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Antioxidant activity of an isomer of gossypitrin (gossypetin-3'-*O*-glucoside) isolated in the petals of *Taliparitti elatum* Sw., and determination of total phenolic content of the total flower

Frantz François-Haugrin, Max Monan, Emmanuel Nossin, Juliette Smith-Ravin and Odile Marcelin

Abstract
Objective: Isolation and identification of the main flavonoid from petals of *Taliparitti elatum* Sw. (*Hibiscus elatus* Sw), comparison of antioxidant properties, determination of total phenolic content of the flower.
Method: The main flavonoid was precipitated using 1, 2 - dimethoxyethane, followed by two serial recrystallizations. Analysis of the precipitate was done with UV spectrum, MS-NMR ^{1}H and MS-NMR ^{13}C spectroscopy. Antioxidant activity was carried out using 1,1- diphenyl -2-picrylhydrazyl (DPPH) and β-carotene.
Results: The main flavonoid of the petals is gossypetin-3'-*O*-glucoside. Until now Cuban research workers argued that major flavonoid was gossypitrin (gossypetin-7-*O*-glucoside). DPPH test leads to an EC50 value of 0,11 mg/L, however β-carotene bleaching test (BCB test) didn't give positive results.
Total phenolic content of ethanolic extract from dried flower is 163 ± 5 mg GAE/g.
Conclusion: *Taliparitti elatum* Sw. is a high source of natural antioxidants. Precedent publications revealed also the presence of various flavonols or flavones (quercetin, rutin). Antimicrobial, anti-hepatotoxic, anti-catarrh, anti-cough, anti-asthma properties of this specie show it great interest in health's range.

Keywords: Antioxidant; gossypetin-3'-*O*-glucoside; gossypitrin; *Taliparitti elatum* Sw.; DPPH; phenolic content

1. Introduction
Today search of natural bioactive substances with antioxidant, antimicrobial, anti-inflammatory properties and other therapeutic effects is developing rapidly, because of the growing interest of consumers for this kind of products, and willingness of policy makers to validate and enhance the natural heritage and ancestral knowledge [1, 2].
Initially our study was about gossypritrin, which is an *O*-glucoside in C_7 position of a flavonoid with OH substitutions in 3, 5, 8, 3', and 4'. It takes its name from cotton flowers which it was initially identified as the Egyptian cotton (*Gossypium barbadense*) and yellow Indian cotton (*G. herbaceum*) [38]. Although it has also been isolated in many other types of flowers (*Matricaria Chamomilla, Papaver nudicaule, Papaver radicatum, Chrysantha segetum* L.), plants (*Drosera peltata, Artemisia fragrans*), and spores (*Equisetum telmateia* Ehrh., *Equisetum fluviatile* L.), its prevalence is found in the genus *Hibiscus*, where it joins the red anthocyanins producing a copigmentation effect found in the extracts of these flowers [7, 8, 14, 16]. Some works on the biological properties of the genus *Hibiscus* and gossypitrin were conducted and resulted in several applications. Thus in 1978, the Japanese Singo has studied the inhibitory action of gossypitrin on rhizome formation, and *Fucus evanescens* eggs division [35], and in 1979 its inhibitory action on the development of fertilized eggs of sea urchins (*Stronlocentrotus intermedicus*) [36]. Several Cuban teams have published many works on gossypitrin extracted from *Taliparitti elatum* Sw. *(Hibiscus elatus* Sw.) and its flowers. Pérez-Trueba *et al*. (2003) [27] have demonstrated its protective effect against hepatotoxicity induced by carbon tetrachloride in rats. Cuéllar-Cuéllar and Gonzalez-Yaqué (2010) [7] have isolated it in the petals of the flowers using different methods, and evaluated its toxicity and its antioxidant capabilities. Cuéllar-Cuéllar and Rojas Hernandez (2011) [8] have evaluated the flowers drying (weight loss, residual moisture), the performance of different flower extracts (toluene, ethanol) according to the parts (petals, pollen, chalice), the isolated chemical constituents, as well as the antimicrobial activity of the flavonoid gossypitrin. *Taliparitti elatum*

Sw. is a big tree of the mallow family that grows naturally in wet mountain forests of Cuba and Jamaica. It produces all year beautiful flowers orange to red, and was domesticated and planted in almost all the islands and countries of the Caribbean basin.

In Cuba, where one of the common names is *Majagua azul*, flowers are traditionally used against cough, asthma, catarrh, researchers attribute their properties to the presence of flavonoid gossypitrin [7]. In Martinica, under the name *Blue Mahoe* or *Mountain Mahoe*, the uses are less known and therefore less common, and a complete exploration of the biochemical and biological properties remains to be done.

This present work initially aimed to reproduce on the petals of flowers of *Taliparitiy elatum* Sw. harvested in Martinica the extraction and purification processes of gossypitrin implemented by the Cubans researchers, and especially to perform antioxidants tests on this molecule. According to Cubans researchers gossypitrin would be the main bioactive substance of the flowers of *Taliparitiy elatum* Sw. Moreover, in order to rationalize an envisaged production of phytomedicines, determination of total polyphenols of a dry extract of the whole flower has been carried out.

