

Pharmacognostical and phytochemical studies of flowers from *Talipariti elatum* S.w

José González^{1*}, Armando Cuéllar², Max Monan³, Enmanuel Nossin⁴, Frantz François-Haugrin⁵

Department of Pharmacy, Faculty of Pharmacy and Foods, Havana University, ARVARNAM, Martinica.

Abstract— The flowers of *Talipariti elatum*, commonly named Blue Mahoe is used as antasthmatic and expectorant in phytotherapy, although the plants' composition has not been determined in detail so far. Hence, the pharmacognostic and phytochemical investigations on *Talipariti elatum* S.w has been carried out in this research, for the development and utilization of the promising medicinal plant. The characteristic physicochemical values like total ashes (10.48 %), water soluble ashes (8.5 %), moisture content (10.85%) and extractable matter in ethanol (33.68 %). The ethanolic extract mainly contained flavonoids in the flowers, also contained triterpens and steroids, amino acids, tannins, anthocyanidins and absence of alkaloids, coumarins, saponins, quinones and cardiotonic glycosides.

Keywords— *Talipariti elatum*, pharmacognostical identification, phytochemical, ethanolic extract.

I. INTRODUCTION

Talipariti elatum S.w Fryxell (Malvaceae), a tree with a wide distribution in Cuba, that growth in any type of soil particularly in swanpyones. It can get about 25 m of height (Fig. 1). It shows peciolated leaves, to mentose-velvet like and heart-shaped at the basis (Fig. 2). The flowers are big, yellow flower leaves that become reddish when old (Fig. 3). The flowers are used as appetitive, emollient, sudorific and as excellent pectoral combined with the flowers of *Hibiscus rosa-sinensis*. The mixture is used in traditional medicine as expectorant and antasthmatic (Roig, 1988). It is reported that flowers of *T. elatum* contains flavonoids (quercetin and rutin), sugars, phenols and tannins, triterpens and steroids and saponins (Acosta & Rodríguez, 2006).

The flower of the plant is an important source of bioactive compounds, such as organic acids, phytosterols, and polyphenols, some of them with antioxidant properties. The phenolic content in the flowers mainly consists of flavonoids like gossypitrin, rutin and quercetin and besides the known flavonoid gossypitrin; the presence of more than 40 different kinds of chemical compounds such as β -sitosterol, γ -sitosterol, red anthocyanin, phenolic acids such as propionic acid, pentatonic acid, hydroxypropionic acid, hydroxyacetic acid, 2-hydroxypropionic acid and hexanoic acid was reported. Gossypetin -3'-O-glucoside was isolated for the first time from the flowers of the plant in Martinic Island by maceration with methanol (24 h), and Soxhlet extraction with methanol, ethyl acetate and 1,2-dimethoxyethane as solvents (Françoise-Haugrin, 2015; Yaque *et al.*, 2016). The aim of this research was to establish the pharmacognostic and phytochemical control methods for the flowers of this plant that grows in Cuba and Martinica.



FIGURE 1. PLANT OF *T. ELATUM* S.W.



FIGURE 2. LEAF OF *T. ELATUM* S.W.



FIGURE 3. FLOWER OF *T. ELATUM* S.W.

II. MATERIAL AND METHODS

The methodology was carried out according to the Quality control methods for medicinal plant materials, WHO, 1998 and Miranda & Cuéllar, 2000.

2.1 Plant material and reagents

Flowers were collected in January 2015 in the gardens of the Faculty of Pharmacy and Foods at Havana University, and identified at the herbarium of National Botany Garden of Havana, where the voucher specimen no. HAJB 82587 has been deposited. The collection of the flowers in Martinic was realizing at the same time. A voucher specimen is deposited and registered in French Pharmacopeia as Fournet 1752 (4232 Guad). Both, Cuban and Martinican specimens are registered as *Hibiscus elatus* S.w.

Analytical grade ethanol (Merck), analytical grade acetic acid (Merck), analytical grade n-butanol (Merck), analytical grade methanol (Merck) and analytical grade water (Merck) were used in the analysis work. All solvents were degassing previously before used in an ultrasonic bath without filtration.

