



Quality Control, Extraction and Identification of Main Components from the Flowers and Fruits of *Talipariti elatum* Sw. Fryxell (Malvaceae)

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Abstract

To determine the presence of components with antioxidant effects in the flowers and fruits of *Talipariti elatum* (S.w) Fryxell (Malvaceae) and to get an increase of data that allow to complete the record file for the flowers and fruits of the spice whose extracts are very use in the treatment of bronchial asthma, motivated the carry out of the present work. A study of different parameters was realized, that let to settle down quality index of the flowers and fruits of the plant in their use like crude drug. In the flowers of the spice, characteristic physicochemical values like total ashes (10.48%), water soluble ashes (8.5%), moisture content (10.85%) and extractable matter in ethanol (33.68%). In the fruits these values were, moisture content (13.47%), alcohol extractive value (4.07%), water extractive value (7.36%) and total ash (3.58%) Techniques of phytochemical screening suggest the possible presence of various groups of metabolites en both vegetable organs such as flavonoids, triterpens end steroids, reductant sugars, anthocyanidins and tannins. The study of the petals from the flowers using ethanol at 95% in serial extractions, permitted the crystallization of a wide range of products, mainly flavonoids. In ethanolic extract from the petals the product that was characterized with more facility is the flavonoid glucoside gossypitrin, while in the case of fruits were characterized two alkaloids related with tetrahydrocannabinol.

Keywords: *Talipariti elatum*; Flowers; Fruits; Ethanolic Extract; Phytochemical Screening; Chemical Characterization

Introduction

Talipariti elatum Sw. Fryxell (Malvaceae) is a tree that grow abundantly across our country in swamps, marshy costs and low terrains. The tree reaches up to 18 m of height and have largely petiolate leaves, limbo between 7 and 20 cm of wide, almost orbicular, heart-shape at the base, acuminate at the apex, with little teethes in the edge and prominently venal in the adaxial surface

[1]. *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 t.) and is often used in reforestation. It is the national tree of Jamaica [2].

The plant is quite attractive with its straight trunk, broad green leaves and hibiscus-like flowers. The flower changes color as it

matures, going from bright yellow to orange-red and finally to crimson (Figure 1). 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 according to [3].



Figure 1: Flowers of *T. elatum* (Sw.) Fryxell.

The petals of the flowers of this medicinal tree are used in Cuba as anti-asthmatic, expectorant and against flu. Up today, four different kinds of biological activities have been demonstrated by our research group: antimicrobial, antioxidant, transition metal quelating and neuroprotective against chemical hypoxia-induced PC₁₂ cell death [4,5]. Recently, was assessed the protective action of gossypitrin (Gos), a naturally occurring flavonoid, on iron-induced neuronal cell damage using mouse hippocampal HT-22 cells and mitochondria isolated from rat brains [6].

The results of the test to determine the oral acute toxicity (OAT) of gossypitrin demonstrated that the glucoside flavonoid classified as non-toxic according to the classification system of the Commission of the European Communities. The flavonoid belongs to class CTA₀ in which the Mortality > 2000 [7].

Figure 2 shows the macromorphological characteristics of the fruits. Fruits are dehiscent capsules, globular or ovoid, 5(-10)-lobulated. Several seeds in each locale, reform, subglasses, pubescent or tomentoses. Capsules are ovoid, from 2 - 4.2 mm long, with simple and long trichrome, antors and small start trichrome. Seeds have kidney form, 4 - 5 mm long, and tomentoses with brown-yellowish trichrome taking into account the results in [8]. The aim of this research was to determine the quality of ethanolic extract and identify the main components of flowers and fruits from *Talipariti elatum*.



Figure 2: Fruits of *T. elatum* (Sw.) Fryxell (Malvaceae).

Material and Methods

Plant material

Flowers and fruits were collected in January 2018 in the gardens of the Faculty of Pharmacy and Foods at Havana University after their mature. They were identified at the herbarium of National Botany Garden of Havana, where the voucher specimen no. HAJB 82587 was deposited and registered as *Talipariti elatum* (S.w). Petals were separated manually from the rest of components of the flowers and packaged in a nylon bag strictly closely. The fruits were collected after their mature and dried at shadow on the ground around the trees and kept into the nylon bag at room temperature.

Selection

Each flower and fruit was selected taking into account their appearance, free of any damage and without the attack of insects. 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. Fruits were crushed in the same way.

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%, FeCl₃, AlCl₃, and gallic acid. Quercetin, rutin, and gossypitrin were used as standards. All solvents were degassed prior to use in an ultrasonic bath without filtration.

Physicochemical parameters of the powders

Total ash, acid insoluble ash, water soluble ash, extractable matter and moisture content was determined according to the standard procedures mentioned in the general rule of WHO and Miranda y Cuéllar [9,10]. Another two determinations were done to the powder of the drugs: drying and residual humidity.

Extracts preparation

The extracts, in both cases, 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 according to [10]. A second extraction from petals was done using 1,2-dimethoxyethane (DME) ($\geq 99\%$) using the same conditions related previously. This process was repeated 3 times. After that, a sustained orange yellow extract was obtained from the petals. 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.

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. Two serial purifications were 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 a 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. The same procedure was done using EtOH 95% [11].

