Macrothink Institute™

Journal of Agricultural Studies
ISSN 2166-0379
2017, Vol. 5, No. 2

Iron Chelating Activity of Gossypitrin Isolated from the Petals of Taliparitielatum Sw. (Fryxell) Malvaceae

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Received: March 30, 2017 Accepted: May 3, 2017 Published: May 4, 2017

doi:10.5296/jas.v5i2.11174 URL: https://doi.org/10.5296/jas.v5i2.11174

Abstract

A Fe(III)-gossypitrin complex was prepared with a metal to ligand molar ratio of 1:1; 2:1 and 3:2 by mixing stoichiometric amounts of gossypitrin and $FeCl_3$. The isolated complex was characterized by visual observation, UV/Vis, IR and ESI-MS spectroscopy. In this study, we selected the major flavonoid glycoside present in the petals of the flowers of Taliparitielatum Sw. (Fryxell) Malvaceae that grows in Cuba, gossypitrin, to characterize the chelating activity of it chemical compound with $FeCl_3$, to find alternative sources with lower side effects in asthmatic patients. A high chelating activity can be observed as a good source of new agents for asthmatic patients.

Keywords: Iron chelating, gossypitrin, petals, Taliparitielatum, flavonoids

1. Introduction

Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plants. They are synthesized by phenylpropanoid pathway. Available reports tend to show that secondary metabolites of phenolic nature including flavonoids are responsible for the variety of pharmacological activities (Mahomoodally et al., 2005; Pandey, 2007). Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions (Kumar et al., 2013a; Kumar et al., 2013b). The chelation of metals could be crucial in the prevention of radical generation which damage target biomolecules (Leopoldini et al., 2006; Kumar et al., 2013c). Because of their capacity to chelate metal ions (iron, copper, etc.),

flavonoids also inhibit free radical generation (Mishra et al., 2013a; Mishra et al., 2013b). Quercetin in particular is known for its iron-chelating and iron-stabilizing properties. Trace metals bind at specific positions of different rings of flavonoid structures (Van et al., 1996). The binding sites are shown in Figure 1.

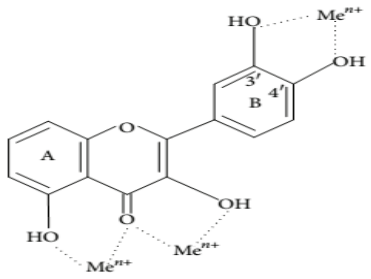


Fig. 1. Binding sites for trace metals where Men+ indicates metal ions

Free metal ions enhance ROS formation by the reduction of hydrogen peroxide with generation of the highly reactive hydroxyl radical. Due to their lower redox potentials flavonoids (Fl-OH) are thermodynamically able to reduce highly oxidizing free radicals (redox potentials in the range 2.13–1.0 V) such as superoxide, peroxyl, alkoxyl, and hydroxyl radicals by hydrogen atom donation. Because of their capacity to chelate metal ions (iron, copper, etc.), flavonoids also inhibit free radical generation (Mishra et al., 2013a; Mishra et al., 2013b). The flavonoid heterocycle contributes to antioxidant activity by permitting conjugation between the aromatic rings and the presence of a free 3-OH. Removal of a 3-OH annuls coplanarity and conjugation which compromises scavenging ability (Bors et al., 1990).

It is proposed that B ring OH groups form hydrogen bonds with the 3-OH, aligning the B ring with the heterocycle and A ring. Due to this intramolecular hydrogen bonding, the influence of a 3-OH is enhanced by the presence of a 3', 4'-catechol, elucidating the potent antioxidant activity of flavan-3-ols and flavon-3-ols that possess the latter feature. Generally O-methylation of hydroxyl groups of flavonoids decreases their radical scavenging capacity (Rice-Evans et al., 1996).

Occurrence, position, structure, and total number of sugar moieties in flavonoid (flavonoids glycosides) play an important role in antioxidant activity. Aglycones are more potent antioxidants than their corresponding glycosides. There are reports that the antioxidant properties of flavonol glycosides from tea declined as the number of glycosidic moieties increased (Ratty and Das, 1988). Though glycosides are usually weaker antioxidants than aglycones, bioavailability is sometimes enhanced by a glucose moiety. In the diet, flavonoid glycosidic moieties occur most frequently at the 3- or 7-position (Hollman et al., 1999).