2.2 Extract and Samples Preparation

Dark red flowering types were collected daily. The isolated petals used were dried by three different methods: in an oven with controlled temperature, at 40°C, during 5 days (Cuban sample #1); in an oven with controlled temperature, at 45°C, during 5 days (Martinican sample #2); at shadow at room temperature during a week (Martinican sample #3). The extracts were prepared with the ground material (60 g) without screen extracted in a Soxhlet apparatus with 675 mL of ethanol at 95% during 20 hours. The ethanolic extracts were concentrated and evaporated under vacuum to 200 mL at 120 rpm, a temperature of 70°C and 500 mbar.

For to the purification, 1g of solid was dissolved in 25 mL of diethyl ether and the volume was completed to 100 mL with ethanol. The sample was refrigerated until an abundant solid appear and it was recuperated by filtration (Figures 4 and 5). 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).



FIGURE 4. ABUNDANT SOLID AFTER REFRIGERATION.



FIGURE 5. SOLID SAMPLE RECUPERATED BY FILTRATION.

2.3 Procedures, Instrumentation and Parameters

The total ash, acid insoluble ash, water soluble ash, extractable matter and moisture content were determined according to the standard procedures mentioned in the general rule of WHO, 1998.

The bioactive compounds such as alkaloids, flavonoids, saponins, tannins, and terpenoids were screened to ascertain their presences in the 95% ethanol extract according to Chhabra *et al.*, 1989.

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 analysed 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.

The IR spectrometric experiments were carry 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).

The total ash, water soluble ash, extractable matter and moisture content were determined according to the standard procedures mentioned in the general rule of WHO, 1998.

Conditions for thin-layer chromatography (TLC): TLCP (thin-layer chromatography plate) were activated under 100~105 $^{\circ}\text{C}$ for 30 min; TLCP on silica gel with fluorescent indicator 254 nm on aluminum cards (layer thickness 0.2 mm) (10 $\times$ 20 cm) using n-butanol: acetic acid: water (4:1:5) as eluent (v/v/v), concentrated sulfuric acid was a chromogenic agent. The TLCP examined under ultraviolet (254 nm) and ordinary light.

III. RESULTS AND DISCUSSION

Moisture content (10.85 %) was less than the limited index (8-14 %). Total ash (10.48 %) and water soluble ash (8.5 %) were all higher than the standard of medicinal plants; the possible reason is that flower medicine is not clean, containing soil and another inorganic impurity, although may be the reached values are the characteristics for this spices. Extractable matter in ethanol (33.68 %) was higher than extractable matter in water (22.63 %), suggesting that the chemical components have medium polarity.

The dry and fresh flowers showed principally the presence of triterpens and steroids, fats and essential oils, sugars, tannins and phenolic compounds, aminoacids, flavonoids, anthocyanidins, and mucilage due to the positive reaction with Liebermann-Burchard test, Ferric chloride test, Ninhydrin, Shinoda test, Fehling test and Sudan test. Negative reaction with Baljet test, Börntrager test, Dragendorff's reagent test, Foam test and Keddetest indicating the absence of coumarins, alkaloids, saponins and cardiotonic glycosides.

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

crystallization. Another one was recuperated after Soxhlet extraction with 1, 2-dichloromethane under the same conditions described previously.

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. 6). The R_f 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).

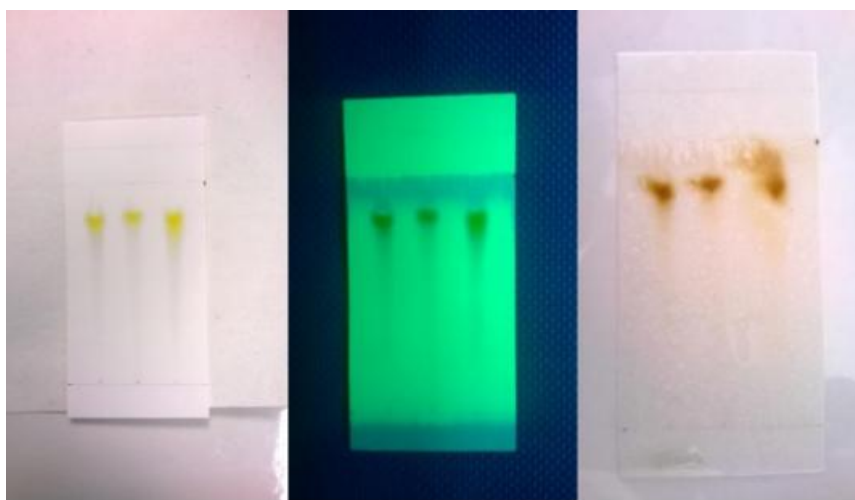


FIGURE 6. TLC OF ISOLATED SAMPLES UNDER ORDINARY LIGHT, UV LIGHT AT 254 NM AND AFTER REVELATION WITH H_2SO_4 AND HEAT.