From fruits extracts after their concentration and kept 10 days in refrigeration was observed a solid that precipitated from the extract. This solid was recuperate by decantation and dried at room temperature during two days after it was submitted to chemical characterization.

Physicochemical parameters of the extract

The extracts were prepared with the ground material getting the physico-chemical parameters like organoleptic properties (odor and color), pH, refraction index, relative density and total solids as discussed in [10] [12].

TLC and capillary analysis

TLCP (thin-layer chromatography plate) 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 (BAW 65:25:10) as developing agent (v/v/v), concentrated sulfuric acid plus heat. The TLCP were examined under ultraviolet (254 nm and 365 nm) and ordinary light. Vanillin at 1%, rutin (R), quercetin (Q) (Merck) and gossypitrin (G) were used as standard [13].

Capillary analysis was developed according to NRSP 312 and Miranda y Cuéllar, 2001, using 20 mL of the extract and Whatman paper #1 (4 cm × 20 cm). The experiment was carry on during 2 hours in a dark chamber with a temperature of 25°C (± 2), taking into account the height, and description of the parts and possible changes under ammonia vapors.

Determination of total phenolic and total flavonoid contents

Total phenols were calculated by the Folin-Ciocalteu method, using as reference the gallic acid (Sigma-Aldrich) at concentrations of 10, 20, 30, 40 and 50 mg/mL as referred by Chlopicka, *et al.* 2012 [14]. On the other hands, the content of total flavonoids was carried out by the colorimetric method according to Pourmorad, *et al.* 2006 [15], using aluminum trichloride and quercetin (Sigma-Aldrich) as reference substance at the concentrations of 10, 15, 25, 50 and 100 µg/mL. In each case, calibration lineal curve was constructed with absorbance read in a spectrophotometer Rayleigh UV-1601 (China) at 715 nm vs. concentration of reference compound, which was then obtained respective concentration of total content of phenol or flavonoids and SD in the studied extract (mg/mL).

Chemical elucidation

Each and every one extract from each plant component was characterized using two different elucidation method. Flower's petals were characterized using GC-MS procedures and HPLC-DAD-ESI-MS/MS procedures, while fruits where characterized only by GC-MS procedures.

GC-MS procedures, instrumentation and parameters of flowers and fruits

A sample of solid powder from ethanolic extract of the petals was subjected to chromatographic analysis in equipment GC/MS, brand FISON TRIO 1000 (UK), using the direct introduction methodology by electron impact technique, with an ionization energy of 70 eV and a variable temperature gradient, according to the case [16].

Fruits samples were subjected to chromatographic analysis in equipment GC/MS, brand Shimadzu QP2010, equipped with a splitter split/splitless. With a BP5 (30 m × 0.25 mm × 0.25 microns) capillary column under the following chromatographic conditions: Helium gas carrier obtained by electron impact fragments to a

power of 70 eV rate of 1.2 mL/min, 1:50 split flow and the volume of injected sample of 1 µL. Programmed oven temperature: initial temperature was 70°C with a heating ramp of 10 °C/min to 300°C and remained stable at this temperature for 10 minutes. Subsequently the temperature was increased at a rate of 10 °C/minute to 300°C for a total time of 78 minutes with an injector temperature 250 °C and the interface temperature 300 °C. The compounds were analyzed using GC/MS NIST21 and NIST107 library and having into account the results obtained after phytochemical screening according with González, *et al.* 2018 [17]. Silylation agent was *N,O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA), CAS 25561-30-2, Lot: 0901-1 Macherey-Nagel GmbH and C. KG.

HPLC-DAD-ESI-MS/MS procedures, instrumentation and parameters of flower extract

UPLC has been used for the profiling and characterization of the metabolites contained in the extracts. The system used is a Dionex U3000 equipped with a DAD detector having a C₁₈ analytical column (100 x 4.6mm particles, i.d: 3µm). Solvent systems: H₂O-0.1% Formic Acid (H₂O) and Acetonitrile-0.1% Formic Acid (Table 1).

Tiempo	Disolvente	Gradiente
0	H ₂ O:CH ₃ CN	80:20
5	H ₂ O:CH ₃ CN	80:20
10	H ₂ O:CH ₃ CN	0:100
25	H ₂ O:CH ₃ CN	0:100
30	H ₂ O:CH ₃ CN	80:20
34	H ₂ O:CH ₃ CN	80:20

Table 1: UPLC gradient used in the research.

The HPLC is coupled to a Varian 500MS Mass Spectrometer (USA) equipped with an electrospray ionization chamber (ESI) used in negative mode at 5 KV and a capillary temperature of 250 °C. The UV detector and the Mass Spectrometer are used in parallel. A split allows the post column eluent flow to be separated into two parts when the flow rate used is greater than 500 L/min. From a flow rate of 1 mL/min, 400 µL/min are sent to the mass spectrometer and around 600 µL/min to the trash [18].

Data was acquired in positive or negative mode using the TDDS option ("Turbo Data Dependent Scanning") to automatically

obtain ion fragmentation spectra that allow the identification of compounds. These mass spectrometry data were compared with free access databases such as "Massbank", "Spider mass DB", the "in-house" database or data from the literature. If this procedure did not allow identification, an attempt to elucidate the structure was carried out manually. Fragmentation spectra (MS²) in negative mode were carried out for the structural elucidation of each of the compounds.

