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Total Phenolic Content in the petals and Oral Acute Toxicity of gossypitrin on Wistar Rats

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Abstract

Taliparitielatum Sw. (Fryxell) Malvaceae is a quite attractive tree with its straight trunk, broad green leaves, and hibiscus-like flowers. This medicinal plant is use in various traditional medicine therapeutic applications in Cuba, especially in treatment of bronchial asthma and flu. From ethanolic and toluene extracts of the flowers our research group have characterized more than 50 different chemical compounds as secondary metabolites. One of them, gossypitrin, a glucoside flavonoid isolated and purified from ethanolic extract of the petals had been reported as antibacterial and antifungal and to possess a remarkable antioxidant activity against a lot of amount of different ROS and RNS. Here, in our study, we realized the determination of total phenolic content from the total ethanolic extract using the petals of the flowers and the measurements of the oral acute toxicity of the main flavonoid in the petals of the flowers of *T. elatum.*

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Keywords

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Phenolic content,
Oral acute toxicity,
Gossypitrin.

Introduction

Medicinal plants utilized as alternative therapy are generalizing again as faster as possible not only in underdevelopment countries, but, in development countries too, not only for their efficacy, due to their security and their indocility during the treatments and Cuba is not an exception.

Taliparitielatum 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. Taliparitielatum 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 (1).

From the petals of their flowers our research team have isolated and characterized 30 different chemical compounds by UV, IR, UHPLC-MS/MS and NMR. The main chemical components in the ethanolic extracts of

this part are flavonoid and their derivate such as gossypitrin, gossypetin-3'-*O*-glucoside, quercetin-3-*O*-glucoside and isomer, kaempferol, protocatechuic acid and quercetin-*O*-sambubioside, etc. (2) (3) (4).

The aim of the present work was to determine the total phenol content in an ethanolic extract from the petals of the flowers of *Taliparitiy elatum* S.w and to evaluate the oral acute toxicity of the main chemical compound in this flower part in order to rationalize an envisaged production of phytomedicines.

Materials and Methods

Plant Material

Flowers were collected in January 2016 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 flowers were collected after their mature and separated into their constituent parts: petals, chalices and pistils with pollen.

Chemicals

All chemicals were purchase from Merck (Darmstadt, Germany). Analytical ethanol, Folin-Ciocalteu reagent, gallic acid, sodium carbonate and distilled water were used in the analysis work. All solvents were degassing previously before used in an ultrasonic bath without filtration.

Extract and Samples Preparation

Dark red flowering types were collected daily. The isolated petals used were dried in an oven with controlled temperature, at 40˚C, during 5 days. The extracts were prepared with the ground material (60 g) without screen extracted in a Soxhlet apparatus with 675 mL of ethanol at 95% during 20 hours. The ethanolic extracts were concentrated and evaporated under vacuum to 200 mL at 120 rpm, a temperature of 70˚C and 500 mbar.

For to the purification, 1g of solid was dissolved in 25 mL of diethyl ether and the volume was completed to 100 mL with ethanol. The sample was refrigerated until an abundant solid appear and it was recuperated to filtration. This process was done twice, to obtain only a yellowish-green solid monitoring by TLC on silica gel with fluorescent indicator 254 nm on aluminum cards (layer thickness 0.2 mm) (10 × 20 cm) using n-butanol: acetic acid: water (4:1:5) as eluent (v/v/v) (2).

Total phenols

Total phenols were measured in triplicate from an ethanolic dry extract of the petals, according to the method of NOA, 2008 (5), using the Folin-Ciocalteu reagent and gallic acid as standard. Absorbances were read at 765 nm on spectrophotometer Jenway 6705 UV/Vis (UK). Results are expressed as gallic acid equivalents (GAE)/g of dried extract.

Oral Acute Toxicity

The acute toxicity test was measured according to the method of Commission of the European Communities Nº 423, 1996 (6). Six young adult rats (3 males and 3 females) of Wistar line from Center of Production of Laboratory Animals (CENPALAB) in Bejucal, Mayabeque Province, Cuba. For this assay two groups of animals (3 per gender) were treated with gossypitrin using a gastric cannula with a single dosage at 2000 mg/Kg of weight (2 mL/200 g of weight constantly).

After 2-3 hours of finished the administration of the substance the animals were feed again. All animals were observed and the behavior registered in individual records several times during the first day and at least once a day during 13 days more to complete the procedure. At the end of the test the animals were sacrificed using diethyl eter. The organs (lungs, hearts, kidneys, stomachs, etc.) were extracted and observed if they are affected. If some of the organs were affected were taken samples for the histopathology analyses. The weight of the animals was processed statistically to register the mean and standard deviation.

Results and Discussion

Determination of total polyphenols

For this assay an ethanolic dry extract from the petals of the flowers of *Taliparitiy 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 (Fig. 2) is a straight line, which equation is: A = mC + n were m is the pendent; C is the concentration and n is the intercept (Table 1).

Calculation of total polyphenols in Gallic Acid Equivalent (GAE) of the dry extract of *Taliparitielatum* Sw. from the petals of the flowers was made taking into account the three determinations with gallic acid, and the ethanolic dry extract assay.