3.1 Scan of the ultraviolet spectrum

The different types of flavonoids have different UV spectra, which can be used to differentiate isomers possessing similar mass spectra. Flavonols had a characteristic UV spectrum with two bands at 260 and 370 nm, and shoulders occurring at 270 and 300 nm. Flavonols with a free hydroxyl at position 3 shows a UV band I maximum at 350-380 nm, while flavonols with a blocked hydroxyl at aglycone position 3 showed lowest UV band I maximum. Band II reduce maximum absorption to 250-280 nm, in this case (Abad-García et al., 2009; Vukics & Guttman, 2010).

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 below 350 nm, with catecholic 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)(Fig. 7 and Table 1).

TABLE 1
VALUES OF λ MAX (NM) AND ABSORBANCE OF ANALYZED SAMPLES

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

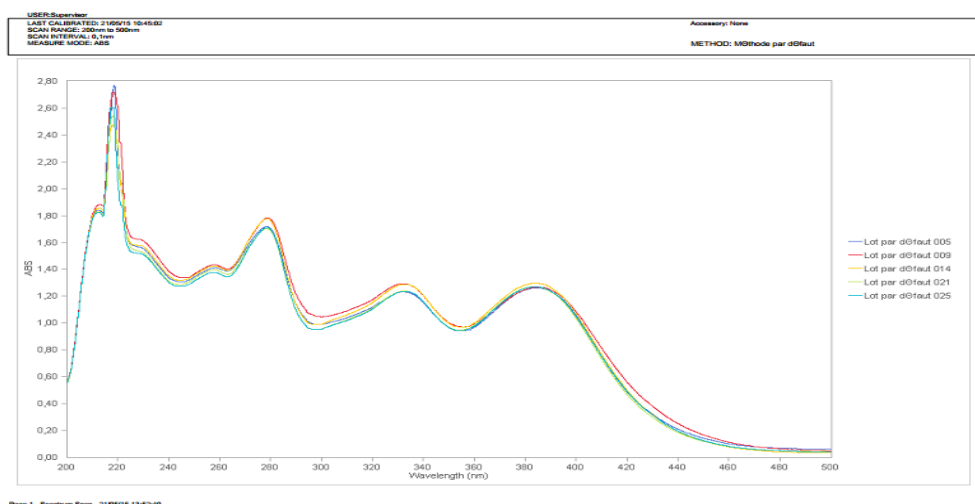


FIGURE 7. UV SPECTRUMS OF THE SOLID SAMPLES.

3.2 Scan of the infrared spectrum

IR spectrums showed typical presence of associated hydroxyl groups^a, alkyl groups^b, carbonyl group^c and aromatic rings^d due to different bands at $3\,375\text{ cm}^{-1a}$; $3\,000 - 2\,980\text{ cm}^{-1b}$; $1\,656\text{ cm}^{-1c}$; $1\,610$, $1\,600$, $1\,569$ and $1\,519\text{ cm}^{-1d}$. Stretching C-O band is around 1200 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).