Statistical analysis

Results are presented as mean ± SD. Statistical analyses were performed by Student's t-test. The values of $p < 0.05$ were considered significant. Duncan test was used utilizing the Statgraphics® Plus, version 5.0 program. The mean effective concentration (IC₅₀) was determined with the help of the Graphprism 5.0 statistical program.

Results and Discussion

Physicochemical parameters of the drugs

Three different methods of drying for each organ of the plant were realized in this research: in an oven, at sun and shadow. After that, were determined the residual humidity of each part of the plant using the azeotropic method (distillation with toluene).

Table 2 summarized the results of drying to each part of the part studied in the research.

Method	Flowers		Fruits	
	Lost in weigh of water (%)	Days of drying	Lost in weigh of water (%)	Days of drying
In oven	73.40	5	73.5	3
Sun	69.60	12.5	70.0	12
Shadow	65.29	18.5	65.0	18

Table 2: Results of drying of flowers and fruits using different methods.

The drying at shadow was the method that less water extracted in both cases (65.29 and 65.0%, respectively) and with the higher time to get an apparent constant weight (18 days). Comparing the drying of the drug at sun the obtained value of loss water was higher (69.60 and 70.0, respectively) and a number of days relatively high [12]. Only in the case of a drying by oven both results

were encouraging, but in only five days to the flowers and three to the fruits was obtain the constant weigh, missing as average 73.40 and 73.5%, respectively. Without any doubt, this one was the better method, and all the drug used in phytochemical analysis was dried in the oven at 35 °C according to Miranda y Cuéllar, 2001. After this time, the drug fracture manually, indicating the efficacy of the method.

Moisture content

Determine the residual humidity in vegetable material is one of the numerical index that complement the quality of the dried method evaluated. The presence of an excess of water in the drug be able to promote the fungus growth, insect proliferation and hydrolysis and/or oxidation of chemical constituents, suggesting an unreal weight.

Water limit established by pharmacopoeia, fluctuate between 8-14%, without few exceptions. To determine the water content in vegetable drugs, the gravimetric method (loss drying) and volumetric or azeotropic method (toluene distillation) (WHO, 1998).

Table 3 shows the results after six replica using the volumetric method taking into account each dried method for each plant's part.

Dried Method	Flowers	Fruits
Shadow	19,96	19,80
Sun	16,16	16,10
Oven	10,85	10,70

Table 3: Results from determination of moisture content (%).

Only the value reached using the oven in both cases is inside the limit established for this assay, come closer the lower accepted rule (8%) according to WHO, 1998 and Miranda y Cuéllar, 2001.

Extractive matter or soluble substances

One of the best numerical index to select the better extraction solvents and to dose the extracts is the determination of extractive matter or soluble substances. To this evaluation, the used drug was dried by oven and milling later on. Water and ethanol were the solvents used to get each extract. Non hydroalcoholic mixture was used in the assay. Table 4 exhibit the results of this evaluation.

Solvent	Flowers	Fruits
Water	22,63	7,36
Ethanol	33,68	4,07

Table 4: Results of the determination of soluble substances (%).

Ethanol show a greater extractive power than water, with an average value about of 11 units, reason why indicate that chemical composition of petals is inside the range of middle polarity, while in fruits, the difference is only about 3 units, being water the solvent with a bigger average, having polar components in its extracts [19].

Ashes

Three determinations of ashes were realized in both plant organs: total ashes, water soluble ashes and insoluble acid ashes. This numerical parameter indicates the composition of the drug, not only organic but inorganic too. The content of "physiological ashes" or "non-physiological ashes" are related with the components in the tissues of the plant and inorganic components or mineral substances (i.e., sand or soil). The results of ashes determination are summarized in Table 5.

Organ	Total ashes	Water soluble ashes	Acid insoluble ashes
Flowers	10.48	8.5	6.6
Fruits	3.58	0.70	1.56

Table 5: Results of ashes determination in both plant's organs (%).

As an average of six determinations the obtained value to the flowers was 10.48%, while for fruits was 3.58%. The accepted value for medicinal plants is around 3-5%, according to USP 40; NF 35, 2017. Regarding our results are considering high, even though, this value could be the characteristic value for this plant, taking into account that plant was under bloom period, manifesting inner physiological changes and increasing it absorption of nutrients from the soil [20].

Water soluble ashes and acid insoluble ashes were found high in the case of the flowers, taking into account the average value accepted for medicinal plants by WHO ($\leq 2\%$), but in the fruits, both values are according to the general rules of WHO, because both results are under accepted value for vegetable drugs.

A lot of researchers have reported that many medicinal plants have high values of ashes that overcome the rules established for those determinations and they are proposing a review of those values, just as, in the case of *Cissus sicyoides* L., and *Cecropia peltata* L., in Cuba, *Trichodesma indicum* L., in India, *Brunfelsia americana* L., and three spices of *Phyllanthus* [21-25].

In the case of *Crotalaria lachnosema* Stapf., and *Viola tianschanica* Maxim., total ashes values were 6.40 and 12.0%, respectively [26,27]. The last mentioned researcher said that the Pharmacopoeia of Popular Republic of China accept like maximal limit the value of 15.0% for that determination [28]. In the same situation are the water soluble ashes and acid insoluble ashes.