2. Materials and methods

2.1 Plant material

Two crops of flowers of *Taliparitiy elatum* Sw. were conducted in Martinica along Route de la Trace. Flowers were washed before drying in the laboratory at room temperature for about ten days. Dried flowers were preserved in a refrigerator and used throughout the work. Grinding concerned only the flower petals. These were crushed with a crusher Prep'Line - SEB, until a relatively fine powder, or crushed with fingers.

2.2 Chemicals

All chemicals were purchase from Sigma-Aldrich Chemical Co., Ltd., at least with analytical grade: absolute ethanol in 5 liters, R.G methanol, 1,2-dimethoxyethane>= 99%, β-carotene~1600 U/mg, Tween 40 (Polyoxyethylene sorbitan monopalmitate), oleic acid, linoleic acid, chloroform for analysis (GBA), 2,2-Diphenyl-1-pricylhydrazyl, Folin and Ciocalteu reagent, standards of L-ascorbic acid BioXtra, Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), DL-Alpha tocopherol, and deionized water.

2.3 Soxhlet extractions with 1,2 dimethoxyethane

The extraction technique was developed on petals of flowers according to Cuéllar-Cuéllar and González-Yaque (2010) [7]. Eighty grams (80 g) of crushed petals were extracted from 1.2 liter of 1,2-dimethoxyethane in a Soxhlet apparatus during 12 hours. This process was repeated 3 times. A sustained orange yellow extract was obtained. Each extract was concentrated in a rotary evaporator (RE Start 300) until a volume of about 50 ml, and then stored at 4 °C for crystallization.

2.4 Crystallization and purification

2.4.1 Crystallization

After 24 hours at 4 °C a green yellow precipitate was collected by filtration on a filter of porosity 3, and dried in the dark at room temperature.

2.4.2 Purification

Two serial purifications was done by dissolving each time dry extract in 35 mL of 1,2-dimethoxyethane at boiling temperature (85 °C). The solution was placed at 4 °C. After few hours characteristic green yellow precipitate appeared. It was filtered on a porous glass filter 3, dried with a hair dryer, and stored in the dark at laboratory temperature.

2.5 Controls by TLC and HPLC analysis

2.5.1 Procedure of the *TLC*

The plate was 0.2 mm silica gel with fluorescence developer. The solvent was a mixture of n-butanol-acetic acid-water in the proportions 4: 1: 5. The developer was of 50% sulfuric acid, and drying was done with a hairdryer.

2.5.2 Conditions of HPLC

Eluent: acetonitrile/double-distilled water (1/1); Column: C 18; Flow rate: 1 mL/min; Temperature: 25 °C; Detection: UV at 272 nm; Injection volume: 20µL.

HPLC procedure: The chromatograph was prepared several hours in advance with the eluent. A sample of 4 mL at 20 mg/L was prepared in the eluent, and then centrifuged 15 minutes at 3000 t/min. The supernatant was recovered, filtered through paper (10-15µm) and after with a syringe filter of 0.45 µm.

2.6 Analysis of purified extract by LC/MS, NMR and UV

First NMR spectra were recorded on a Bruker Avance III 400 MHz BBFO+ probe spectrometer for 1H and 100 MHz for 13C in DMSO-d6. HRMS were obtained on an Agilent Technologies 6520 Accurate-Mass Q.Tof LC/MS apparatus using electrospray ionization mode and time-of-flight analyzer (ESI-TOF). Column C18 -2,1x50 mm x1,8 µm.

Second NMR experiments were performed on a Bruker Advance III, 600 MHz (1H), delivering a magnetic field of 14.1 Tesla.

The UV spectrum was made in methanol using spectrophotometer UV-Vis JENWAY 6715 (UK) between 200 and 500 nm with a spectral bandwidth of 1.5 nm and a spectral resolution of 0.1 nm.

2.7 Total phenols

Total phenols were measured in triplicate from an ethanolic dry extract of the flowers, according to the method of Wong *et al*. (2010) [40] with slight modifications, using the Folin-Ciocalteu reagent and gallic acid as standard. To a sample of 0,3 ml at 300 mg/L in distilled water were added 1.5 mL of Folin-Ciocalteu reagent 1:10, and 1.2 mL of sodium carbonate at 7.5%. The tubes were shaken with a Vortex and left 30 minutes in the dark at laboratory temperature. Absorbances were read at 765 nm on spectrophotometer Jenway 6705 UV/Vis. Results are expressed as gallic acid equivalents (GAE)/g of dried extract.

2.8 Diphenyl picryl hydrazyl (DPPH) test

DPPH method was done according to Srikanth and Muralidharan (2010) [34].

