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HPLC Analysis of the Total Extract and Gossypitrin Isolated from the Petals of *Taliparititi elatum* (S.w) Fryxell (Malvaceae)

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A B S T R A C T

Taliparititi elatum (S.w) Fryxell (Malvaceae) is a tropical Caribbean tree with a reported wide range of healing properties such as appetitive, emollient, sudorific, antasthmatic and excellent expectorant combined with the flowers of *Hibiscus rosa-sinensis*. In the present study, an extract from the petals of *T. elatum* collected in Cuba was analyzed to determine the chemical composition using an HPLC equipment with RP-18 flash chromatographic column, MeOH (A) and H_2O (B) as eluents and gossypitrin as standard. These results provide scientific support for the empirical use of *T. elatum* extracts as an antasthmatic medicine.

Introduction

Taliparititi elatum (S.w) Fryxell (Malvaceae) is a tree with a wide distribution in Cuba and Jamaica that grows in any type of soil, particularly in swampy ones, with a reported wide range of healing properties such as appetitive, emollient, sudorific, antasthmatic and excellent expectorant. It can get about 25 m of height. Its leaves are heart shaped at the basis and peciolated, with yellow flowers that change to red (Acosta and Rodríguez, 2006). In Cuba, this specie is known as Majagua, Majagua común and Majagua macho. In Jamaica it is known as Majó, Blue Mahoe, Cuba Bark and Mountain Mahoe. The mixture is used in traditional medicine as expectorant and antasthmatic (Roig, 1974).

There is an ever-increasing interest in the biological effects of the bioflavonoids, members of the large group of plant polyphenols. Because of the aromatic character of these compounds, they have been analyzed by several chromatographic methods. In the case of high-performance liquid chromatography, they are readily detected by their ultraviolet absorbance or electrochemical properties (Valls y col., 2009).

More evidence that the bioflavonoids undergo extensive metabolism during uptake from the gut and distribution around the body and in specific tissues is accumulating.

In addition, free radicals products at sites of inflammatory processes react with bioflavonoids and their metabolites, generating important new compounds of as yet unknown properties. For these reasons, careful examination of the chemical nature of bioflavonoids and their products in biological systems is absolutely required (Lambert y Elias, 2010; Ablajan, 2011).

Combination of mass spectrometry with the various chromatographic methods has proved to be highly successful in this regard (Prasain *et al*., 2004). A number of analytical techniques have been used to evaluate the metabolism and bioavailability of flavonoids in vitro and in vivo (Pinheiro y Justino, 2012). These methods include gas chromatography (GC), reverse-phase high-pressure liquid chromatography (HPLC), and capillary electrophoresis (CE) in combination with UV absorbance, fluorescence, electrochemical detection, and mass spectrometry (MS) (Vukics y Guttman, 2010). The aim of the present study was to evaluate the amount of chemical components of an extract elaborated from the petals of the flowers of *Taliparitielatum*.

Material and Methods

Plant material

Plant material (petals of the flowers) was collected in January 2013 in the gardens of the Faculty of Medical Sciences "Salvador Allende" at Havana City, and identified at the herbarium of National Botany Garden of Havana, where the voucher specimen no. **HAJB 82587** has been deposited.

Chemicals

All the chemicals and reagents were of analytical grade. MeOH, EtOH, $CHCl_3$ and HPLC grade methanol were purchased from Merck (Darmstadt, Germany). Reversed phase silica gel for flash chromatography, RP-18 chromatography column, and TLC silica gel with fluorescent indicator 254 nm on aluminium cards (layer thickness 0,2 mm) (10x20 cm) DC-KARTEN SI F and silica gel 60 for chromatographic column 0,063-0,200 mm (70-230 mesh ASTM) were purchased from Merck (Darmstadt, Germany). All materials were used without further purification.

