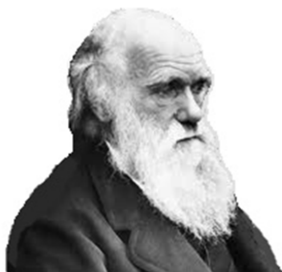
Available online at www.worldnewsnaturalsciences.com



World News of Natural Sciences

An International Scientific Journal

WNOFNS 24 (2019) 100-116 EISSN 2543-5426

Chemical characterization by TLC, UV, IR, HPLC-MS/MS and NMR of gossypetin-3'-*O*-glucoside from *Talipariti elatum* (Sw.) Malvaceae

José González[1,*], Armando Cuéllar[1], Marc Gaysinski[2], Lionel Massi[2], Max Monan[3], Enmanuel Nossin[3], Frantz François-Haugrin[3]

[1]Faculty of Pharmacy and Food, Havana University, Havana, Cuba

[2]Faculty of Science, Nice Sophia-Antipolis University, Nice, France

[3]ARVARNAM, Martinica, France

*E-mail address: jgyaque@ifal.uh.cu

ABSTRACT

Talipariti elatum (Sw.) Malvaceae, also known as *Blue Mahoe* or *Mountain Mahoe*, commonly named as Majagua azul or Majagua, with red or crimson flowers, is a medicinal tree traditionally used against cough, asthma, catarrh, and expectorant. Its flowers contain a lot different chemical compounds, mainly flavonoids. From red petals of the flowers a flavonol glucoside was isolated and characterized by TLC, UV, IR, HPLC-MS/MS and NMR spectroscopy. Structure analyses of that chemical component revealed that It have the identical glucoside moiety attached to a flavonol skeleton like gossypitrin (gossypetin-7-*O*-β-glucoside) but in different position for which the structure of gossypetin-3'-*O*-glucoside was deduced from HSQC, HMBC, COSY and NOESY correlations.

Keywords*: *Talipariti elatum, flavonoids, TLC, UV, IR, MS, NMR

1. INTRODUCTION

Juan Tomás Roig y Mesa (1877-1971), the main researcher in the Cuban traditional medicine, have an important publication about the medicinals, aromatics and poisonous plants in our country, in which him mentioned that *Talipariti elatum* Sw. (Malvaceae) where one of the common names is Majagua azul, are traditionally used against cough, asthma, catarrh, etc.

(Received 10 March 2019; Accepted 26 March 2019; Date of Publication 29 March 2019)

The flowers of this medicinal tree are used by Cuban population as cataplasm and infusions mixed with sugar cane or honey bee [Roig, 2014].

The spice was known as *Hibiscus elatus*, but in 2007, Cuban Government considered to make the reconversion of the Cuban flora, renamed the plant as we know until day. The main considerations that the researchers proposed to do so were: arborescent habit, prominent stipules that close the terminal yams, foliar lamina coriaceous, with entire margin, capsule 10-locular and chromosomal number relatively high [Areces and Fryxell, 2007].

T. 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 1200 meters (3900 Ft.) and is often used in reforestation. It is the national tree of Jamaica. Talipariti 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 (Figure 1). It grows quite rapidly, often attaining 20 meters (66 Ft.) or more in height. 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 [US DA, 2013].

Figure 1. Flowers of *Talipariti elatum* (Sw.)

Until now, only gossypitrin (gossypetin-7-*O*-β-D-glucoside) has been reported from the red petals of Majagua tree (Márquez et al., 1999; Cuéllar y González, 2010). Gossypitrin was first taken out in 1916 from the flower of *Gossypium* flowers (Parkin, 1916). A research team elucidated this flavonoid glucoside using LC–MS, LC–NMR, and offline NMR experiments after isolation from the herb of *Drosera peltata* (Braunberger et al., 2013). From yellow petals of Iceland poppy (*Papaver nudicaule* hort. non L.), besides the known flavonoid gossypitrin, seven kaempferol derivatives were isolated (Schliemann et al., 2006). The purpose of this investigation was to elucidate the structure of the constituent found in the hydroethanolic extract from *T. elatum* by combining data obtained by TLC, UV, IR, HPLC-MS and NMR experiments.

2. MATERIAL AND METHODS

2. 1. Plant Material

Flowers were collected in January 2018 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 Martinica 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* (Sw.) Malvaceae.

2. 2. Solvents

DMSO *d6* analytical grade and TMS analytical grade (Merck), 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 in an oven with controlled temperature, at 40 ºC, 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 ºC and 500 mbar. For to the purification, 1 g 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. TLC

Yellowish-green solid was 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) according to Wagner and Bladt, 1996.

2. 5. UV

The UV spectrometric experiments were carried out on a UV-VIS JENWAY UV Vis 6715 (UK). The scan range was 200 to 500 nm; absorbance 0.000-3.0000, spectral band 1.5 nm, spectral resolution 0.1 nm and the analyzed samples were diluted in methanol, into quartz cuvettes, comparing the obtained spectrum with the original spectrum of gossypitrin. The cuvettes thickness was d = 1 cm.

2. 6. IR

The IR spectrometric experiments were carried out on a Brucker IR model ALPHA (Germany) with a scan range 7500-360 cm^{-1}, on KBr disks, spectral resolution 2 cm^{-1}, diode laser 850 nm (Laser Class 1).

2. 7. 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 Thermo Finnigan (Thermo Electron, San Jose, CA) 3D ion trap mass spectrometer 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 the compound was recorded from 130,0 to 650,0 m/z in negative mode.

2. 8. NMR Procedures, Instrumentation and Parameters

NMR spectra were recorded on an Advance 500 spectrometer in DMSO d6 at 298K. Qualitative analyses were recorded by ^1H (500 MHz) and ^{13}C (125 MHz) and homonuclear and heteronuclear experiments like DEPT135, DEPT90, COSY, HSQC, HMBC, ROESY and TOCSY. Chemical shifts are reported in ppm relative to TMS and coupling constants in Hz.