A solution of green yellow precipitate at 1 mg in 4 mL of methanol was prepared and filtered on a paper filter. Then seven (7) tubes of successive dilutions from 0,75 to 0,02 mg in 4 mL of methanol were made. Eight (8) whites containing 4 mL of every dilution and 1 mL of methanol were prepared. A white with 5 mL of methanol, and a tube control containing 4 mL of methanol and 1 mL of DPPH 1 mM establishing the zero time was made. Eight (8) tubes tests containing each 4 ml of every dilution and 1 mL of DPPH 1 mM added every three minutes were prepared. Tubes were left in the darkness

and the readings made on the spectrophotometer JENWAY 6715 at 517 nm after 30 minutes exactly (controlled in the chronometer). The tests were made in triplicate. The standards (L-ascorbic acid, BHT, α-tocopherol) were realized in the same way as the tests. The EC50 (effective concentration 50) of the solution of the green yellow precipitate was determined.

Percentage of the radical trapping = $(A_0 - A_x / A_0)*100$

A_0 = absorbance of the reference solution (only DPPH)

A_x = absorbance of DPPH in the presence of different concentrations of standard or of extract

2.9 Evaluation of degradation of β-carotene in the presence of linoleic acid

This test was carried out according to Jayaprakasha *et al* (2001) [18].

An emulsion was prepared by dissolving 4 mg of β-carotene in 20 mL of chloroform ($CHCl_3$). This solvent was removed by evaporation in vacuum using a rotary evaporator at 40°C to a volume of about 4 mL. Those 4 mL were placed in a round bottom 200 mL flask, and then 80 mg of linoleic acid, 800 mg of Tween 40 and 200 mL of H_2O_2 0.01 $mol.L^{-1}$ were added. The emulsion was shacked vigorously for 1-2 minutes. An identical emulsion without β-carotene was prepared for white. Ten (10) tubes for each set of absorbance measurements were prepared according to the selected measurement times. The absorbance of standards (BHT, α-tocopherol), control (without antioxidant) and solution of the green yellow precipitate in ethanol was measured at 470 nm. All the tubes were placed in a water bath at 50 °C (corresponding to the time t = 0). Absorbances were read every 15 min to 90 min, and then every 30 minutes up to 180 minutes (using glass cells). Antioxidant capacity is expressed as percentage of inhibition of bleaching according to the formula: (%) = $[1-(A_0 – A_t)/(A^0_0 – A^0_t)] \times 100$, with:

A_0 and A^0_0 are absorbances for standard and control respectively at t=0

A_t and A^0_t are absorbances for extract or standard and control respectively at t=180 min.

3. Results and discussions

3.1 Harvest of flowers.

The harvest of flowers, raw material, though of primary importance, did not pose any particular difficulties. Indeed, the main plantations are located along Route de la Trace, easy to access. As in Cuba, the *Taliparitti elatum* Sw. also flourished here in Martinica throughout the year with two flowering peaks in January and July-August [23-25]. The flowers were fallen from the trees or harvested by hand, transported in plastic bags and left to dry the same day.

3.2 Drying of flowers

Drying was carried out in air at laboratory temperature. The petals, initially red, took a brown tint. Drying quality was estimated as satisfactory, after ten days, for crushing by hand and mechanical milling. However González-Yaque [nd2] [15] recalls that the drying method is fundamental in pharmacognosic studies to determine the quality of the plant extract. Indeed drying plant material removes enough water to keep the plant, prevent mold and action of enzymes and bacteria.

Evaluation of drying methods performed by Cuéllar-Cuéllar and Rojas-Hernandez (2011) [8] is shown in table 1.

Table 1: Results of flower drying assessments Taliparitti elatum Sw. (according to Cuéllar-Cuéllar and Rojas-Hernandez, 2011) [8]

Drying method	Drying days	Lost per 100 g	Residual moisture
Oven at 45 °C	3	73,5	10,7
Shadow	18	65,0	19,8
Sun	12	70,0	16,1

3.3 Soxhlet Extraction

The development of the Soxhlet extraction with 1,2 dimethoxyethane was one of our main goals. Indeed this molecule was identified by Márquez-Hernández *et al.* (1999) [22] as one of the specific solvents (with 1,2 dichloroethane) for gossypitrin. It was used by Cuéllar-Cuéllar and González-Yaque, (2010) [7] repeatedly. With this solvant a clear extract sustained orange yellow color was obtained.

3.4 Cold crystallization

To pass from a liquid extract to a product in solid form the liquid must be oversaturated [5]. We observed a green yellow precipitate when amount of solvent was reduced from 200 ml to 35 ml, and temperature close to room temperature. Yield after the first crystallization was 2.5%, and decrease to 0.1% after the second purification.

3.5 Control of the purity of the extracts by HPLC and TLC

HPLC after the first crystallization reveals three peaks with retention times of 1, 82; 2, 21 and 2,41 minutes respectively. TLC shows three spots Rf1 = 0.67**,** Rf2 = 0.80**,** Rf3 = 0.89. After first purification, HPLC reveals two peaks with retention ties of 2,1 and 2,8 minutes respectively. TLC shows two clearly visible stains, Rf1 = 0.68**,** Rf2 = 0.79. After second purification, HPLC only one peak remains with a significant intensity and with retention times of 2, 0 minutes. TLC shows only one visible spot, Rf = 0.78. As a result, TLC and HPLC show that two successive recrystallizations led to a substantially pure compound, then purifications were arrested. At this stage further analyzes were performed in order to accurately characterize the molecule present in the sample: that is LC-MS, UV spectrum, NMR.