Extract preparation

The petals were separated from the rest of the components of the flower and dried in an oven with controlled temperature, at 30 ^{0}C, during 4 days. The extracts were prepared with ethanol 95 %, during ten days, utilizing the maceration process. 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 aluminium cards (layer thickness 0,2 mm) (10x20 cm) using n-butanol: acetic acid: water (4:1:5) as eluent.

Isolation and HPLC determination of gossypitrin

Experiments were carried out on a KNAUER spectrometer (Germany) equipped with an UV detector at 280 nm of length wave. A portion (1 mL) of this extract was dissolved in 50 mL of MeOH and separated on a reversed phase flash chromatography column (C-18, 60 Å, 5 µm, 250 x 4 mm, Eurospher), using as eluent A MeOH and eluent B H_2O. A gradient of 15-85% B during 30 min at 1mL/min was

followed by holding the gradient, then increasing to 50 % A during 10 min, holding 10 min, reversing to 0 % B during 5 min, and reequilibrating for 5 min. The determination of gossypitrin (**G**: compound 1) was also carried out from the solid residue dissolved in 50 mL of MeOH, using the above described procedure.

TLC

TLC analysis of the extract and the solid yield, including gossypitrin, were performed on normal silica gel plates with fluorescent indicator 254 nm on aluminium cards (layer thickness 0.2 mm) (10x20 cm) using as eluent (n-butanol: acetic acid: water (4:1:5); developing agent was H_2SO_4/ $CeSO_4$ reagent and heat. TLCP (thin layer chromatography plate) were activated under 100~105 ^{0}C for 30 min and were examined under ultraviolet (254 nm) and ordinary light.

Semi-preparative HPLC

Compound 1 was isolated for further analytical and pharmacological studies adopting the already established method for the separation of phenolics and ellagic acid derivatives in *T. elatum* (Cuellar & González, 2010). Briefly, an HPLC-system consisting of two Knauer HPLC WellChrom K1001 pumps (Berlin, Germany), a Bischoff UV-VIS detector Lambda 1000 (Leonberg, Germany), and a dynamic mixing chamber was employed. The purified ethanolic extract (20 mg per injection) was loaded onto a Polaris Amide C18, 250 mm × 10.0 mm, 5 μm column (Varian) and eluted with 0.05% (v/v) aqueous TFA (A) and acetone (B) at room temperature using a two-step gradient (24% B to 44% B in 80 min and 44% B to 64% B in 30 min, 3 mL/min). All collected fractions were lyophilized. A yellow residue (7.8 g) was obtained after concentration under reduced pressure (40 °C) and a portion (2 g) was purified by semi-preparative High Pressure Liquid Chromatography, HPLC in order to obtain around 0.8 g of compound 1 (purity: 96 % by HPLC) and its structure was confirmed as **G** by UV and MS (Yaque *et al*., 2016).

UV spectrum

The UV spectrometric experiments were carried out on a JASCO ultraviolet-visible spectrometer (Japan). The scan range was 200 to 500 nm, absorbance 0 to 2.5, and the analyzed samples were diluted in methanol, into quartz cuvettes, comparing the obtained spectrum with the original spectrum of gossypitrin (Cuéllar & González, 2010). The cuvettes thickness was d = 1 cm.

Results and Discussion

TLC check

Chromatoplate presented three obvious yellow single spots, from each application, examined under ordinary light, UV_{254} and colorimetric detection with concentrated sulphuric acid (Fig.1), probably due to the presence of flavonoids.

Identification of main flavonoid by HPLC

The chromatographic analysis of total extracts from the petals of the flowers of *T. elatum* by HPLC allowed the identification of 48 different chemical constituents (Fig.2), including gossypitrin, the flavonoid glucoside present in the petals of the flowers of *Taliparitiy elatum* and in the solid residue.

The chromatographic analysis of 50 mL of total extract after elution in 50 mL of MeOH (Fig. 3) showed, at least, 29 different peaks

with a remarkable reduction in the number of chemical constituents.