Phytochemical screening

Before the extraction with any solvent, in our case ethanol 95%, is necessary to realize some kind of simple, easy and preliminary assays to detect qualitatively certain constituent's groups. Through simple test with the help of microchemistry we can to probe the presence of those groups by means of precipitate formation, changes in coloration, etc.

Tables 6 and 7, summarize the results of phytochemical screening for both vegetable organs, indicating that in the case of the flowers we have the presence of triterpenes and/or steroids, fat and/or esencial oils, reductant sugars, tannins and/or phenols, aminoacids and/or amines, flavonoids, anthocyanidins, mucilage and bitter principles and/or astringents.

Test	Diethyl ether	Ethanol	Water
Dragendorff	-	-	-
Liebermann-Burchard	+	+	
Börntrager	-	-	
Baljet	-	-	
Sudan III	+		
Fehling		+	+
Ninhydrin		+	
FeCl ₃		+	+
Shinoda		+	+
Foam test		-	+/-
Kedde		-	
Anthocyanidins		+	
Mucilage			+
Bitter principles and/or astringents			+

Table 6: Phytochemical screening of the flower's petals from *T. elatum* (Sw.).

Test	Diethyl ether	Ethanol	Water
Dragendorff	+++	+++	-
Wagner	+++	+++	-
Liebermann-Burchard	+	-	
Börntrager		-	
Baljet	+	-	
Sudan III	+		
Fehling		+	+
Ninhidrina		-	
FeCl ₃		+	+
Shinoda		+	+
Foam test		-	-
Kedde		-	
Anthocyanidins		+	
Bitter principles and/or astringents			+

Table 7: Phytochemical screening of fruits from *T. elatum* (Sw.).

Fruits mainly contain alkaloids, fat and/or esencial oils, reductant sugars, flavonoids, anthocyanidins, pirochatecolic tannins, and bitter principles and/or astringents. It is the first time that our research group report the presence of alkaloids in the plant, because up to now even in the stem bark any alkaloid is present in the extracts of this medicinal plant. Only in phytochemical screening in diethyl eter, alcohol and water the test was very positive using Dragendorff test as well as in Wagner test, but only in alcohol and water extracts [17].

In 2016, researching the chemical composition of three samples of petals from *T. elatum* collected in Cuba and Martinica, our research group found out the presence of one chemical compound with an impair molecular mass (*m/z* 479), probably an alkaloid [29].

Physicochemical parameters of the extract

Both ethanolic extract from each vegetable organ were submitted to different analysis to determine the physicochemical parameters. Table 8 shows the results of physicochemical parameters in each part of the plant. Figures 3 and 4 show the color and general characteristic of both ethanolic extracts.

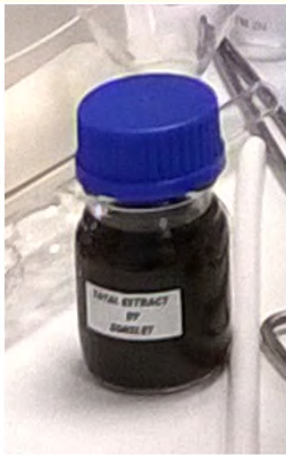


Figure 3: Ethanolic extract of the flowers.



Figure 4: Ethanolic extract of the fruits.

Physicochemical parameter	Flowers	Fruits
Odor	Characteristic	Characteristic
Color	Dark liquid Orange-reddish	Translucent liquid Yellowish
Relative density	0,929 ± 0,0005	0,8197 ± 0,0024
Refraction index	1,5863 ± 0,003	1,3605 ± 0,0002
pH	3,1 ± 0,07	5,55 ± 0,02
Total solids	4,34 ± 0,1	1,07 ± 0,03
Capillary analysis	6,3 ± 0,15	5,3 ± 0,14

Table 8: Physicochemical parameters of extracts from the flowers and fruits.

The value of total solids to the ethanolic extract of the flower (4.34%) is below to the value reported by Márquez., *et al.* 1999, that was (5.57%). The difference is due to they used an hydroalcoholic mixture (50% v/v) and the extraction method was percolation. Relative density (0.929) was similar to that reported by [30], while refraction index (1.5863) was lightly superior to that reported by the same authors (1.365). The measure value of pH is equal to 3.1, suggesting that the extract is acid and this result is identical to the obtained by [31].

Capillary analysis showed an image vividly colored, reason why classify like fairly high (5.0-8.0 cm), the fringe (superior limit of ascension) classify like regular jagged, sub-fringe is orange-reddish in color, band shows tonalities between clear orange-reddish and orange, sub-band shows a clear orange color (Picture is not shown).

Fruits exhibit a value of relative density of 0.8197, with a refraction index of 1.3605, pH equal to 5.55 (), lightly acid, the value of total solids is low (1.07%), but is in correspondense with the result obtained in soluble solids in ethanol, suggesting that its components are in the range between few and middle polar.

Capillary analysis showed an image few colored, classifying as fairly high (5.0-8.0 cm). The fringe is between lineal and festinated; sub-fringe is yellow in color, the band exhibit tonalities between clear yellow and cream, sub-band showed a clear cream color. Under ammonia vapors the image intensify its color (Figure 5).