3. RESULTS AND DISCUSSION

3. 1. TLC analysis

To characterize the qualitative chemical profile, the samples were initially analyzed via thin-layer chromatography (TLC) (Wagner and Bladt, 1996). Dried TLC plates were sprayed with specific reagents and heated to observe the color reaction. The presence of phenolic hydroxyl groups was observed through positive reaction with ferric chloride and aluminum chloride.

TLC analysis, under previously describe conditions, showed three yellow spots typically of flavonoid compounds, that varying in size and shape, being the biggest and largest the No. 3, follows by No. 2 and for last No. 1. All spots change colorations from yellow to green-yellowish under UV lamp at 254 nm and to brown with H_2SO_4 and heat (Fig. 2). The Rf calculated values were 0,808 (1); 0,807 (2) and 0,780 (3), respectively (**from left to right**). These results suggesting that the isolated powders content at least only one kind of chemical

compound. The presence of phenolic hydroxyl groups was observed through positive reaction with ferric chloride and aluminum chloride (Wagner and Bladt, 1996), probably due to the presence of flavonoids (Cuéllar & González, 2010).

According to Markham in 1989, the interpretation of spot colour in terms of flavone or flavonol structure allow to inferred that the samples developed a bright yellow under UV light without NH_3. The spot appearance corresponded to Flavonols with a free 3-OH with or without 5-OH, often 7-glycosides.

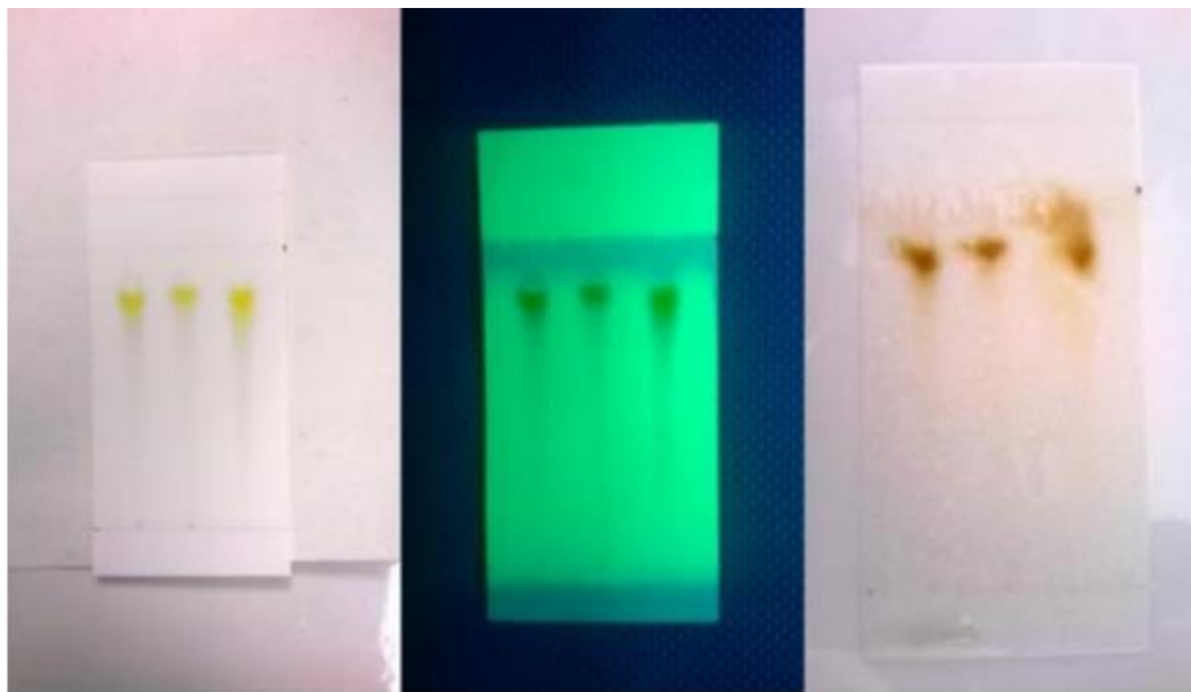


Figure 2. TLC of the samples (from left to right: ordinary light; under UV_{254} and positive reaction with H_2SO_4).

3. 2. UV analysis

Ultraviolet and visible spectroscopy was one of the earliest techniques routinely used for flavonoid analysis due to the existence of two characteristic UV/Vis bands in flavonoids, band I in the 300 to 550 nm range, arising from the B ring, and band II in the 240 to 285 nm range, arising from the A ring. The different types of flavonoids have different UV spectra, which can be used to differentiate isomers possessing similar mass spectra (Abad-Garcia et al., 2009; Vukics and Guttman 2010). Flavonols had a characteristic UV spectrum with two bands at 260 and 370 nm, and shoulders occurring at 270 and 300 nm (Abad-Garcia et al., 2009).

Compounds 1, 2 and 3 showed UV spectra characteristic of flavonols. Compounds 1, 2 and 3 showed UV spectral data that are in agreement with those of gossypetin, gossypitrin and gossypetin-3'-*O*-glucoside with a maximum absorption at 384 nm. The products are flavonols with a free hydroxyl group at 3, supporting by the existence of a band bellow 350 nm, with cathecolic groups in ring B (substitution in 3', 4') because a band at 277 nm and an inflection at 257 nm, and three oxygenated positions on ring A like gossypitrin (Márquez et al., 1999).