The chromatographic analysis of solid residue (Fig. 4), showed the presence of at least 15 different chemical components, a quantity lesser than the previous result, indicating an effective isolation method.

After purification by semi-preparative HPLC column, chromatographic profile showed that compound G reduces its retention time, but still remain the presence of several chemical constituents in the sample (Fig. 5). The unequivocal identification of these constituents required isolation by semi-preparative HPLC and MS and NMR experiments.

Scan of the ultraviolet spectrum

There were three absorption peaks at 278 nm, 332 nm and 382 nm. There may be the presence of flavonoids in 95 % ethanol extracts (Fig. 6).

For the first time the results showed that the extracts and solid residue of *T. elatum* exhibited a high content of metabolites after HPLC analysis of the extracts on reversed phase flash chromatography column RP-18. The MeOH/H_2O and MeOH fractions revealed complex mixtures of chemical constituents as indicated by TLC and UV analysis.

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 H2SO4 and heat (Fig. 2). The Rf calculated values were 0,808 (1); 0,807 (2) and 0,780 (3), respectivally. 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 aluminium chloride (Lu *et al*., 2007).

HPLC of solid residue indicated the presence of 15 chemical components, and gossypitrin showed a retention time of 37.9 minutes (Fig.2), 37.6 minutes (Fig. 3) and 36.4 minutes (Fig.4), respectively. This result was corroborated when the corresponding peak of gossypitrin (R_t: 31.2 min) was collected after carry out on a KNAUER chromatographer with a semi-preparative column (Fig.5) and analysed on a JASCO UV spectrometer. The UV spectrum (Fig.6) showed that the flavonoid has three characteristics bands at 278, 332 and 382 nm, and one inflection at 257 nm (Márquez *et al*., 1999).

There is a big difference among these results and the results of the research on the same extraction utilizing a GC-MS technique because of the amount of chemical components. It was only of 16 for the crude extract of the petals using toluene and ethanol as eluents (González, 2007). It could be possible, due to the high sensitivity of HPLC analysis method compared with GC (Pinheiro & Justino, 2012), or the possibility because in the presence of more or less metabolites due to the difference in time of collection of the flowers of the plant (hour, week day, etc.). Maybe the difference in the growing area or growing conditions of *Taliparitі elatum* plantation (geographic zone, type of soil, humidity, nutrients, etc.), provoque differences in the dynamic accumulation of metabolites into the flowers.

Fig.1 TLC of the products isolated from the ethanolic extracts of *T. elatum*.

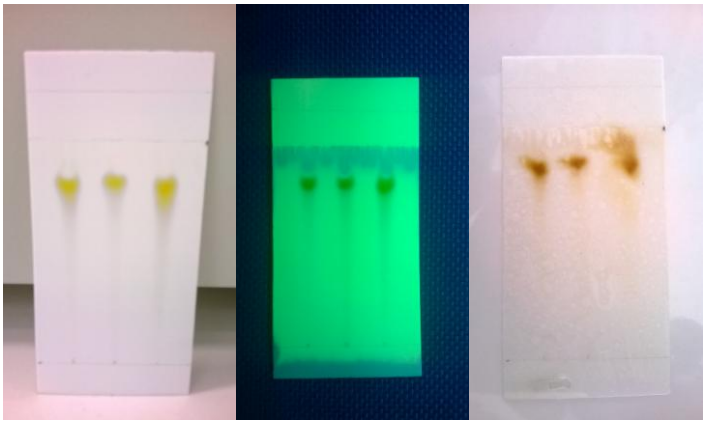


Fig.2 Chromatogram of the chemical constituents in the total extract from the petals of the flowers of *T. elatum*.