3.6 Analysis of the green yellow precipitate by LC-MS and UV spectrum

As can be seen in figure 1, m/z of the compound obtained by this extraction process is equal to 481.09725. We can confirm the presence of a molecule of same molecular weight than gossypitrin (mass 480 g/mol). This result is also consistent with those obtained by Cuban researchers Gonzalez-Yaque and Cuellar-Cuellar (2010) [15]. Pics of m/z 319.1 are found. Both UV spectra performed in Martinica and in Cuba (figures 2 and 3) show a great similarity, tending to confirm the presence of two isomeric molecules. Those UV spectra are compatible with a flavonol presenting a free hydroxyl group in 3, supported by the existence of band above 350 nm. Existence of catecholic groups is confirmed by the band at 278 nm, and the inflection at 257 nm.

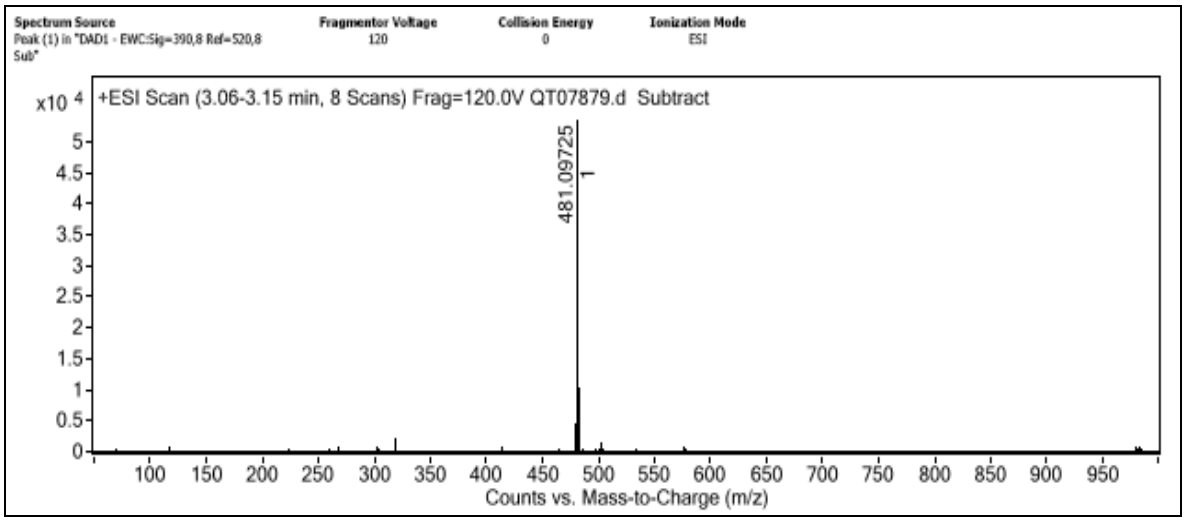

Fig 1: Result of LC-MS of the purified extract.