Flavonoids are good chelating agents towards metal ions and, in the case of iron and copper, the favoured places of chelation are catechol groups, hydroxyl groups adjacent to oxo groups, and 1-oxo-3-hydroxyl-containing moieties (Fernandez et al., 2002; Ren et al., 2008). This ability to chelate metals has been used to enhance the capabilities of MS; it has been used to assist the elucidation of flavonoid glucuronides (Davis et al., 2006; Davis et al., 2007) and

various diglycosides (Pikulski et al., 2007). This is a result of the spectral changes observed when flavonoids are complexed with metals, giving rise to simpler yet more intense spectra (Satterfield & Brodbelt, 2000).

For thousands of years, mankind has known about the benefit of drugs from nature. Plant extracts, for the treatment of various ailments, were highly regarded by the ancient civilizations. Even today, plant materials remain an important resource for combating illnesses. Some medicinal plants traditionally used for management of diseases were selected and their phenol and flavonoid content and iron chelating activities were evaluated, including gossypitrin, the major flavonoid glycoside present in the petals of the flowers of *Taliparitielatum* Sw. (Fryxell) Malvaceae that grows in Cuba.

The structure of the last-mentioned compound remains questionable: gossypitrin was first taken out in 1916 from the flower of Gossypium by Parkin (Parkin, 1916). The aim of this research was to characterize the chelating activity of it chemical compound with $FeCl_3$ to find alternative sources with lower side effects in asthmatic patients.

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. Specimen is registered as *Taliparitielatum* Sw. (Fryxell) Malvaceae (*Sin*. *Hibiscus elatus* Sw.).

2.2. Chemicals

Analytical grade ethanol (Merck), analytical grade acetic acid (Merck), analytical grade n-butanol (Merck) and analytical 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 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. Procedures, Instrumentation and Parameters

The checking of the behaviour compared with the Fe^{3+} ions, was enhanced evaluating an extract that contain 12 mg/mL of ethanol with the ion solutions at 1 % in distilled water, adding 1 mL of each solution and 3 drops of the reference extract in a test tube, through a visual observation, utilizing water as negative control.

2.4.1. UV

The UV spectrometric experiments were carried out on a JASCO ultraviolet-visible spectrometer (Japan). The scan range was 200 to 500 nm, absorbance 0 to 2.5, and the analysed samples were diluted in methanol, into quartz cuvettes, comparing the obtained spectrum to the recrystallized gossypitrin with and without $FeCl_3$. The cuvettes thickness was d = 1 cm.

2.4.2. IR

The IR spectrometric experiments were carried out on a JASCO FT/IR-460 (Japan), Detector TGS, Light Source Standard (Laser Neon), and Resolution 2 cm^{-1}, Scanning Speed 2mm/sec; Scan range 3800-650 cm^{-1}, comparing the obtained spectrum to the recrystallized gossypitrin with and without $FeCl_3$.

2.4.3. HPLC-UV-ESI-MS

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. For MS analysis only the positive ion mode of ESI were examined with the scan range from m/z 0 to 2000. 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.

3. Results and Discussion

In the visual checking of the gossypitrin behaviour in from of the Fe^{3+} ions, the yellow-green colour disappear and the solution become deep black instantaneously. The complexing power of the gossypitrin gave positive values from 25 mg to 2, 81 mg (minimum visual reaction limit).

UV spectral results of gossypitrin in MeOH shows two absorption maximum bands at λ = 332 and 382 nm, and show another band at λ = 278 nm with an inflexion at 257 nm (Fig.2). In contrast with this, the absorption bands in the electronic spectrum of a mixture of

gossypitrin with Fe (III) chloride changes, and shows new bands at λ = 249 and 366 nm, undergoes a bathochromic shift of 64 nm. The third band disappears. The results suggest a reaction with the iron ion of the reactive (Fig. 3).