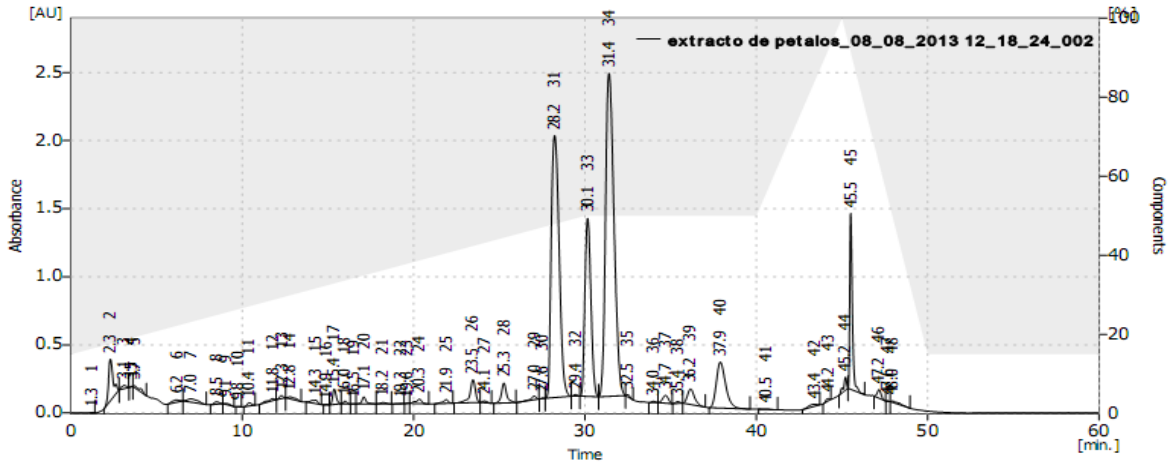


Fig.3 Chromatogram of the chemical constituents in the total extract from the petals of the flowers of *T. elatum* after elution on a reversed phase flash chromatographic column.

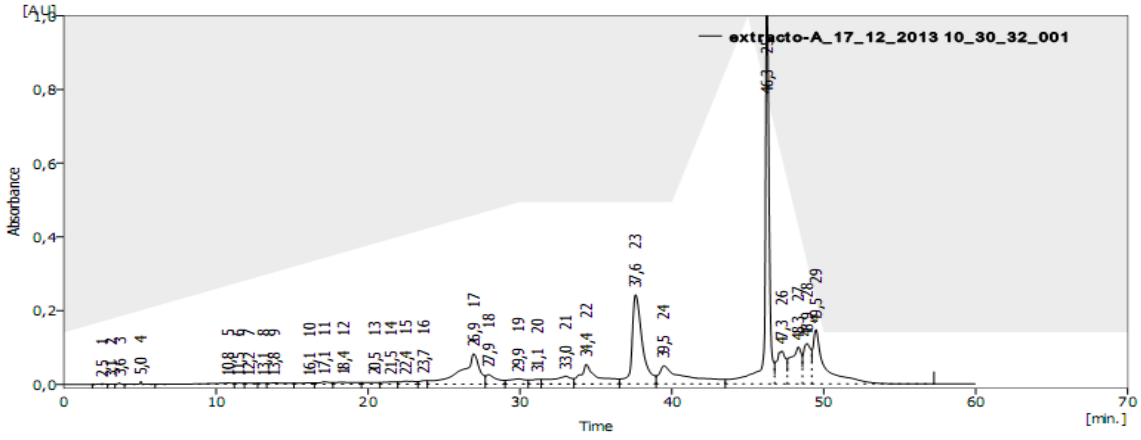


Fig.4 Chromatogram of the chemical constituents of solid residue from the petals of the flowers of *T. elatum*