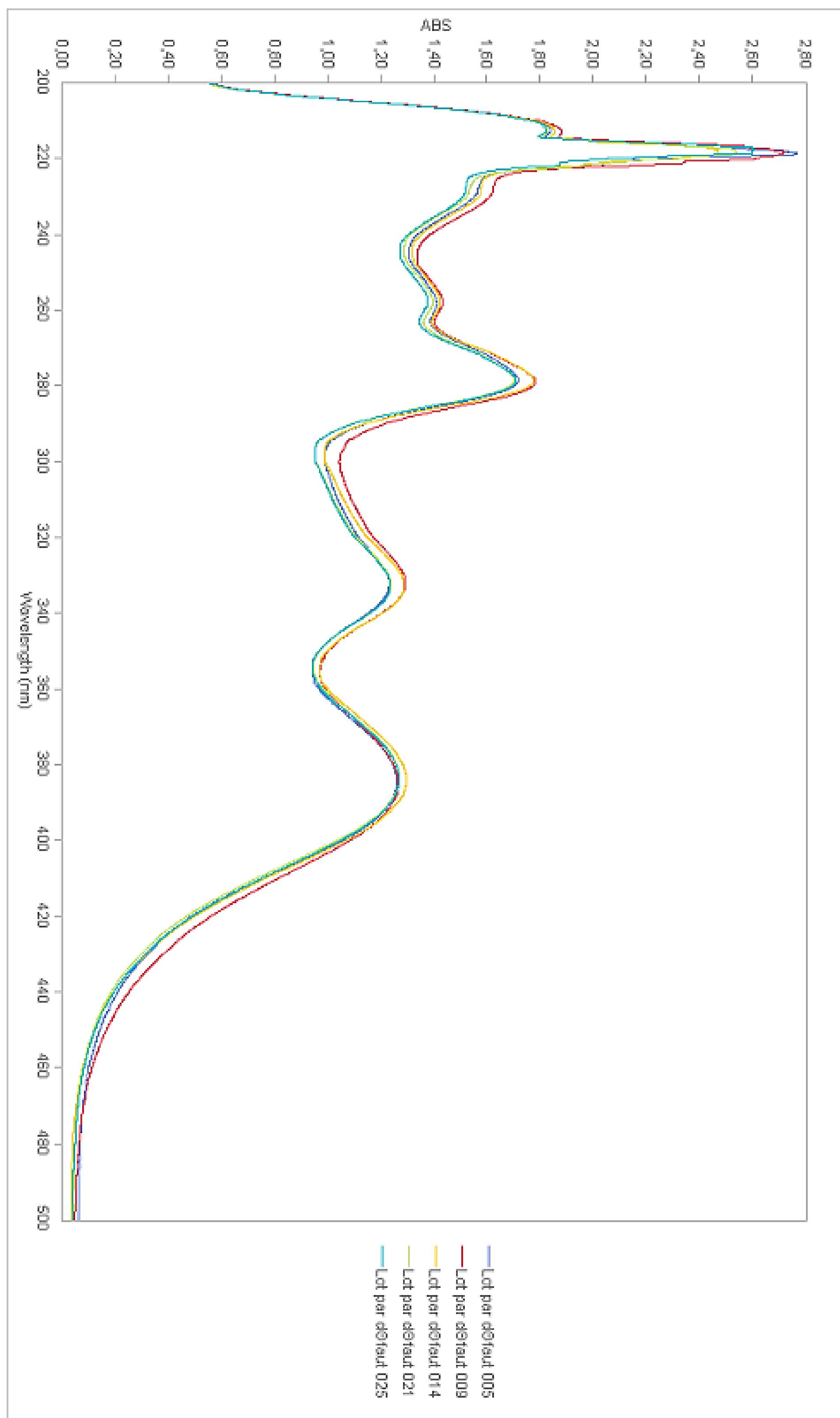


Figure 3. UV Spectrums of solid samples.

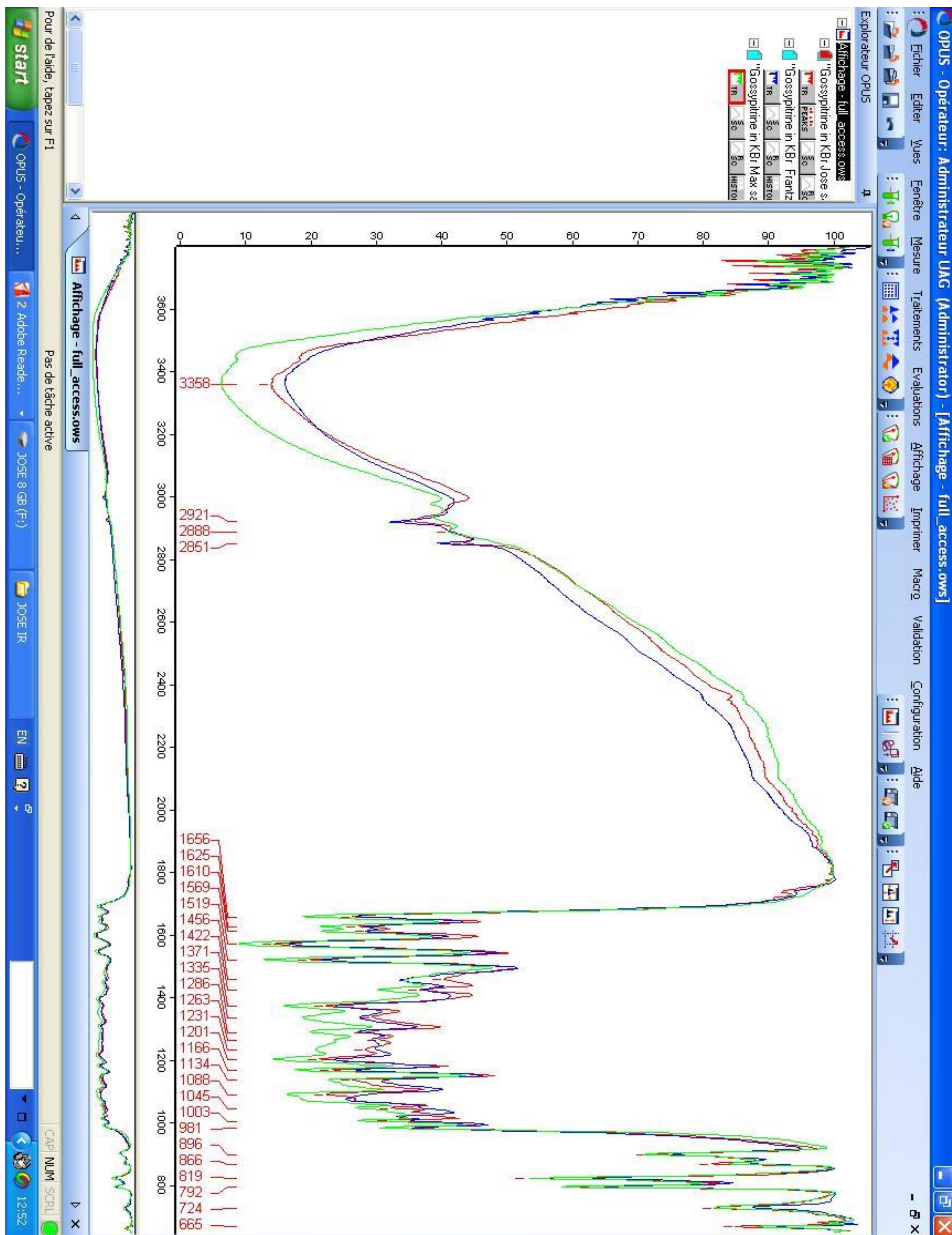


Figure 4. IR spectrums of solid samples.