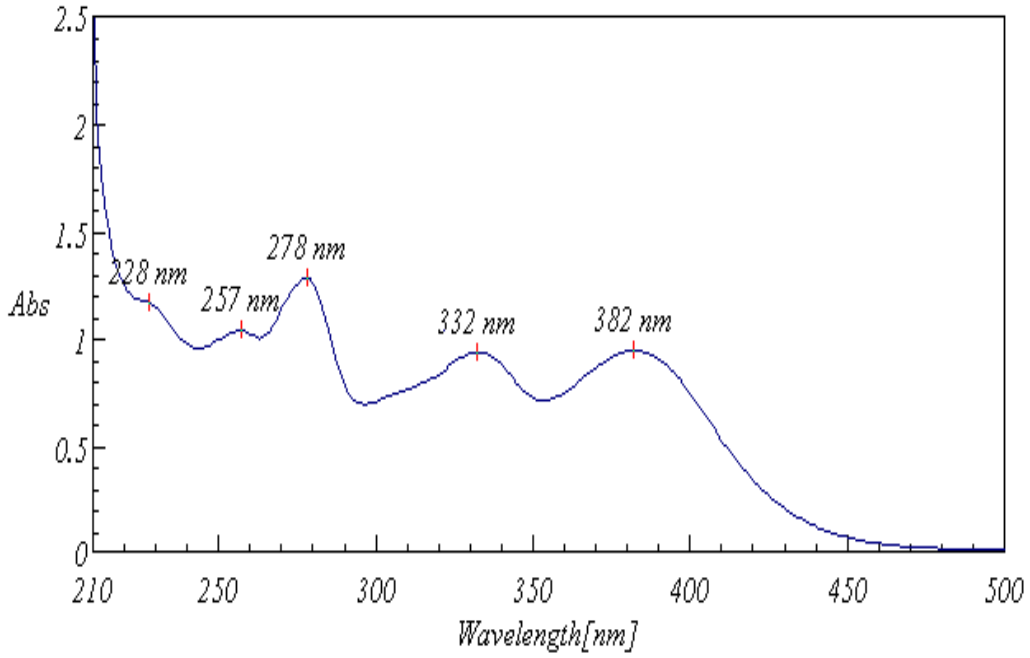


Fig. 2. UV spectrum of gossypitrin without $FeCl_3$

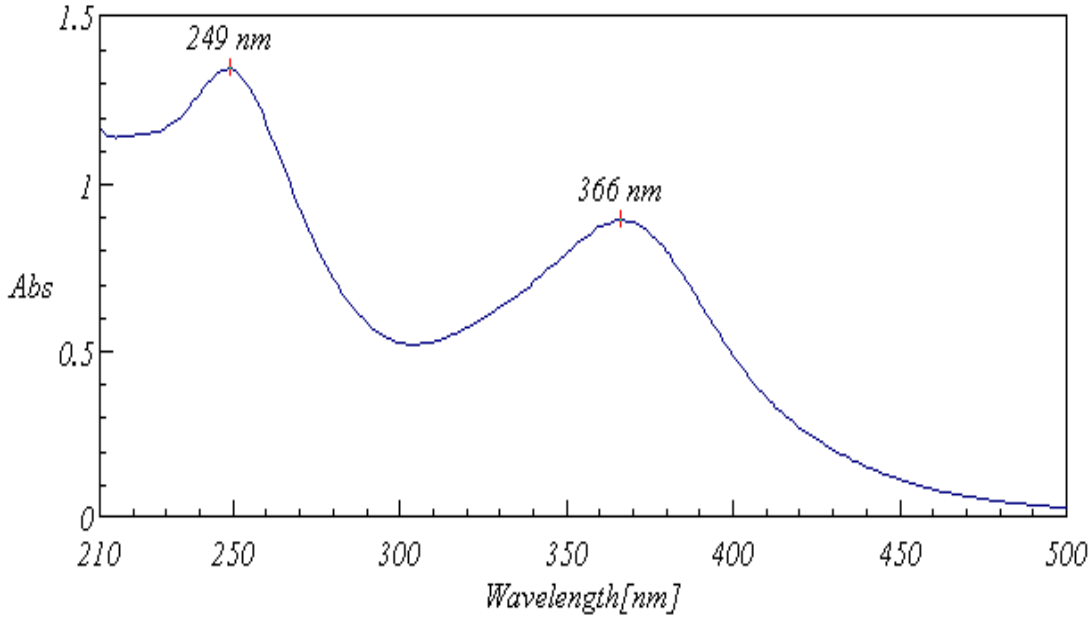


Fig. 3. UV spectrum of gossypitrin with $FeCl_3$

Studies on flavonoids by UV spectroscopy have revealed that most flavones and flavonols exhibit two major absorption bands: Band I (320–385nm) represents the B ring absorption, while Band II (250–285 nm) corresponds to the A ring absorption.

Electronic spectroscopy provided information about the structure and composition of flavonolate complexes. The literature indicates that changes in the visible spectrum (370-500 nm) depend more on the nature of the salts, complexants present, metal salts used to maintain ionic strength, and other factors than those in the UV spectrum (Abad-Garc á et al., 2009). As a rule, changes in the range 250-270 nm are insignificant (region affected by ring A) whereas those in the visible range (350-500 nm) reflect changes in the chromone structure