The rate of total polyphenols in the extract of *Taliparitielatum* Sw. equal to 168 ± 1 mg GAE/g of dry extract. Comparing this value with those obtained with the same method by Wong *et al.*, (2010) (7) for other species of *Hibiscus*, we can see in Table 2 that total polyphenols of our sample is higher. Our result is close to that of Martinica where François-Haugrin *et al.*, 2016 (8) found that in the same plant, but using the whole flowers with a value of 163 ± 1 mg GAE/g of dry extract.

Determination of Oral Acute Toxicity of Gossypitrin

The results of the test to determine the oral acute toxicity 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 CTA_0 in which the Mortality > 2000.

Fig.1 *Taliparitielatum* Sw. (Tree, flower and leaf)

Fig.2 Curve of determination of total polyphenols of the ethanolic dry extract of *Taliparitielatum* Sw

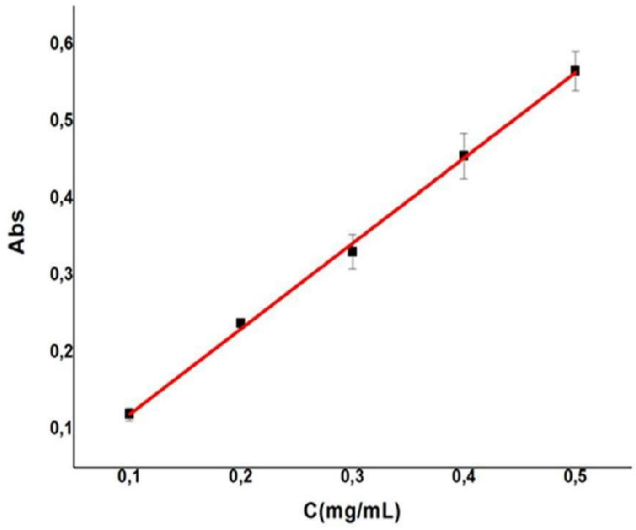
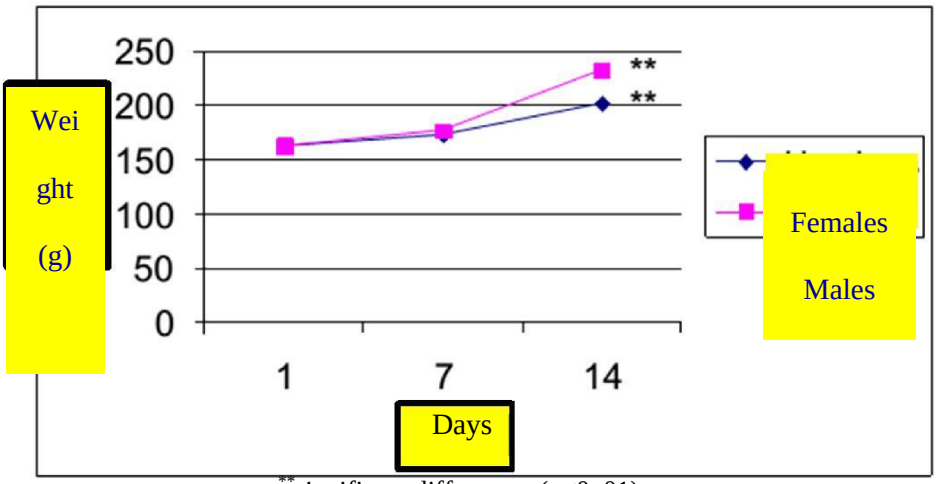


Fig.3 Variation of weight during the assay



** significant differences (p<0, 01).

Table.1 Parameters of total polyphenols determination

Lineal Equation	A = mC + n	R-Square: 0.99814
Pendent (m)	1.11 ± 0.02	Concentration of ethanolic extract (mg/mL)
Intercept (n)	0.008 ± 0.008	8.4 ± 0.1

Table.2 Total polyphenols concentration of flowers of Hibiscus according to Wong *et al.*, (2010) 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

Oral administration of gossypitrin no lethality was produced at the employed dosage (2000 mg/Kg) in the used animals. During the 14 days of the experiment the animals do not manifested toxically signs on their skins, eyes, mucosal membranes and none system taken into account. Not evidence of convulsion, diarrhea, salivation, lethargy, sleep and coma were observed. At macroscopic level non pathologic changes were observed after realize the autopsy of the animals.

Moreover, the animal non experimented retard in the growth and the increase of corporal weights was normal during all the time of assay. Significant differences (p<0, 1) were found (for both sex) in the weight between the first and the last day of the experiment and between the 7 and 14. All these results permit that the flavonoid classified as non-toxic. None significant differences (p>0, 01) were found between the increase of weight of the animals under assay according to the typical behavior inside the spice, sex and age (9) (10). Figure 3 show the variation of weight during the assay (1, 7 and 14 days).

For the first time determination of total polyphenols from an ethanolic extract of the petals of the flowers of *T. elatum* Sw. was developed. The rate 168 ± 1 mg EAG/g of dry extract is notably higher than those obtained with other species of *Hibiscus (tiliaceus, rosa-sinensis, taiwanensis, schizopetalus, mutabilis*), and closed to this

spice cultivated in Martinica (163 ± 5 mg GAE/g of dry extract). OAT (oral acute toxicity) of gossypitrin extracted and characterized from ethanolic extract of the petals demonstrated that the glucoside flavonoid tested is non-toxic at the unique dosage of 2000 mg/Kg of weight. The animals used in the assay no showed any kind of unusual symptoms in their behaviors and it was not necessary to do the histologic analysis because the observed organs were in good conditions.

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