Table 1 list the absorbances and maximal ultraviolet wavelength (λ max) for the chemical constituents found in the extract. UV scan is shown in Fig. 3.

Table 1. Maximal ultraviolet wavelength (λ_{max}) and absorbances for the chemical constituents found in the hydroethanolic extracts from flowers of *T. elatum*.

Sample 1		Sample 2		Sample 3	
λ_{max} (nm)	absorbance	λ_{max} (nm)	absorbance	λ_{max} (nm)	absorbance
384.3	1.261	383.5	1.293	384.0	1.269
331.9	1.233	333.6	1.257	333.6	1.270
278.5	1.719	278.8	1.737	278.7	1.748
258.1	1.411	257.7	1.410	257.8	1.399

3. 3. IR analysis

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 (Li et al., 2011; Erdogdu et al., 2010).

IR spectrums of the samples showed typical presence of OH stretching vibrations, strongly and broad band of associated alcohols and phenols (3358 cm^{-1}); typical C-H stretching, weak intense band (2921-2851 cm^{-1}); stretching C=O band, that in the flavonoids appear so displaced by the high conjugation of the carbonyl group (1656 cm^{-1}); stretching C-C bands of aromatic compounds (1610-1519 cm^{-1}); and stretching C-O band (1201 cm^{-1}). According with these results and data in literature we suggested that compounds 1, 2 and 3 to be flavonols (Li et al., 2011; Erdogdu et al., 2010; González et al., 2016) (Fig. 4).

3. 4. HPLC-ESI-UV-MS/MS analysis

The total current chromatogram of sample 1 is represented in Figure 5, showing the UV and the MS detection in both scan modes. In this case, only the highest peak is concerning to the flavonol under analysis. Data relative with samples 1, 2 and 3 are summarized in Table 2. All samples presented in general the same behavior. All solid samples are comparable, and they exhibits two majoritarian peaks at 14.68 and 16.70 min, respectively. Preliminary HPLC-ESI-UV-MS/MS allows us to recognize the presence of two chemical compounds with different retention times (26.014 min; 26.414 min) but with the same molecular mass after filtration of peak m/z 481 in positive scan mode. The base peak was in both cases m/z 319 as is shown in Figure 6. Data related with the samples is summarized in Table 3.

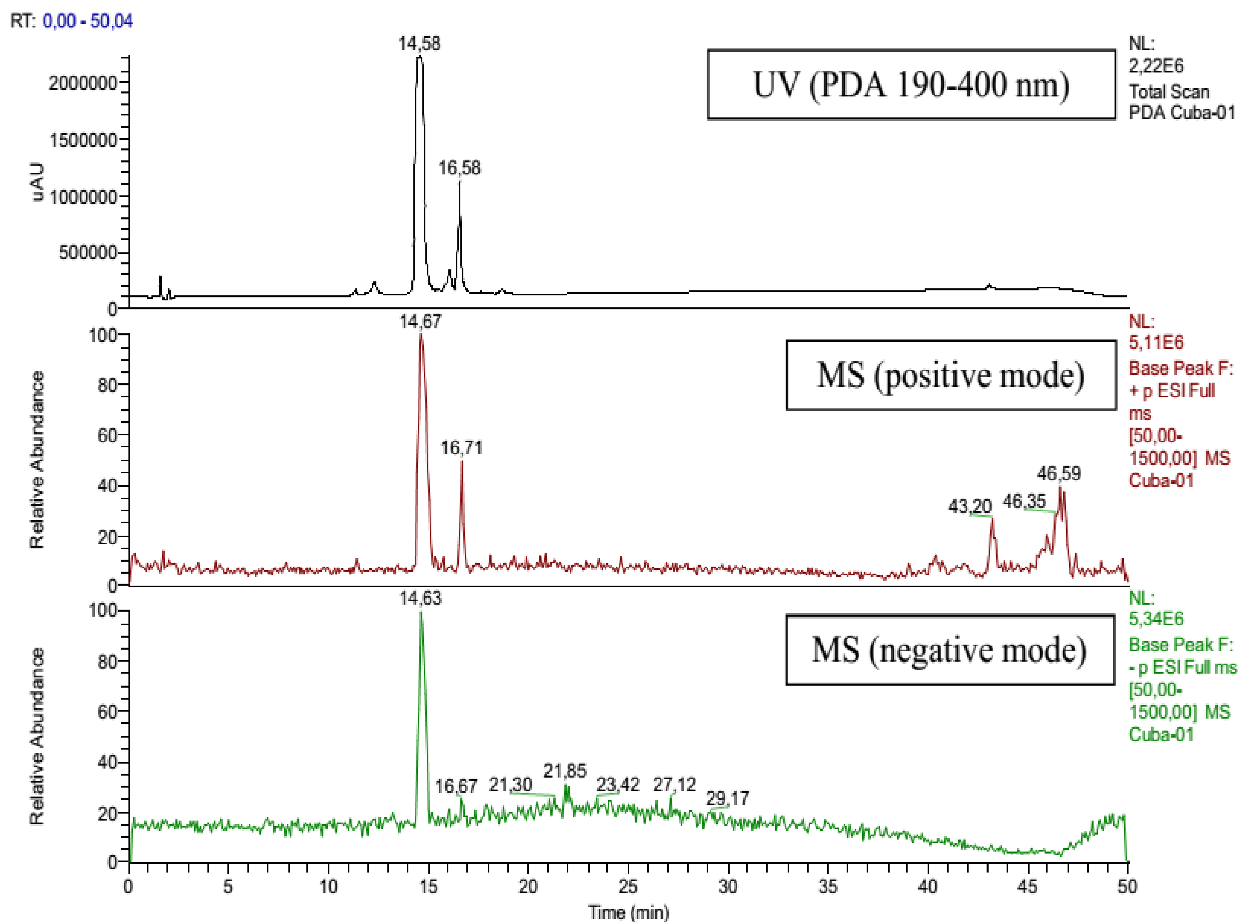
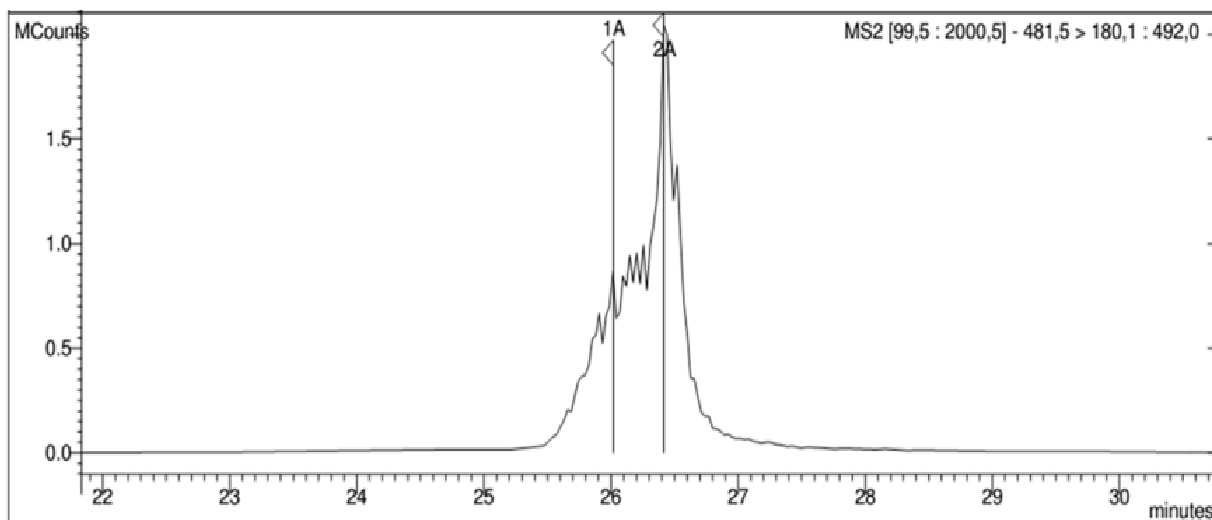