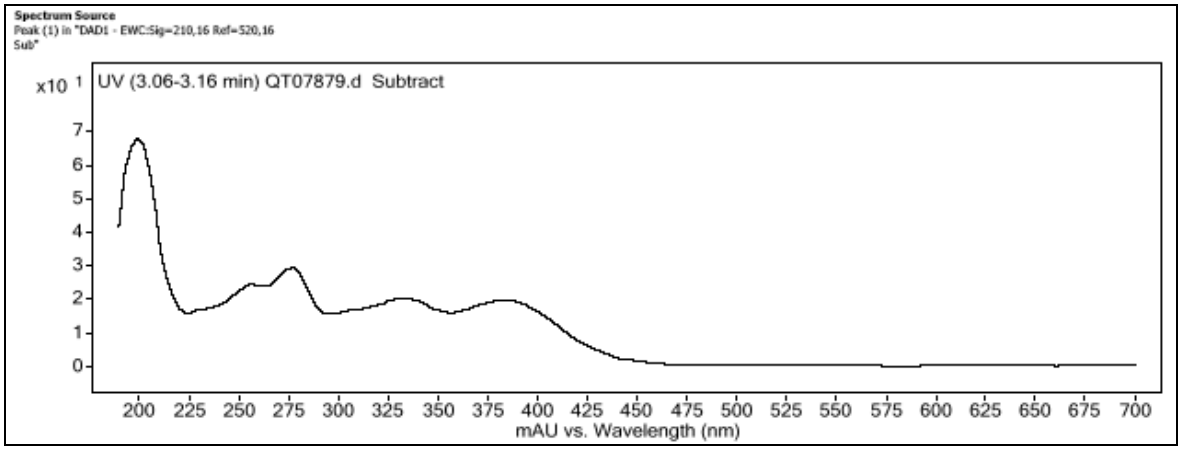

Fig 2: UV spectrum of our sample of Martinica

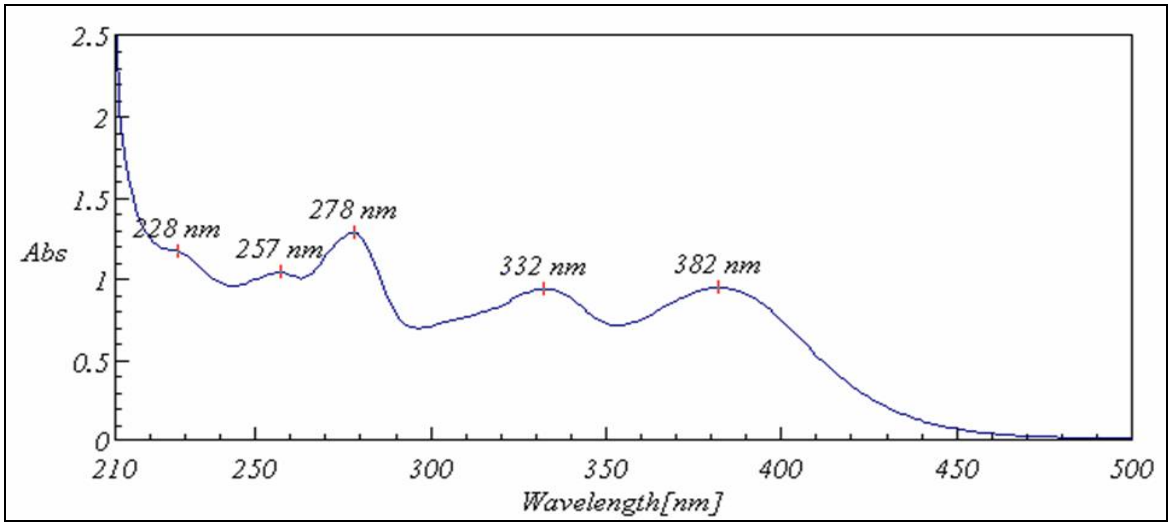

Fig 3: UV spectrum of a Cuban sample

3.7 Analyses of the purified extract by SM-NMR

The NMR analyzes were performed on a Bruker 400 MHz spectrometer with a probe 5 mm Pabbo BB-1H / D Z-GRD 210861. The high resolution mass spectrum reveals the presence in the fraction of an ion $[M + H^+]$ of exact mass M = 481.09725 g.mol^{-1} corresponding to the formula $C_{21}H_{21}O_{13}$. Fragmentation of this ion gives a fragment $M^{\bullet+}$ of exact mass M = 319.04403 g.mol^{-1} corresponding to the formula $C_{15}H_{11}O_8$. These results allow us to consider a pentahydroxy led flavonol structure substituted with an hexose [11]. Data of monodimensional NMR of ^1H and ^{13}C flavonol core are

identical to those of the gossypetin published in the literature [4]. They are presented in Table 2.

Table 2: Data of monodimensional NMR of ^{1}H and ^{13}C flavonol core

No.	^{13}C	^{1}H
4	176.61	-
5	153.57	-
7	152.83	-
4’	149.11	-
2	146.52	-
3’	145.54	-
8a	145.49	-
3	136.16	-
8	124.96	-
1’	123.81	-
6’	122.92	7.91 (dd ^{3}J (6’H, 5’H) = 8.6 Hz ^{4}J (6’-H, 2’-H) = 2.1 Hz)
5’	116.50	6.99 (d ^{3}J (5’H, 6’H) = 8.6 Hz
2’	116.23	8.07 (d ^{4}J (2’H, 6’H) = 2.1 Hz
4a	103.18	-
1"	102.26	4.83 (d ^{3}J (1"H, 2"H) = 7.3 Hz)
6	98.59	6.26 (s)
3"	77.21	3.33*
5"	76.49	3.32*
2"	73.63	3.33*
4"	69.73	3.33*
6"	60.74	6"$_{a}$3.75 (m) – 6"$_{b}$3.58 (m)
5-OH		11.89
3-OH		9.35
4’-OH		8.62

The chemical shifts of the protons of the glycoside fragment were deducted from the HSQC spectrum. The proton δ = 5.53 ppm, δ = 5.12 ppm, δ = 5.06 ppm and δ = 4.59 ppm correspond to the protons of the hydroxyl of the sugar without being able to assign them unambiguous. It is the same for aromatic hydroxyl δ = 9.38 ppm and δ = 10.40 ppm not present in HSQC spectrum (figure 4).