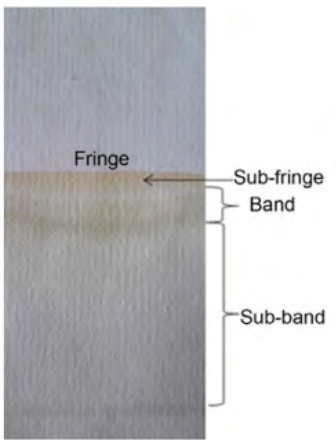


Figure 5: Capillary analysis of ethanolic extract from the fruits.

TLC of samples and extracts

To define the qualitative chemical profile each powder sample was analyzed by TLC according to [32]. After dried the plates, were sprayed with specific reagents and heating to observe possible changes in coloration. The presence of phenolic hydroxyl groups was note through the reaction with FeCl_3 and AlCl_3 .

Under those conditions the plates showed characteristic yellow spots of flavonoid compounds. Spots change the color from yellow to green-yellowish when they are exposed under UV light at 254 nm, and to brown with H_2SO_4 (Figure 6). Calculated R_f values were 0.808 (1), 0.807 (2) and 0.780 (3), respectively (from left to right).

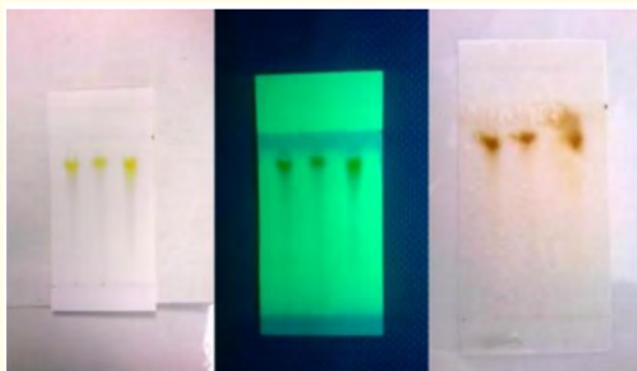


Figure 6: TLC of petal powders (from left to right: ordinary light; $\text{UV}_{254\text{ nm}}$; H_2SO_4 and heat).

Those results suggest that the isolated compounds contain at least only one kind of chemical component. The presence of phenolic hydroxyl groups (-OH) is remarked through a positive reaction with FeCl_3 and AlCl_3 . According to [33,34], interpretation of spot color in term of structures that belongs to flavone or flavonols, allow to infer that samples develop a yellow bright color under UV light with or without NH_3 . The appearance of the spot belongs to a flavonol with a free -OH in 3, with or without 5-OH, often glycosylates in position 7.

After several days in refrigeration was sight a solid that precipitated from ethanolic extract of the fruits. This solid, ethanolic extract, standard od gossypitrin, rutin and quercetin, were dot on chromatoplates. Under ordinary light were observe typical yellow spots with a long tale that belongs to flavonoid compounds, which increase their color under ammoniac vapor, changing the tonalities under UV light at 356 nm to pale yellow (Figure 7).

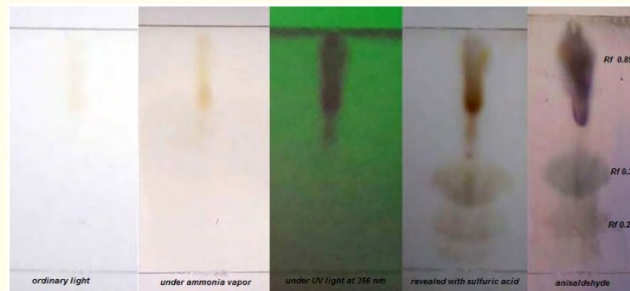


Figure 7: TLC of ethanolic extract from fruits of *T. elatum* (Sw.).

Spots change its color after revelation with H_2SO_4 and heat, and three different spots were observed, the bigger with a R_f 0.89 (yellow-orange), the second with R_f 0.36 (green-purple) and the third with R_f 0.21 (green). The last two mentioned spots almost circular, typically from triterpenoid structures. The tale of the major spot was surround by a blue or purple coloration typical of alkaloidal compounds. None evidence of the presence of gossypitrin, rutin or quercetin were observing, according to their respective R_f , main spot after its revelation with anisaldehyde showed a yellow-orange just as is showed in Figure 87.

Determination of total phenolic and total flavonoid contents

To check the total phenolic and total flavonoid content in the extracts of each organ of this medicinal plant, both determinations were done using Folin-Ciocalteu and colorimetric method with AlCl_3 , according to Chlopicka., *et al.* 2012 and Pourmorad., *et al.* 2006, respectively. Table 9 illustrate the results of both determinations to each investigated organ.

Extract	Total phenol content (mg EAG/g)	Total flavonoid content (mg/mL)
Flow- ers	168 ± 1	63,84
Fruits	0.92 ± 0.01	0.33 ± 0.005

Table 9: Total phenol and flavonoid content in ethanolic extracts.

The value reached in total ethanolic extract from the petals of the flowers of the plant is high if is compared with those values reported to another Hibiscus spices, according to [35] (Table 10).