and ring B of the flavonoid (Vukics & Guttman, 2010). The bathochromic shift of the gossypitrin in this spectral region is probably indicative of the formation of the Fe complexes with the 3´, 4´-*o*-phenols of ring B (**Scheme 1**). In general, the UV spectral results confirm the structure **(3', 4'-Fe^{+3})** for the gossypitrin complexes with $FeCl_3$ in solution.

IR spectrum of gossypitrin shows the characteristics bands of this bioflavonoid (Fig. 4):

1. 3228.25 cm^{-1} OH stretching vibrations, strongly and broad band of associated alcohols and phenols.
2. 2927.41 cm^{-1} typical C-H stretching, weak intense band.
3. 1653.66 cm^{-1} stretching C=O band, that in the flavonoids appear so displaced by the high conjugation of the carbonyl group.
4. 1608.34-1523.49 cm^{-1} stretching C-C bands of aromatic compounds.
5. 1195.65 cm^{-1} stretching C-O band.

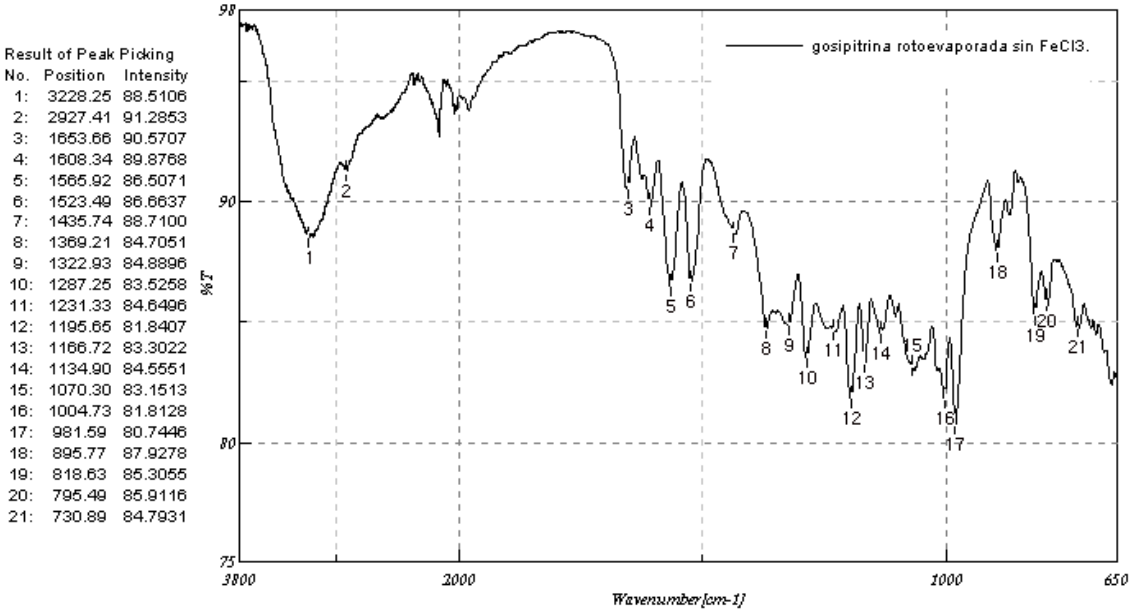


Fig. 4. IR spectrum of gossypitrin without $FeCl_3$.