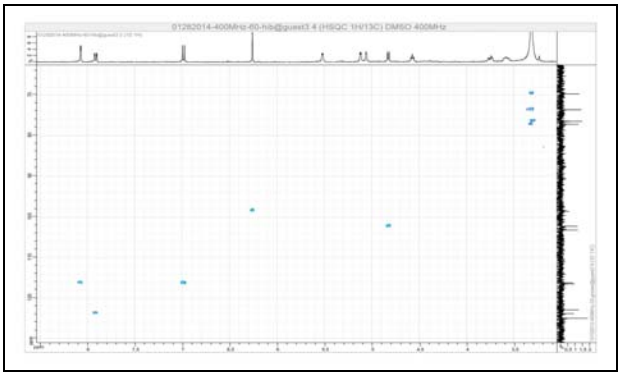

Fig 4: HSQC spectrum

The COSY and HMBC correlations highlighted well confirm the structure of the gossypetin linked to an hexose in 3' position of ring B (Figures 5 and 6).

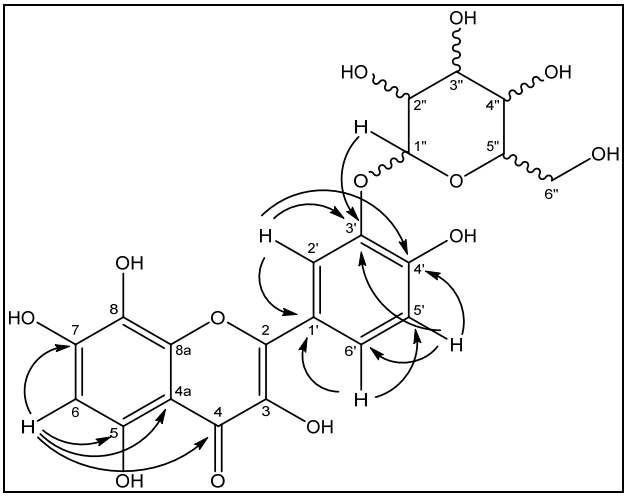

Fig 5: HMBC correlations highlighted

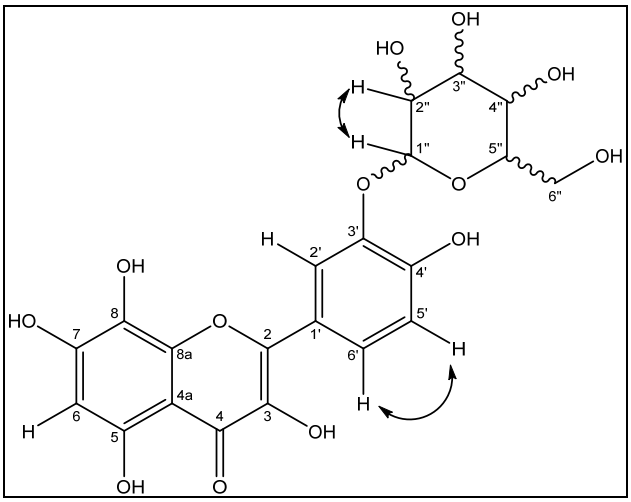

Fig 6: COSY correlations highlighted

The NOESY correlations (Figure 7) between the proton 2'H at δ = 8.07 ppm and the anomeric proton 1"H at δ = 4.83 ppm unambiguously confirmed the substitution by the sugar 3'-position. The hydroxyls of the sugar in turn correlate with the protons of the hydroxyls 4'-OH and 3-OH and between them.

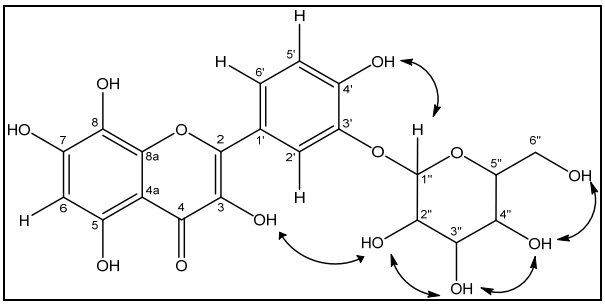

Fig 7: NOESY correlations highlighted

Then the formula of the molecule is clearly present in the figure 8.

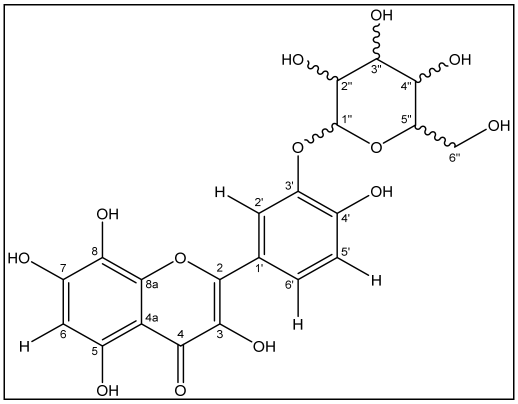

Fig 8: Molecule of Gossypetin 3'-O-glycoside

3.8 Determination of total polyphenols

For this assay an ethanol dry extract of whole flowers of *Talipariti elatum* Sw. was achieved, and three sets of measures performed. Three determinations with gallic acid as standard were carried out in five points. Absorbances were measured at 765 nm.

The curve of the average absorbance versus concentrations (Figure 9) is a straight line, which equation is: y = 0.0018x + 0.0033 (R^2 = 0.9961).