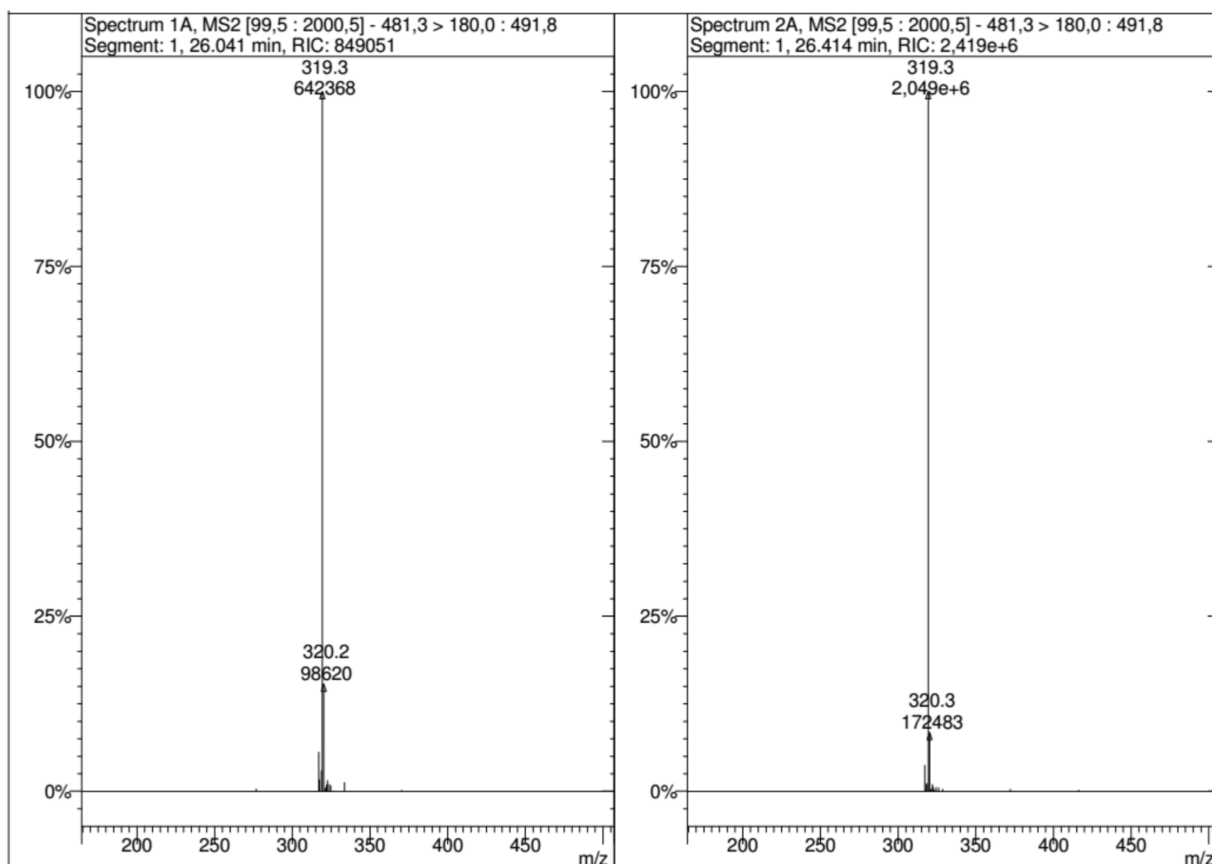
Figure 5. Total ion current chromatogram obtained through the analysis of hydroethanolic extract from red petals of *T. elatum* in both ion modes.