IR spectra of the reaction products (Fig. 4) exhibited new strong bands with maxima at:

1. 3228.25 cm^{-1} OH band, more intense and broad.
2. 1608.24 cm^{-1} for the stretching vibration of carboxylate. The stretching C-H band disappears.
3. 1576.25 cm^{-1} weak band, overlap by the band before where it situated the stretching C-C band of aromatic compounds, which disappears.

4. 1375.96 cm^{-1} band that groups the stretching C-O with the rest of the bands.

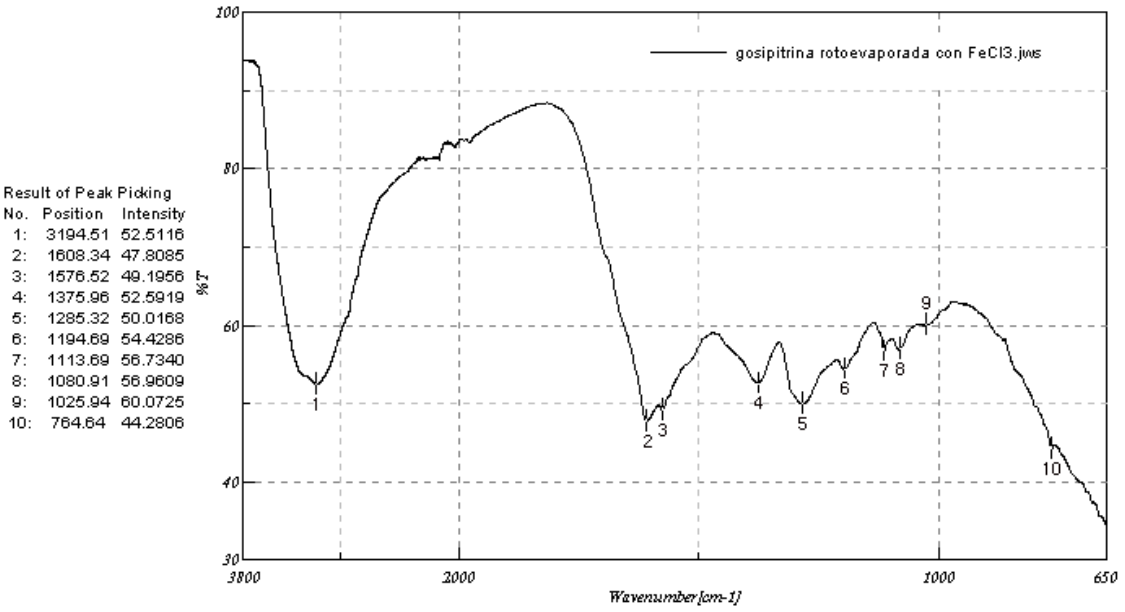
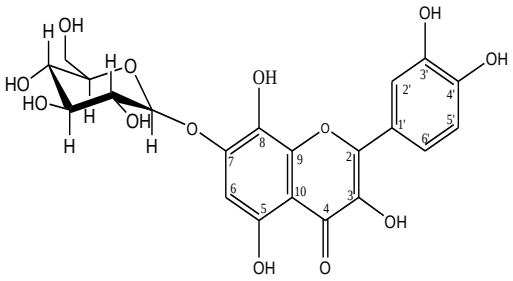


Fig. 5. IR spectrum of gossypitrin with $FeCl_3$.

Comparison of the spectrum of the reaction product with that of pure gossypitrin indicates that the reaction product is a complex of gossypitrin and Fe (III) chloride. The absorption maxima corresponding to ν_{as} of carboxyl group (1608.34 cm^{-1}) for this product are shifted by about 45 cm^{-1} to low-frequency compared with ν_{as} of the carboxyl group (1653.66 cm^{-1}) in starting gossypitrin.