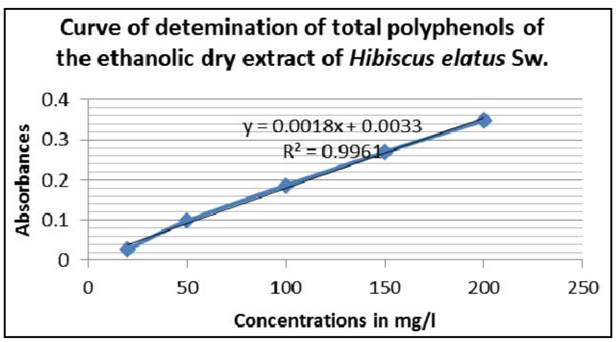

Fig 9: Curve of determination of total polyphenols of the ethanolic dry extract of Taliparíti elatum Sw. whole flowers

Calculation of total polyphenols in Gallic Acid Equivalent (GAE) of the dry extract of *Talipariti elatum* Sw. whole flowers was made taking into account the three determinations with gallic acid, and the ethanolic dry extract assay. The absorbance per 100 mg of dry extract after the dosage of total polyphenols curve is 0.187, and then mean value in GAE and the standard were performed. So rate of total polyphenols in the extract of *Talipariti elatum* Sw. equal to 163 ± 5 mg GAE/g of dry extract. Comparing this value with those obtained with the same method by Wong *et al.* (2010) [40] for other species of Hibiscus, we can see in Table 3 that total polyphenols of our sample is higher.

Table 3: Total polyphenols concentration of flowers of Hibiscus according to Wong *et al.* (2010) [40] in GAE

Hibiscus species	Total Polyphenols (mg GAE/g)
H. tiliaceus	24,20 ± 1,67
H. rosa-sinensis	7,35 ± 0,46
H. taiwanensis	5,80 ± 0,79
H. shizopetalus	5,16 ± 0,3
H. mutabilis	4,95 ± 0,23

3.9 Diphenyl picryl hydrazyl (DPPH) assay

3.9.1 DPPH test curves

The scavenging percentage curves of the DPPH radical depending on the concentration of ascorbic acid, BHT, α-tocopherol, and gossypetin-3'-*O*-glucoside were plotted (Figure 10). Scavenging percentage of DPPH radical increases with standard antioxidants concentration and also with gossypetin-3'-*O*-glucoside concentration. Figure 10 also shows that our sample scavenging capacity is slightly lower than that of L-ascorbic acid, almost identical to that of α-tocopherol, but greater than that of BHT.

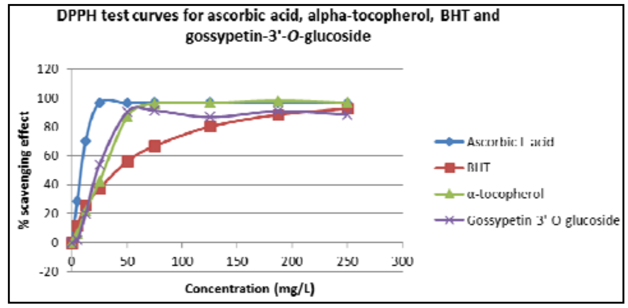

Fig 10: DPPH test curves for ascorbic acid, alpha-tocopherol, BHT and gossypetin-O-3'-glucoside

3.9.2 Calculation of EC50

EC50 is necessary antioxidant concentration to scavenge 50% of DPPH free radicals. The equation of the line corresponding to the rising part of the curve (Figure 11) is y = 490.56 x - 3.9541, so EC50 sample = 0.11 mg/L

For standards, results are as follow: EC50 α-tocopherol = 0.12 mg/L; EC50 BHT = 0.17 mg/L; EC50 Ascorbic acid = 0.04 mg/L

So we get: EC50 ascorbic acid <EC50 gossypetin-3'-*O*-glucoside <EC50 α-tocopherol <EC50 BHT

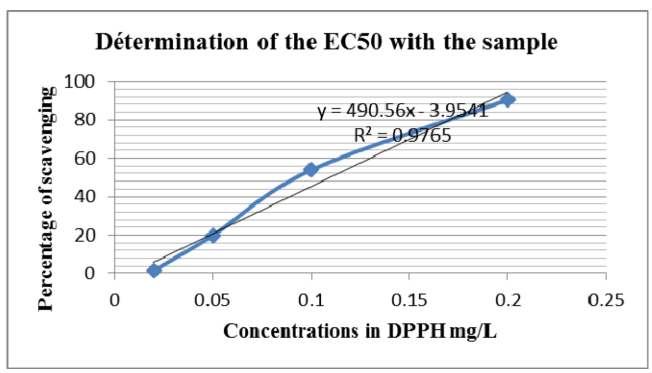

Fig 11: Determination of EC50 with the sample line representing the increasing portion of the curve of the scavenging percentage depending on the concentration of DPPH.