Table 2. Spectral data of samples 1, 2 and 3.

Spectral information	Sample 1	Sample 2	Sample 3
UV (λmax) nm	278	278	276
MS (+) m/z	481.1	481.1	481.1
MS (-) m/z	479.2	479.2	479.2



(a)



(b)

Figure 6(a,b). LC-MS of sample 1.

Table 3. Spectral data of samples 1, 2 and 3 after filtration of peak m/z 481.

Spectral information	Sample 1	Sample 2	Sample 3
MS^2 m/z	481.3	481.3	481.3
Rt m/z 319.3	26.041 min	26.380 min	26.325 min
Rt m/z 319.3	26.414 min	26.694 min	26.534 min

3. 5. NMR

After isolation and purification the samples were submitted to an NMR analysis using the described conditions previously. ^{1}H-^{1}H COSY, HSQC and HMBC correlations were used to assign all proton and carbon atoms within the corresponding substructures and established possible links to other parts of the molecule. Using ^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, TOCSY, HSQC and HMBC experiments, identification of the sugar moiety was performed according to the strategy used for structure elucidation of flavonoid glucosides.

^{1}H NMR spectrum of this flavonoid derivative showed four proton signals in the aromatic region; (δ = 8.08 ppm (*d*, 1H, J4 = 2.21 Hz, 2'-H), (δ = 7.92 ppm (*dd*, 1H, J3 = 8.72 Hz - J4 = 2.21 Hz, 6'-H), (δ = 6.99 ppm (*d*, 1H J3 = 8.71 Hz, 5'-H), consistent with a gossypetin derivative. The observed multiplicity (ABX system) is characteristic of a catechol. Observed chemical shift value of proton 6-H (δ = 6.25 ppm) and carbon 6-C (δ = 97.98 ppm) confirmed the presence of Hydroxyquinol (ring A) (Figure 7) (Lee et al., 2008). The ^{13}C NMR values for this flavonoid (Table 4) were assigned on the basis of ^1JCH, ^2JCH, ^3JCH and ^4JCH correlations observed in the HSQC and HMBC spectra (François-Haugrin et al., 2016; Yaque et al., 2016).

Table 4. NMR data of gossypetin, gossypitrin and gossypetin-3'-*O*-glucoside.

Atom	Gossypetin (2)	Gossypetin (3)	Gossypitrin (3)	Cuban sample*					
N°	^{13}C (ppm)	^{13}C (ppm)	^{13}C (ppm)	^{13}C (ppm)	^{1}H (ppm)	M	I	J (Hz)	OH (ppm)
1	/	/	/	/	/				**/**
2	146.5	146.7	147.8	145.8	/				**/**
3	135.3	135.5	136.1	135.6	/				**9.39**
4	175.9	176.6	176.6	176.06	/				**/**

4a	102.6	103.8	104.9	102.62	/				/
5	152.1	152.7	151.7	152.3	/				**11.89**
6	97.8	98.0	98.1	97.98	6.25	s	1	/	/
7	152.5	152.3	150.6	153.08	/				**10.39**
8	124.6	124.7	127.1	124.3	/				**8.6**
8a	144.76	144.9	143.8	144.9	/				/
1’	122.2	122.3	122.4	122.3	/				/
2’	115.2	115.3	115.5	115.79	8.07	d	1	2.02	/
3’	144.85	145.0	145.4	144.9	/				/
4’	147.5	147.7	148.2	148.6	/				**9.35**
5’	115.3	115.5	115.9	115.86	6,99	d	1	8.71	/
6’	120.1	120.3	120.6	123.28	7.92	d	1	8.71	/
1’’			101.6	101.84	4,83	d	1	7.32	/
2’’			73.5	73.12	3,44-3,34	m	1	**	
3’’			77.5	75.97	3,44-3,34	m	1	**	
4’’			70.0	69.14	3,44-3,34	m	1	**	**
5’’			76.0	76.72	3,44-3,34	m	1	**	
6’’			61.0	60.15	3.76	d	1	11,82	
					3.63	dd	1	11.82 3.58	

*Attribution realized accordance with literature data and the experiences by RMN (^{1}H, ^{13}C) and 2D (COSY, HSQC, HMBC, TOCSY and ROESY).
**Undetermined.

The sugar region showed the presence of one moiety. The ^{1}H and ^{13}C values of this sugar unit were assigned by a combination of 1D ^{1}H NMR, 2D COSY, TOCSY and HSQC experiments. The ^{1}H and ^{13}C resonances were in accordance with β-glucopyranoside confirmed by the presence of 6 carbons sp3 (5 CH and 1 CH_2) where the protons at δ = 4.83 ppm (*d*, 1H, J = 7.32 Hz, 1’’-H), δ = 3.76 ppm (*d*, 1H, J = 11.96 Hz, 6’’-H), δ = 3.59 ppm (*dd*, 1H, *J* = 11.96 Hz, *J* = 4.10 Hz, 6’’-H) δ = 3.44 - 3.34 ppm (*m*, 4H, 2’’, 3’’, 4’’, 5’’-H) resonated in the characteristic zone of glycosylated flavonoid compounds indicates a glucose moiety (Figure 7). Measured coupling constants value for anomeric proton signal 1’’H (*J* = 7.32 Hz) is according to one glycosylated structure type β. The substitution position was determined by ROESY and HMBC. HMBC correlations between 1’’H (δ = 4.83 ppm) and C3’ (δ = 144.93 ppm) and in ROESY between 2’H and 1’’H unequivocally confirmed the ring B substitution in 3' position. Therefore, the substance is consequently determined to be gossypetin-3'-*O*-β-glucopyranoside (Figure 8 and Figure 9) (Françoise-Haugrin et al., 2016; Yaque et al., 2016)