According to the literature, the most probable complexation mechanism of gossypitrin involves reaction with Fe^{3+} with reactive groups in the **3, 4**-positions of the chromone, although reaction at **4, 5**-positions is also possible (Mel'nikova et al., 2002).



Scheme 1. Structure of gossypitrin.

IR is a spectroscopic technique that analyses the vibrational modes of molecules and molecular groups, allowing bond characterization, and, by comparison with known tabulated data, identification of functional groups; in the case of flavonoids, vibrational spectroscopy has been systematically used to study hydroxyl and carbonyl groups, but more recent

technical developments have allowed its application to a broader set of research goals. Similarly, metal complexation by flavonoids is also routinely assessed by vibrational spectroscopy (Siebert & Hildebrandt, 2007; O'Coinceanainn et al., 2004).

Mass Spectrometry (MS) has proved to be one of the most effective techniques in biomedical research, in special when complex matrixes of biological samples must be analyzed. MS gives data about molecular and ion/fragment masses, leading to a more complex and laborious data analyzing. This problem can be overcome with the construction of structural databases, which allow an easier and quicker data annotation, as well for NMR spectroscopy.

The positive scan mode was used throughout the mass spectrometric experiments. In the full-scan mass spectra of all three complexes, protonated, sodium-cationazed, gossypitrin could be found during the whole measurement. The nomenclature of part of the fragments was according to Domon and Costello (Domon & Costello, 1988), and "G" was defined to denote gossypitrin in the whole work.

The full-scan mass spectrum of isolated gossypitrin (bottom) and gossypitrin-Fe complexes is shown in Fig. 6. In these spectrums, five gossypitrin-Fe complexes are obviously found, which are peak A (*m/z* 536), B (*m/z* 592), C (*m/z* 1016), D (*m/z* 1072) and E (*m/z* 1128). According to the molecular weight, it was demonstrated that complex A is $[(G\text{-}H)\ Fe]^+$ (molar ratio of G/Fe = 1:1), complex B is$[(G\text{-}H)\ Fe_2]^+$ (molar ratio G/Fe = 1:2), complex C is $[(2G\text{-}3H)\ Fe]^+$ (molar ratio G/Fe = 2:1), complex D is $[(2G\text{-}3H)\ Fe_2]^+$ (molar ratio G/Fe = 2:2) and complex E $[(2G\text{-}3H)\ Fe_3]^+$ (molar ratio G/Fe = 2:3).