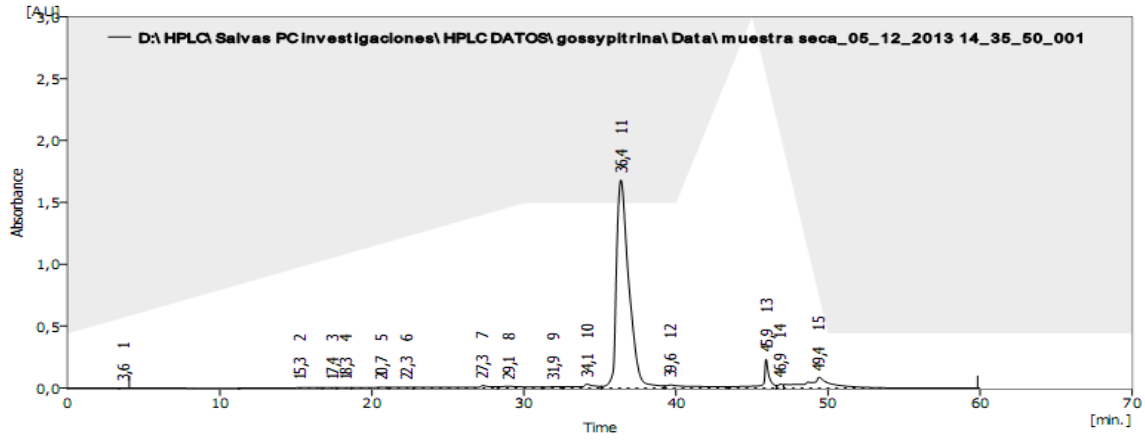
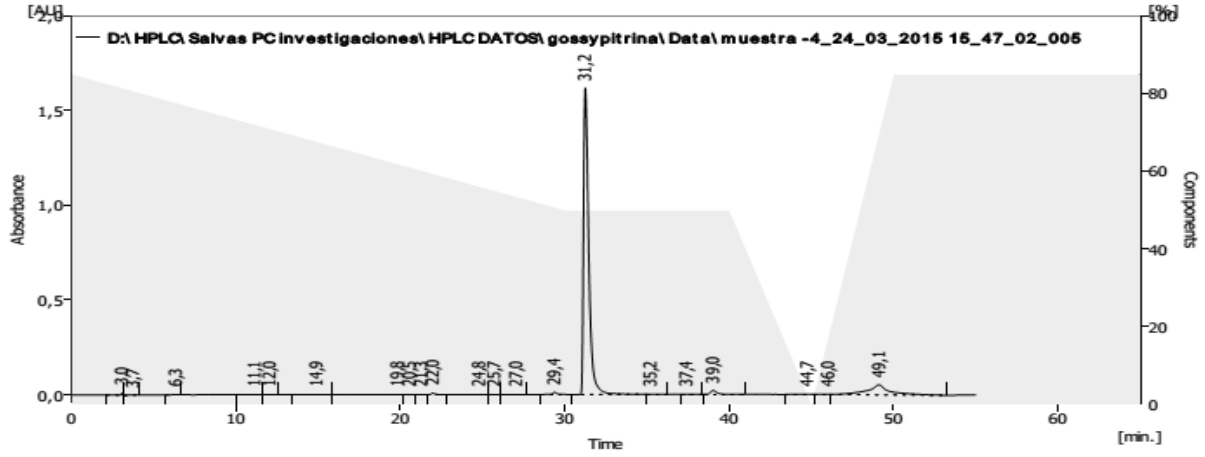


Fig.5 Chromatographic profile of the solid sample after purification by HPLC semipreparative column from the petals of *T.elatum*



Conclusions

The present study provides the most thorough qualitative investigation of flavonoids and its derivatives in *T. elatum* performed so far. HPLC, UV and TLC served for dereplication of several major flavonoids. This research showed that, *T.elatum* can be identified by characterizes profiles of flowers, specific chemical composition can be regarded as distinctive identification character. In identification of ultraviolet spectrum, there are three obvious absorption peaks. There are obvious spots on thin-layer chromatography. It provides reference basis for formulating quality standard of *T. elatum*, authenticity of medicinal flowers and resource utilization. The method enables the appropriate determination of such phenolics, which obviously contribute to the activity, for the quality control of the drug and of herbal medicinal products containing *T. elatum.*

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