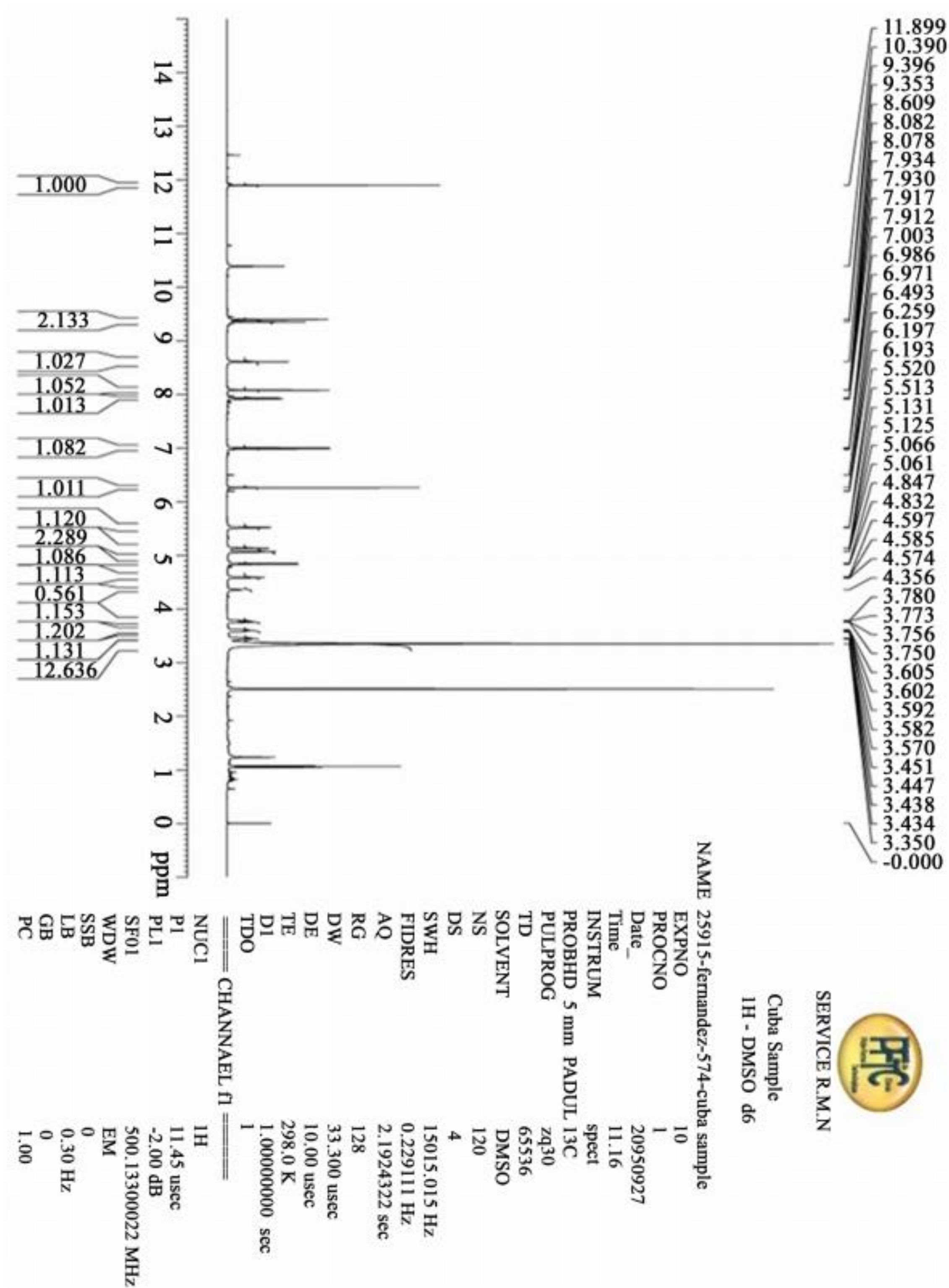


Figure 7. ^{1}H NMR spectrum of compound 1.

Figure 8. HMBC spectrum of gossypetin-3'-*O*-glucoside, showing the correlation between 1''H (δ = 4.83 ppm) and C3' (δ = 144.93 ppm).

The principal difference between gossypitrin and gossypetin-3'-*O*-glucoside is the sugar moiety position in both flavonoids. Gossypetin-3'-*O*-glucoside, whose structure was unambiguously determined from the NMR data, have the sugar moiety in C3' position according to the experiments, while gossypitrin have the sugar moiety in C7 position. Signal at

10.39 ppm (C-7) disappear in the spectrum ^{1}H NMR of gossypitrin, while in ^{1}H NMR spectrum of gossypetin-3'-*O*-glucoside the corresponding signal at 3'-C (9.34 ppm) disappear too (Scoelly and Kapetanidis, 1993; Wind et al., 1998; Braunberger et al., 2013). The UV, IR and MS of both flavonol glycosides do not allow differentiate the structures of the last mentioned compounds (González et al., 2016).

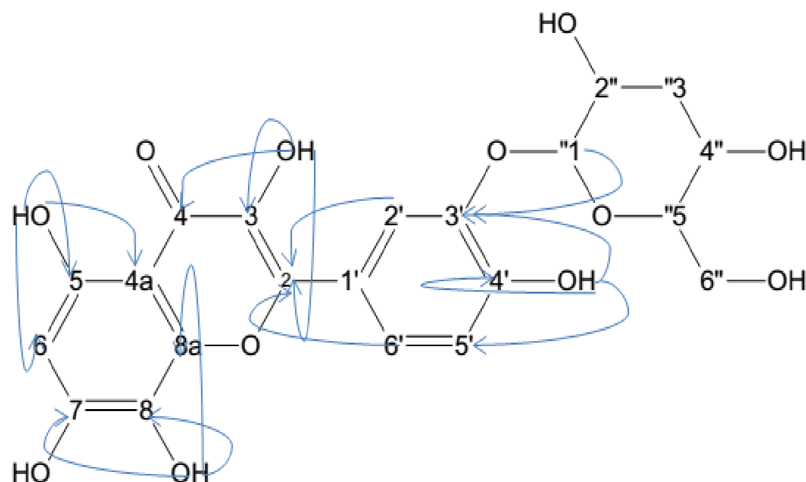


Figure 9. HMBC correlations in gossypetin-3'-O-glucoside.