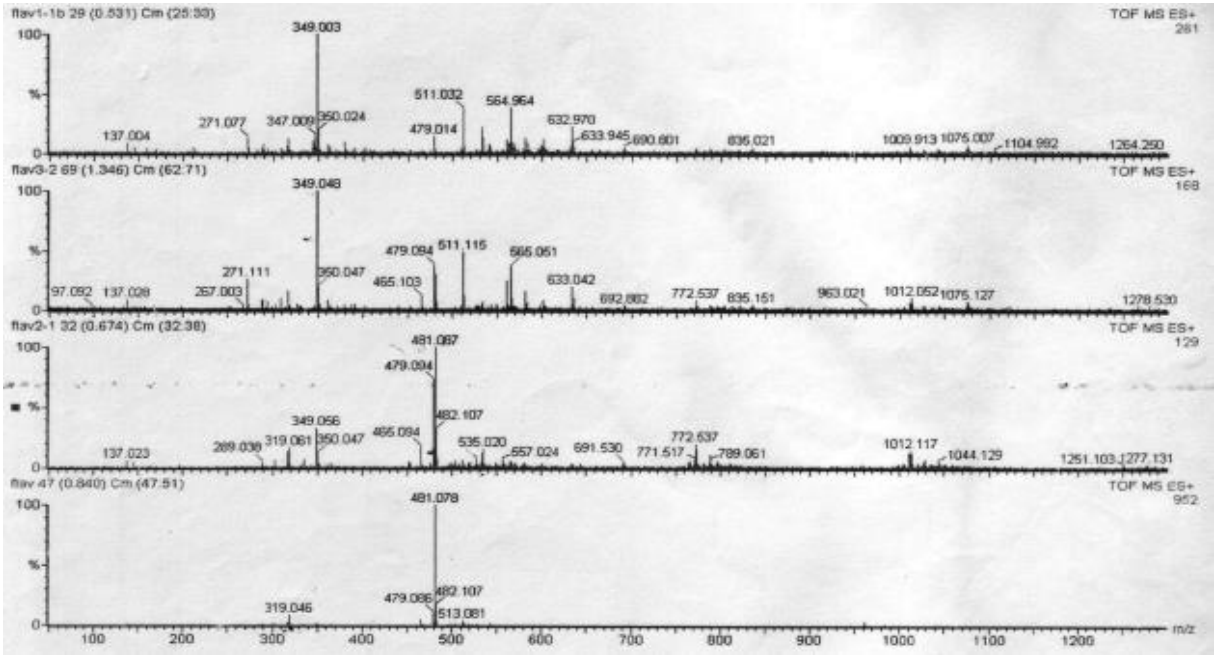


Fig. 6. Full scan mass spectrum of gossypitrin (bottom) and gossypitrin-Fe complexes.

The peaks at *m/z* 479 (M-1), 481 (M), 482 (M+1) and 503, corresponding to protonated gossypitrin ($[G+H]^+$) and sodium-cationazed gossypitrin ($[G+Na]^+$). The main product ions are at *m/z* 319, by the elimination of the one glucose unit (-162 Da), *m/z* 536 from the neutral

loss of one Fe ion (- 56 Da), *m/z* 289 from the loss of CH_2O (-30 Da), *m/z* 271 from the neutral loss of H_2O (-18 Da), *m/z* 137 that corresponding to $^{1,2}A$ arises from a RDA before the neutral loss of *m/z* 134 ($^{1,3}B$), and the ion at *m/z* 97 which is produced by the cleavage of $^{1,2}A$ to loss -40 Da (C_2H_2O).

Sequentially, the signal at *m/z* 536 and *m/z* 592, arises from the addition of one iron ion in each case (+56 Da), respectively. The ion with molecular weight of 1016 is produced by the addition of one molecule of G to produce a dimmer (molar ratio 2:1) with a central Fe ion. In this point of the spectrum we can observe the presence of an *m/z* value of 692 due to the neutral loss of two glucose moieties (-324 Da). The same situation occur when the ion at *m/z* 1072 arise from the sum of another iron ion to the dimmer with molar ratio of 2:1 to produce the neutral fragment at *m/z* 1072 (molar ratio 2:2).

Finally, we suggest that the ion at *m/z* 1278 arise by addition of a glucose residue of *m/z* 150 from the ion at *m/z* 1128 (complex E), fragment that comes from the neutral reaction between D (*m/z* 1072) and another iron ion (+56 Da) (Cavaliere et al., 2005; Satterfield & Brodbelt, 2001).

4. Conclusions

UV, IR and Mass spectrometry played a very important in the structure characterization and fragmentation analysis of gossypitrin-transition metals complexes. Iron ions can form complexes with gossypitrin. For a monomolar gossypitrin-metal complex ($[(G-H)\ metal]^+$), the fragments came from the neutral loss of a glucose unit, aglycone and sugar chain. For complex $[(2G-H)\ metal]^+$, the glucose unit, the aglycone and the gossypitrin molecule could be lost during fragmentation. According with ESI-MS results, we obtained a large amount of structural information and only monomers (1:1; 1:2) and dimmers (2:1; 2:2 and 2:3) are present when gossypitrin react with iron ions, but never trimmers.

It is fair to conclude that although MS methods rarely provide a full molecular determination they are, due to their intrinsic characteristics, the best approach to study flavonoid structures, in complement, when possible, with NMR experiments. For faster cruder screenings, UV absorption data can be used to develop appropriate methods to achieve initial flavonoid class identification.

Acknowledgements

The authors would like to thank to M.Sc. Alen Nills Baeza Fonte for his help in the acquisition of UV and IR spectrums. We extend our sincere thanks to Center of Genetic Engineering and Biotechnology in Havana for supporting to bring out every detail on the ESI-MS spectrums of gossypitrin and gossypitrin-Fe complexes.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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