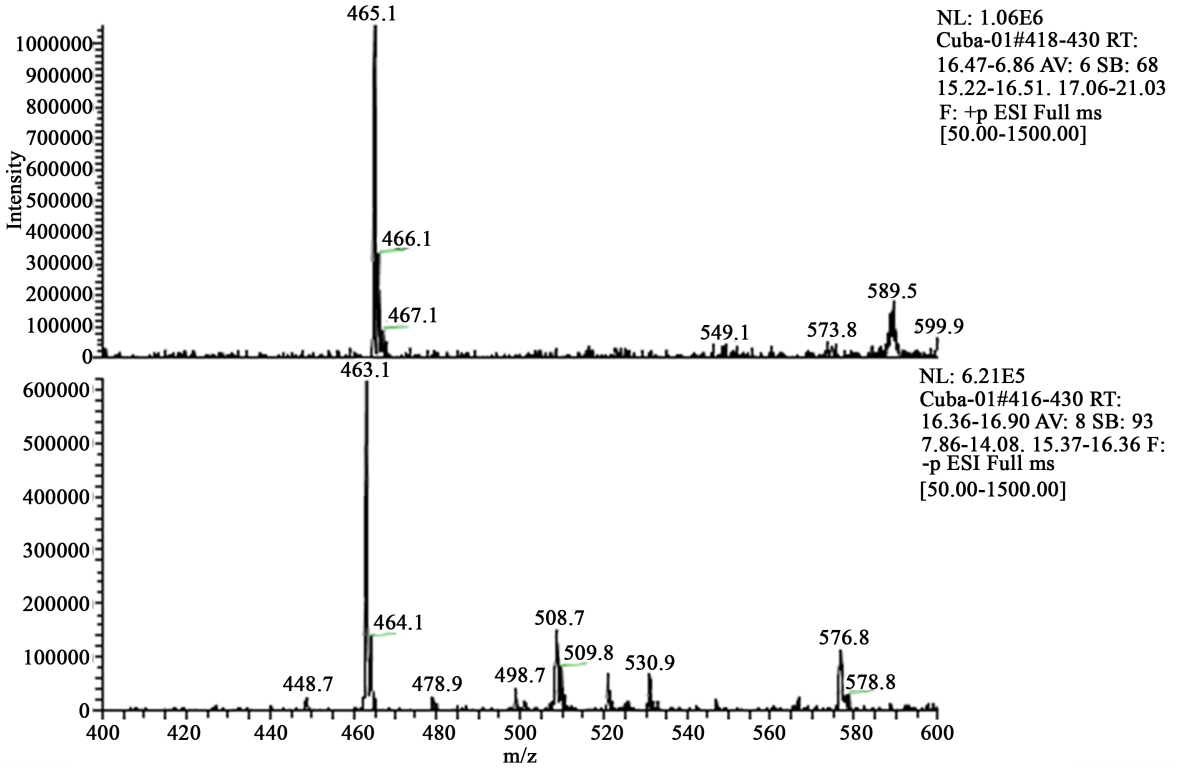
Compound 3 yielded an MS^2 spectrum typical for quercetin. The fragments proposed for quercetin reinforces the discussed hypothesis that these successive CO and CO_2 losses involve first the C ring. The most interesting fragments concern the base peaks at *m/z* 179 ($^{1,2}A^-$) and 151 ($^{1,2}A^- - CO$), 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 [11], this compound was suggested to be Isoquercitrin (quercetin 3-*O*-glucose), which were identified previously in hawthorn [12] [13], 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.

Finally, Peak 1 corresponding to a retention time of 11.38 min with a molecular ion at *m/z* 480 [(MS (positive mode)] producing a MS^2 fragment at *m/z* 467, remains unknown. According with literature, this kind of chemical compound is perhaps to be an alkaloid.



(a)



(b)

Figure 2. (a) Mass spectrum of the compound with molecular mass of 480 in both ion modes; (b) Mass spectrum of the compound with molecular mass of 464 in both modes.