4. CONCLUSIONS

The role of flavonoids in biological systems appears yet to be far from definitively determined, involving a large number of research groups all over the world. Interestingly, although many new actions of flavonoids *in vivo* have been put forward, the previously proposed actions are never dismissed, only relegated to secondary ways of flavonoid action, and usually considered to be important in pathological conditions.

The present study describes the identification of one of the major flavonoids from petals of the red flowers of *T. elatum* as gossypetin-3'-*O*-β-glucoside. The identical backbone skeleton and sugar moiety of gossypetin-3'-*O*-β-D-glucoside and gossypitrin (gossypetin-7-*O*-β-D-glucoside) may be due to broad specificities of glucosyl transferases involved in the biosynthesis of the glycosides from the respective aglycones. The aglycones of flavonols may be formed from a common C6-C3-C6 precursor, e.g. gossypetin flavonol. This is the first report with complete UV, IR, MS and NMR data for this compound found in the flowers of *Talipariti elatum* that grows in Cuba and Martinica.

References

[1] Abad-Garcia, B., Berrueta, L.A., Garmon-Lobato, S., Gallo, B., Vicente, F. (2009). A general analytical strategy for the characterization of phenolic compounds in fruit juices

by high-performance liquid chromatography with diode array detection coupled to electrospray ionization and triple quadrupole mass spectrometry. *J. Chromatogr. A* 1216, 5398-5415

[2] Areces F y Fryxell PA. (2007). Flora de la República de Cuba. Fascículo 13. Malvaceae. p. 5-6.

[3] Braunberger, C., Zehl, M., Conrad, J., Fischer, S., Adhami, H.-R., Beifuss, U. and Krenn, L. (2013). LC-NMR, NMR, and LC-MS Identification and LC-DAD Quantification of Flavonoids and Ellagic Acid Derivatives in Drosera pelttata. *Journal of Chromatography B,* 932, 111-116. http://dx.doi.org/10.1016/j.jchromb.2013.06.015

[4] Cuéllar, A.; González Yaque J. (2010). Obtención del glucósido flavonoide gossypitrina de los pétalos de flores de *Talipariti elatum* S.w y evaluación de su posible efecto antioxidante. *Rev. Colombiana Cienc. Anim.* 2 (2). pp. 338 -348

[5] Erdogdu, Y.; Unsalan, O.; Sajan, D. & Gulluoglu, M.T. (2010). Structural conformations and vibrational spectral study of chloroflavone with density functional theoretical simulations. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 76, 2, 130-136

[6] François-Haugrin, F., Max Monan, Emmanuel Nossin, Juliette Smith-Ravin and Odile Marcelin (2016). Antioxidant activity of an isomer of gossypitrin (gossypetin-3'-*O*-glucoside) isolated in the petals of *Talipariti elatum* Sw., and determination of total phenolic content of the total flower. *Journal of Pharmacognosy and Phytochemistry* 5(5): 200-208

[7] González J, Armando Cuéllar, Max Monan, Enmanuel Nossin, Frantz François-Haugrin. (2016). Pharmacognostical and phytochemical studies of flowers from *Talipariti elatum* S.w. *International Journal of Engineering Research & Science*, Vol. 2, Issue 6, June, 69-74

[8] Lee, S., Park, Y., Moon, B., Lee, E., Hong, S. and Lim, Y. (2008). Substitution Effect of Hydroxyl Groups on ^{1}H and ^{13}C Chemical Shifts in Hydroxyflavonols. *Bulletin of the Korean Chemical Society,* 29, 1597-1600, http://dx.doi.org/10.5012/bkcs.2008.29.8.1597

[9] Li, X.-H., Liu, X.-R.; Zhang, X.-Z. (2011). Molecular structure and vibrational spectra of three substituted 4-thioflavones by density functional theory and ab initio Hartree-Fock calculations. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 78, 1, 528-536

[10] Márquez, I., Cuéllar, A., Martínez J., Alemán A., Lora J., Castro H. (1999). Estudio fitoquímico de la especie Hibiscus elatus Sw. *Revista Cubana de Farmacia* 33(2), 127–131

[11] Roig, J. T. (2014). Plantas medicinales, aromáticas o venenosas de Cuba. Editorial Científico Técnica, La Habana, Cuba, 618-619.

[12] Schliemann W, Schneider B, Wray V, Schmidt J, Nimtz M, Porzel A and Böhm H. (2006). Flavonols and an indole alkaloid skeleton bearing identical acylated glycosidic groups from yellow petals of Papaver nudicaule. *Phytochemistry* 67, 191–201

[13] Scoelly, T. and Kapetanidis, I. (1993). Flavonoids from Drosera rotundifolia. *Scientia Pharmaceutica,* 61, 277-282

[14] U.S. Department of Agriculture. (2013). *Hibiscus elatus* Sw. "mahoe". Natural Resources Conservation Service. Plants Database.

[15] Vukics, V., Guttman, A. (2010). Structural characterization of flavonoids glycosides by multi-stage mass spectrometry. *Mass Spectrom. Rev.* 29, 1-16

[16] Wagner, H., Bladt, S. (1996). Plant Drug Analysis. A thin layer chromatography atlas, 2nd Ed. Springer. Berlin.

[17] Wind, O., Christensen, S.B. and Mølgaard, P. (1998). Colouring Agents in Yellow and White Flowered *Papaver radicatum* in Northern Greenland. *Biochemical Systematics and Ecology,* 26, 771-779. http://dx.doi.org/10.1016/S0305-1978(98)00031-3

[18] Yaque, J.G., Cuéllar, A., Gaysinski, M., Monan, M., Nossin, E. and François-Haugrin, F. (2016) New Reported Flavonol Characterized by NMR from the Petals of *Talipariti elatum* S. w. in Cuba. *American Journal of Plant Sciences,* 7, 1564-1569. http://dx.doi.org/10.4236/ajps.2016.711148

[19] Yaque, J.G., Cuéllar, A., Massi, L., Monan, M., Nossin, E. and François-Haugrin, F. (2016) Isolation and Characterization of Flavonols by HPLC-UV-ESI-MS/MS from Talipariti elatum (Sw.) *American Journal of Plant Sciences,* 7, 1198-1204. http://dx.doi.org/10.4236/ajps.2016.78115