Hibiscus spices	Total polyphenols (mg EAG/g)
<i>H. tiliaceus</i>	24,20 ± 1,67
<i>H. rosa-sinensis</i>	7,35 ± 0,46
<i>H. taiwanensis</i>	5,80 ± 0,79
<i>H. schizopetalus</i>	5,15 ± 0,3
<i>H. mutabilis</i>	4,95 ± 0,23

Table 10: Total polyphenol content of flowers from different *Hibiscus* spices (Wong, *et al.* 2010).

Total flavonoid content is high too for each used sample in the assay because represent about the 38% of the obtained value in total phenol content from the elaborated extracts, due to the extraction method used and the solvent employee (EtOH 95%) that allow the extraction of flavonoids from the cellular vacuoles.

Comparing the total phenol and flavonoid content of flowers and fruits of the plant, the value is around 167 and 63 times higher than those obtained for the fruits, respectively [19]. Fruits values are considered low, suggesting that is possible to increase those values heating the extracts and using a hydroalcoholic solvent.

Chemical elucidation

CG-EM of solid sample from the petals of the flowers

The solid that crystalize from ethanolic extract of the petals was submitted to chemical characterization by EM using direct introduction, showed a molecular mass of *m/z* 318 that belongs to a flavonoid hexahydroxylated, because the sugar supported for the flavonoid get lost in the process as is showed in Figure 8.

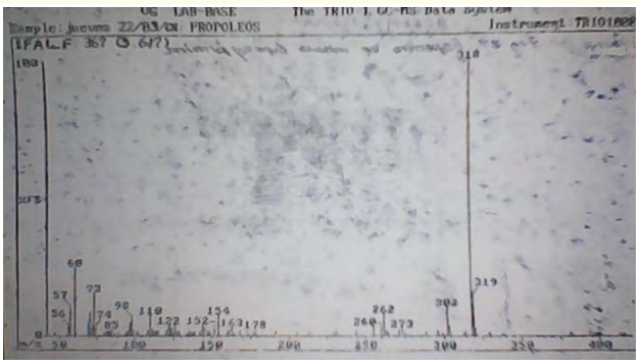


Figure 8: Mass spectrum of gossypitrin.

In the spectrum is possible to observe some relevant peaks belonging to characteristic fragmentations of flavonoids very hydroxylated, like the zone between *m/z* 152-154, that indistinctly could belong to fragments supporting ring A or ring B of the compound's backbone and especially the peak with *m/z* 302/303 that suggest three oxygenated substituents in ring A (Figure 9).

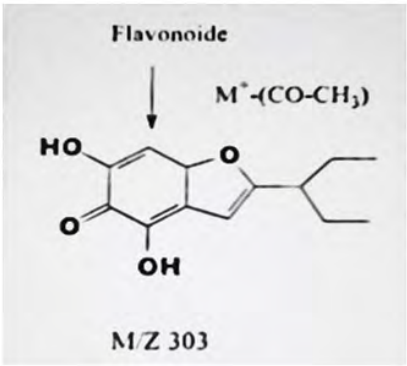


Figure 9: Fragment of ring A with three oxygenated substituents.

Confirmation of all those structural details were confirmed by HPLC-MS and the analysis was done according to [36] and [37], respectively.

HPLC-MS of the powders from the flowers

After refrigerate the flower extract at 4 °C, a precipitate is formed. This characteristic is used to obtain a powder containing gossypitrin. To do this, 3 tests were carried out. A first test carried out by recovering the precipitate from a hydroalcoholic extract, a second test obtained from the supernatant of the first precipitation and a third test carried out from an extract with 1,2-dimethoxyethane (100 mm column, 3 µm, 0.450 µL/min).

Analysis of three chromatograms showed that at least 5 chromatographic peaks have been detected, including a largely predominant peak. So, we are not in the presence of a pure compound but of a mixture of products. The majority peak was identified by MS/MS as being gossypitrin (G7G) or gossypetin-3'-O-glucoside (G3'G) (MS/MS data not shown here).

Its relative amount varies between 61 and 79% depending on the preparation of the extract. This difference is not significant when using DME or EtOH at 95% in the first crystallization (61 and 65%, respectively) but more significant between the first (65%) and the second crystallization (79%) (Figure 10).

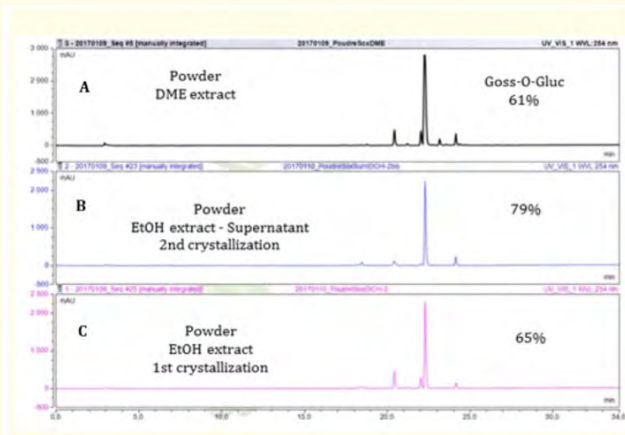


Figure 10: HPLC-UV₂₅₄ chromatograms of powders from the extracts of the flowers.

(A: precipitate from extraction with 1,2-DME; B: precipitate from extraction with EtOH 95%-2nd crystallization; C: precipitate from extraction with EtOH 95%-1st crystallization).