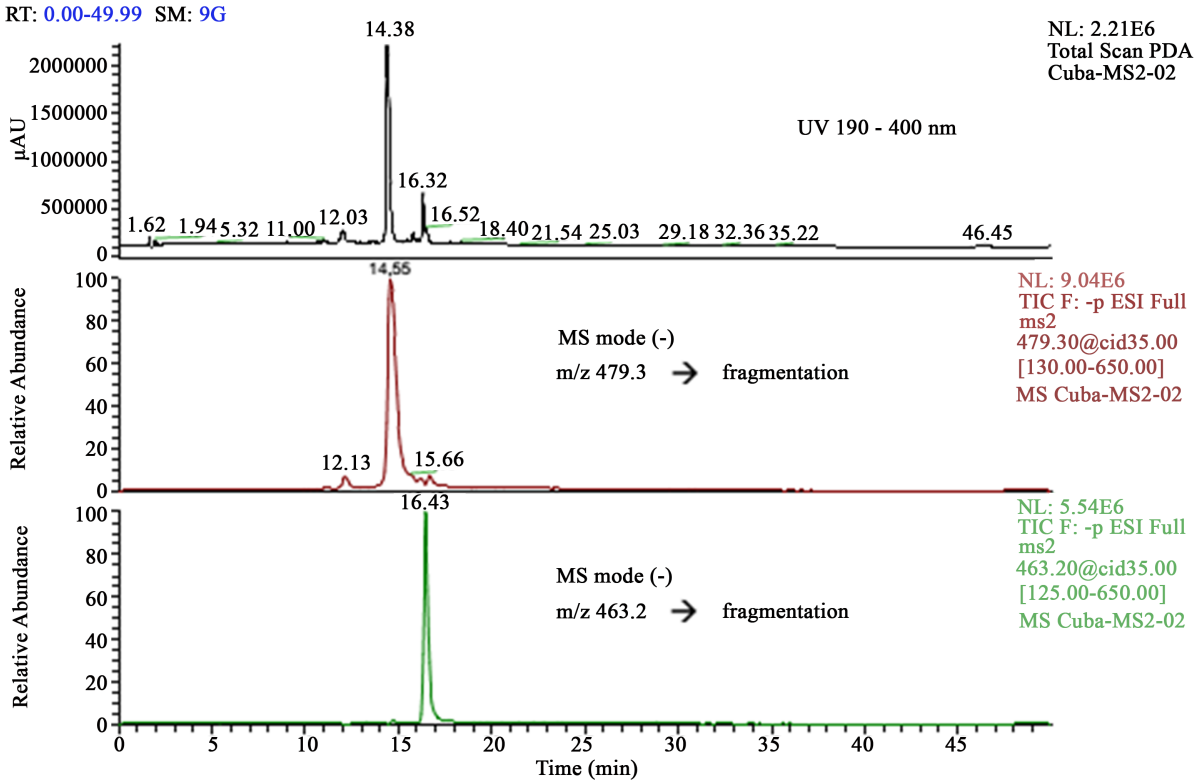


Figure 3. Chromatograms of both compounds in negative ion mode.

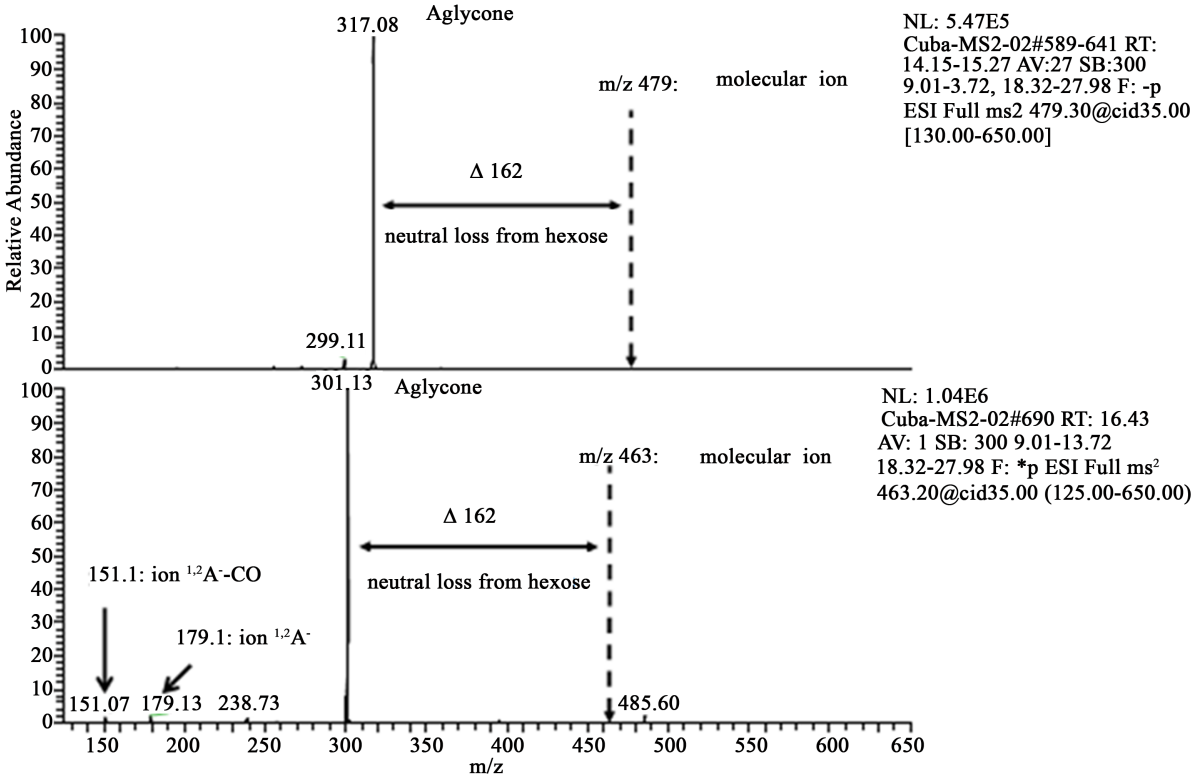


Figure 4. MS^2 spectrums of both compounds in negative ion mode.

4. Conclusion

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 *T. elatum*. The compounds identified can be also used as biomarkers especially for *T. elatum* little research has been published for this species. The phenolic profiles of the petals reveal high predominance of flavonoids, which are antioxidant compounds that modulate a variety of beneficial biological events. Therefore, *T. elatum* edible flowers may 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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