3.10 Beta-carotene bleaching test

The curves of absorbance versus time are shown in Figure 12. It is observed that the absorbance of the control without antioxidant decreases constantly, from 0.536 at 0 minute and falls to 0.308 after 180 minutes. That of the BHT remains virtually unchanged from 0.550 to 0.546, as well as that of α-tocopherol ranging from 0.543 to 0.475, as well as that of α-tocopherol ranging from 0,543 to 0,475. These results indicate that the reagents and materials were correct and the protocol well developed. The β-carotene bleaching caused by the

degradation products of linoleic acid has been stopped or slowed.

Test results are: Capacity of BHT = 98.2%; capacity of α-tocopherol = 70.2%; capacity of gossypetin-3'-O-glucoside = 0%. Test result can be compared with that obtained with ascorbic acid, which is a well-known antioxidant, but doesn't show antioxidant properties when used in the β-carotene bleaching test. The phenomenon is called "polar paradox" [12, 19, 28]. As a polar antioxidant remains essentially in the aqueous of the emulsion, it is too diluted in the lipid phase to protect effectively the carotene molecule.

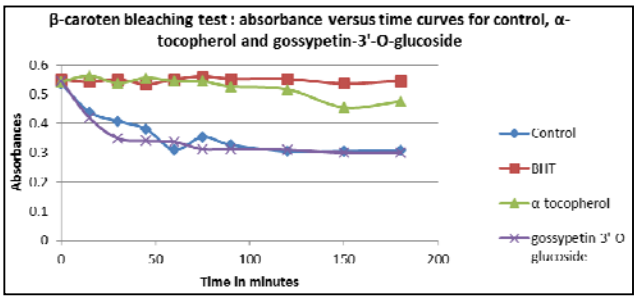

Fig 12: Curves of absorbances versus times are shown here.

3.11 Interest of gossypetin glucosides for medical care

Many recapitulatory works were already done about pharmacodynamics effects of extracts of *Hibiscus* species, especially of their petals or leaves [13, 32]. However study of antioxidant capacity of the chemical compounds that are glucosides of gossypetin reveals specific interest of this type of molecules.

3.12 Four glucosides of gossypetin are known:

- ***Gossypetin-8-O-glucoside or gossypin*:** It can be isolated from flowers or calyces of *Hibiscus vitifolius* [39], or *Hibiscus sabdariffa* L. [37]. It seems to inhibit cell proliferation in human melanoma cell lines [3]. It shows potent neuro protective activity against global cerebral I/R injury-induced oxidative stress in rats [6]. Extracts of the flowers and calyces are used for treatment of many complaints as highblood pressure, leaver diseases and fever, but a lot of other properties were demonstrated, as cancer preventive activity against prostate cancer cells [21].
- ***Gossypetin-7-O-glucoside or gossypitrin or gossypetrin*:** It was found also in *Hibiscus sabdariffa* L. [29, 30, 37], in *Hibiscus tiliaceus* [26], and in the petals of the flowers of *Hibiscus elatus* Sw. [22]. Extracts of the flowers of *Hibiscus elatus* Sw. are used as expectorant and antasthmatic in Cuba Island [31]. Antioxidant and antasthmatic activities of gossypitrin were evaluated [7, 8, 16]. The protective effect on Carbon tetrachloride-induced in vivo hepatotoxicity was shown [27].
- ***Gossypetin-3-O-glucoside or gossytrin*:** It has been isolated from *Hibiscus sabdariffa* L [33], and also from *Hibiscus tiliaceus* [26]. Flowers of *Hibiscus tiliaceus* are used as anti-fertility agent. Boiled in milk they are used for treatment of earache.
- ***Gossypetin-3'-O-glucoside*:** That glucoside is present in flowers of *Abelmoschus manihot* (initially *Hibiscus manihot*) [20, 41]. Extracts of those flowers seems to have interesting properties against myocardial and cerebral anoxia [42], myocardial and cerebral ischemia injury [17]. Among others, anti-inflammatory and antipyretic effects were studied [10]. For the first time our work reveled presence of this molecule in *Hibiscus elatus* Sw. (*Taliparitti elatum* Sw.) harvested in Martinica. Complementary works must be done to determine if properties assigned to gossypitrin molecule can't be attributed to the isomeric molecule that is gossypetin-3'-*O*-glucoside.

In genus Hibiscus, it is noticeable that the specie *Hibiscus sabdariffa* L., in which three glucosides of gossypetin can be found, is one of the most used as medicinal plants [9].

4. Conclusion

For the first time gossypetin-3'-*O*-glycoside was extracted and characterized from petals of flowers of *Taliparitti elatum* Sw. of Martinica. Determination of total polyphenols was developed. The rate 163 ± 5 mg EAG/g, is notably higher than those obtained with other species of *Hibiscus* (*tiliaceus, rasosinensis, taiwanensis, shizopetalus*). DPPH test yielded an EC50 higher than ascorbic acid, but lower than α-tocopherol and BHT. The development of the β-carotene bleaching test shown the ability of BHT and α-tocopherol to inhibit the bleaching of the β-carotene, but the high solubility of gossypetin-3'-*O*-glucoside in aqueous phase doesn't allow inhibition of the bleaching.

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