Therefore, not all G7G or G3'G precipitates from the first precipitation. Further purification by various chromatographic process is needed to obtain a pure compound. Obviously, the extraction with EtOH get a big amount of recovery than DME extraction and four another chemical compounds are present in the samples. The main product is always accompanied by another little peak near to 22 minutes of R_f suggesting the possibility to get in the sample two chemical components that could be isomers, in which case the separation is difficult or little advantageous.

Our research group found the same result when was analyzed a "pure sample" of gossypitrin, where were detected 6 different chemical compounds, mainly flavonoid glycosides, including G7G/ G3'G like major constituent of the petals of the flowers from *T. elatum* (Sw.) (Figure 11) [38].

Between both determinations the retention time of the main product have a big difference (≈ 19 min), but this situation is depending of various factors (kind of column, gradient used, HPLC brand, technician skills, etc.). Main chemical component has a molecular mass of 479 Da, after its fragmentation in negative ion mode, with a base peak at m/z 317 (Figure 12), confirm the result obtained by direct introduction of crystallized sample as is mentioned before.

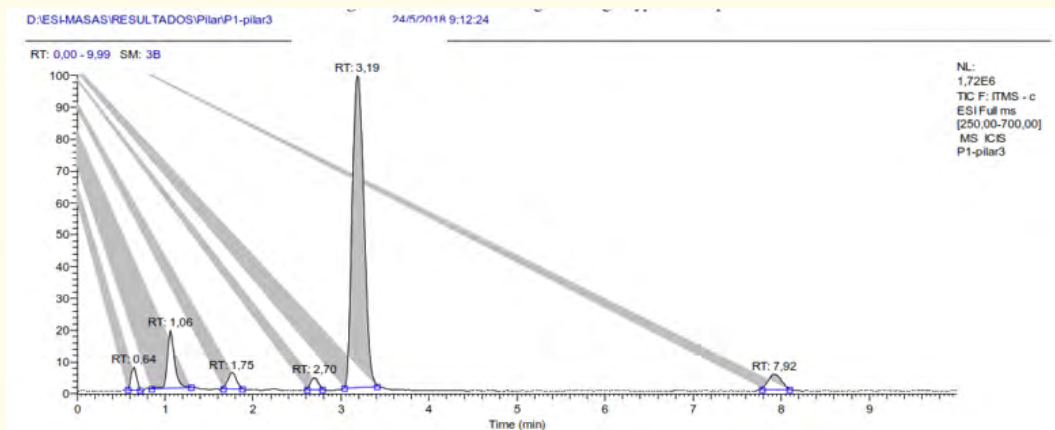


Figure 11: UHPLC-DAD chromatogram of a "pure gossypitrin sample".

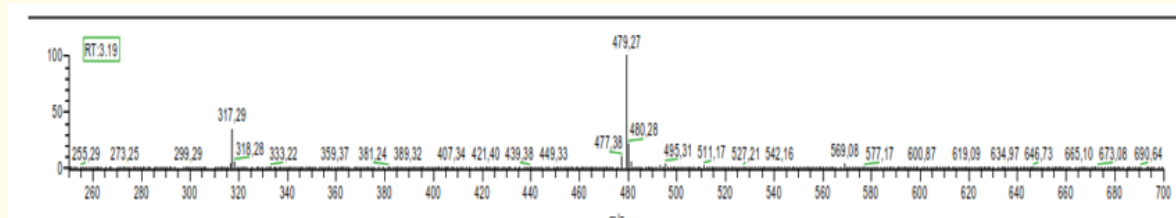


Figure 12: Mass spectrum of gossypitrin and/or gossypetin-3'-O-glucoside.

Obviously, that molecular mass belongs to an hexahydroxylated flavonoid with a sugar moiety attached to the backbone in position 3 and/or 7 mainly, even though is possible to find the location of sugar moieties in another position like 3', 8, etc., just as were found in *Hibiscus* flowers to flavonoid glycosides derived from gossypetin aglycone [11].

UHPLC-MS/MS of ethanolic extracts from the flowers

In ethanolic extracts using UHPLC-DAD-ESI-MS/MS were detected 54 chromatographic peaks, proposing the identification to 30 chemical components, mainly organic acids and flavonoids. Peak # 46 with a retention time of 22.287 min correspond to G7G and/or G3'G (m/z 479 [M-H]⁻) (Figure 13) [18].