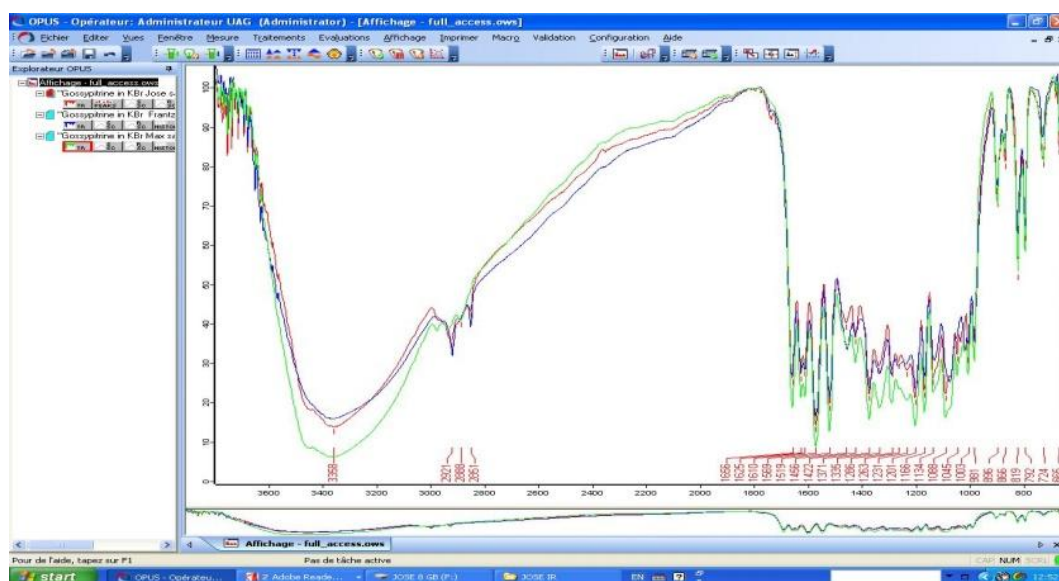


FIGURE 8. IR SPECTRUMS OF THE SOLID SAMPLES.

IV. CONCLUSION

Physicochemical parameters like ash values, moisture content and extractable matter are all indicators of quality flower medicine, which help to determinate the physiological and non-physiological ash, possibility of microbial growth or contamination and presence of impurities respectively. The relative high total ash value and a high ratio of water soluble ash content of *T. elatum* S.w indicates that the crude drug contains plenty of physiological ash and the non-physiological content, it will affect the clinical efficacy of drugs, so it should pay attention to the control quality of medicinal materials in plants production.

The compounds identified can be also used as biomarkers especially for *T. elatum*. The phenolic profiles of the petals reveal high predominance of flavonoids, which are antioxidant compounds that modulate a variety of beneficial biological events.

Therefore, *T. elatum* edible flowers may be considered a source of important phytochemicals (mainly flavonoids and phenolic acids) with bioactive properties to be explored for pharmaceutical applications.

The analysis of medicinal plant materials is not restricted to those methods discussed or recommended here and many techniques similar to those used for the analysis of synthetic drugs are also frequently employed (e.g. volumetric analysis, gravimetric determinations, gas chromatography, column chromatography, high-performance liquid chromatography and spectrophotometric methods). Details of all these methods can be found in The international pharmacopoeia.

REFERENCES

- [1] Roig, J. T. 1988. Plantas Medicinales Aromáticas y Venenosas de Cuba. Editorial Científico Técnica. La Habana.
- [2] Acosta de la Luz, L. L.; Rodríguez Ferradá, C. A. 2006. Plantas medicinales. Bases para una producción sostenible. Agrinfor, Impresiones MINAG, 124-125.
- [3] François-Haugrin, F. 2015. Extraction et purification de la gossypitrine des pétales de fleurs de Mahot bleu (*Hibiscus elatus* Sw.), et évaluation de ses propriétés antioxydantes par différentes méthodes. Perspectives en agroalimentaire, en cosmétique et en santé (confidentiel). Mémoire de diplôme d'ingénieur CNAM, Paris.
- [4] 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* S.w. American Journal of Plant Sciences, 7, 1198-1204.
- [5] WHO. Quality Control Methods for Medicinal Plant Materials. 1998. Typeset in Hong Kong. Printed in England 95/10628-Best-set/Clays-6000.
- [6] Miranda M.; Cuéllar A. 2000. Manual de Prácticas de Laboratorio. Farmacognosia y Productos Naturales. Universidad de la Habana.
- [7] Wagner, H., Bladt, S., 1996. Plant Drug Analysis. A thin layer chromatography atlas, 2^a ed. Springer. Berlin.
- [8] 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.
- [9] Márquez, I., Cuéllar, A., Martínez J., Alemán A., Lora J., Castro H. 1999. Estudio fitoquímico de la especie *Hibiscus elatus* S.w. Revista Cubana de Farmacia 33(2), 127-131.
- [10] 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.
- [11] 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.