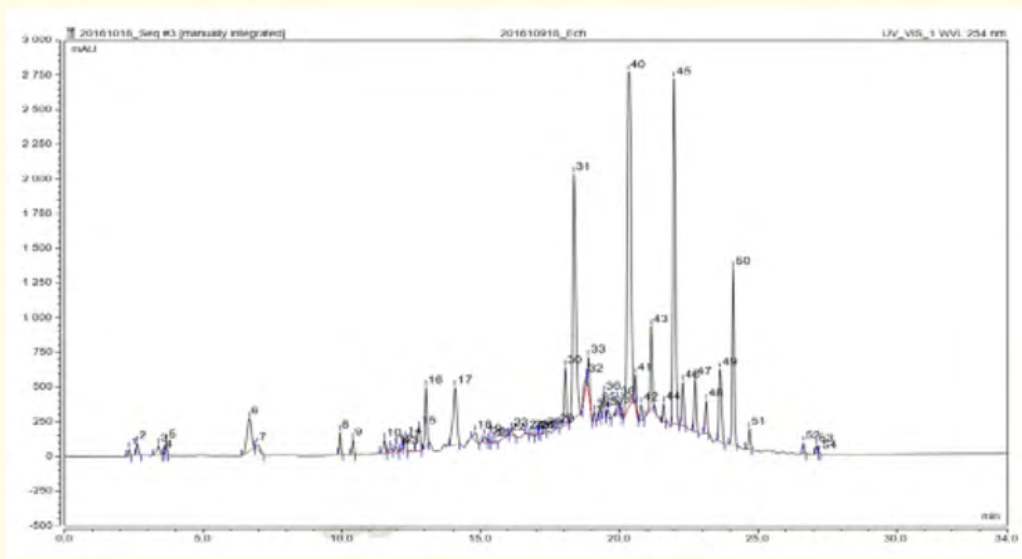


Figure 13: Current chromatogram of hydroethanolic extract of the petals of flowers from *T. elatum*.

As we reported previously in 2021, the 8 We selected the 8 main chromatographic peaks of the flower extracts from *T. elatum* and one by one, they were purified by liquid chromatography. In all cases, each figure corresponds to UV 254nm Chromatograms, as follow: (A) RP-UPLC preparative of the raw sample; (B) Analytical RP-UPLC of the raw sample; (C) Analytical RP-UPLC of the purified fraction. Here we will make the comment about only one fraction related with the flavonoid glucoside G7G and/or G3'G [18].

In such case, as mentioned peak 7 was collected (A, B) harvested and reinjected (C), observing a one single higher peak, that corresponds to G7G and/or G3'G, respectively (Figure 14).

UHPLC-MS/MS of ethanolic extracts from the fruits

Both samples of ethanolic extracts from the fruits (total extract and precipitate) were analyzed after their running by GC-MS. The

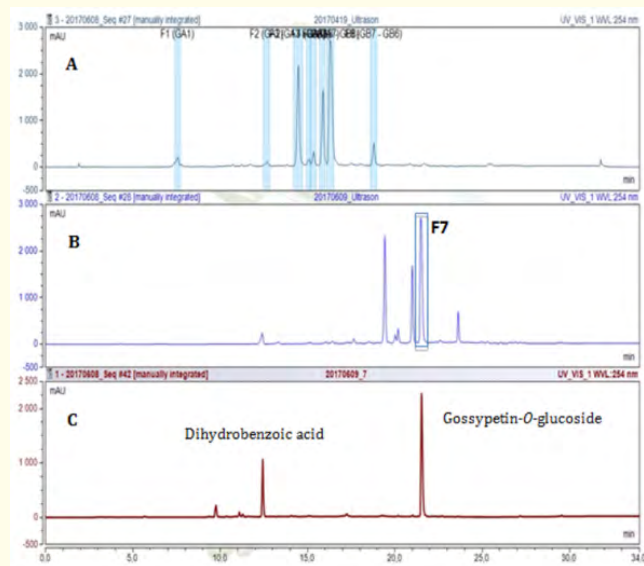


Figure 14: Current chromatogram of fraction 7 isolated and reinjected by UPLC.

final result allows to infer that both samples have a difference in their chemical composition of 7 components, corresponding the greater quantity to solid sample with 163. Chemical constituents from the fruits belong to According to reductants sugars, carboxylic acids, alcohol or derivatives, steroids, DNA derivatives, monoterpene derivatives and alkanes using the NIST Libraries (21 and 107).

At 41.335 min was reported the presence of two chemical compounds relating with alkaloids derivatives with 502 m/z. There is Bis-trimethylsilyl- δ -9-tetrahydrocannabinol acid and Tetrahydrocannabinolic acid-2TMS, respectively as is showed in Figure 15 [19].

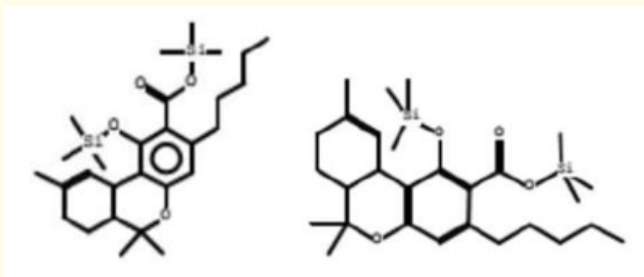


Figure 15: Chemical structures of both compounds relative with alkaloids.

Conclusions

Quality parameters to standardize the raw drug such as residual humidity (water content), ashes, solubles extractives, for both plant organs (flowers and fruits) are, in general, according to the rules of WHO for vegetable drugs. Phytochemical screening mainly suggests the presence of flavonoids, reductant sugars, tannins, anthocyanidins, and alkaloids. Physicochemical parameters of ethanolic extracts at 95% from the petals and fruits of *T. elatum* let us to infer that both extracts have the necessary quality to produce phytomedication, phytocosmetic or nutraceutical products in our country. A lot of chemical components were isolated and identified using different chromatography and spectroscopic techniques, some of them, constitute new evidence for those parts of this medicinal plant.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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