Direct analysis by UHPLC-MS/MS of 8 purified fractions from ethanolic extracts of *Taliparitielatum's* flowers in Martinica

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Abstract— *From ethanolic extracts of the flowers of Taliparitielatum (Sw.) eigth different samples were isolated, purified and analyzed by UHPLC-MS/MS to determine the chemical constituents that they content. Each sample was separated and reinjected to get the most possible information about the chemical compounds that they posses. After an exhaustive analysis 8 different chemical components were tentatively identified according to their MS and literature data. Two compounds until remain unknown.*

Keywords— Taliparitielatum, ethanolic extract, flowers, UHPLC-MS, chemical components.

I. INTRODUCTION

Taliparitielatum Sw. (Fryxell) of the Malvaceae family is a tree native of Cuba and Jamaica. It is called « Majagua azul » in Cuba, « Mahot bleu » or « Mahot de montagne » in Martinique (US Dpt. of Ag., 2013). It produces beautiful orange to red flowers throughout the year (Figure 1). Domesticated then planted in almost all the islands and countries of the Caribbean basin, it is used for ornamental but also for medicinal purposes. Its flowers are widely used in the Cuban pharmacopoeia for coughs, asthma, catarrh, etc., (Roig, 1974; Acosta y Rodríguez, 2006) where two phytomedication"Imefasma" and"Flormaj" have already been manufactured and marketed in that country.

FIGURE 1: Flowers of *Taliparitielatum* (Sw.) from Cuba and Martinica

Despite widespread use in the Caribbean and undeniable pharmacological potential, there is very little information on its phytochemical constituents in the literature.The ARVARNAM and BIOSPHERES research groups with the University of Havana united in collaborative project therefore undertook to study this plant to characterize its secondary metabolites. The first analyzes by UPLC-DAD-ESI-MS/MS of the flower extract were therefore carried out and published in2017 where the structures of 12 chemical compounds were elucidatedafterisolation. The aim of this research was to analyze eight differentes samples to get information about the chemical components present in each one.

II. MATERIALS AND METHODS

2.1 Plant Material

Flowers were collected in January 2016 along the track road in Balata forest located in Martinica. A voucher specimen is deposited and registered in French Pharmacopeia as Fournet 1752 (4232 Guad). Martinica specimens are registered as *Hibiscus elatus* Sw.

2.2 Solvents

LCMS grade water (Merck), LCMS grade acetonitrile (Merck), LCMS analytical grade ethanol (Merck), and formic acid (Merck) were used in the analysis work. All solvents were degassing previously before used in an ultrasonic bath without filtration.

2.3 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 h. 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 (BAW 4:1:5) as eluent (v/v/v).

2.4 HPLC-DAD-ESI-MS/MS Procedures, Instrumentation, and Parameters

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 3 µm). Solvent systems: H_2O-0.1% Formic Acid (H_2O) and Acetonitrile-0.1% Formic Acid (Table 1).

TABLE 1
CHROMATOGRAPHIC CONDITIONS (GRADIENT) USED IN THE EXPERIMENTS

Time	Solvents	Gradient
0	H_2O:CH_3CN	80:20
5	H_2O:CH_3CN	80:20
10	H_2O:CH_3CN	0:100
25	H_2O:CH_3CN	0:100
30	H_2O:CH_3CN	80:20
34	H_2O:CH_3CN	80:20

Debit : 0.450 mL/min
UV Detection : 254nm

The HPLC was coupled to a Varian 500MS Mass Spectrometer equipped with an electrospray ionization chamber (ESI) used in negative mode at 5 KV at 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 2 parts when the flow rate used is greater than 500 µL/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. For the comparison of different powders: Column of 250 mm x ID 4.6 mm, particle 5 µm. The flow rate is 0.800 µL/min. For the product purification: 150 mm x ID 10 mm column, 5 µm particle. Flow rate: 5 mL/min.

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.

III. RESULTS AND DISCUSSION

Fifty-four peaks were detected here. Thirty temporary identifications, 26 of which were described for the first time in the ethanolic extract of *Taliparitiparitielatum* (Sw.). We mainly found organic acids and flavonoids there. Fragmentation spectra (MS^2) in negative mode were carried out for the structural elucidation of each of the compounds (Figure 2).

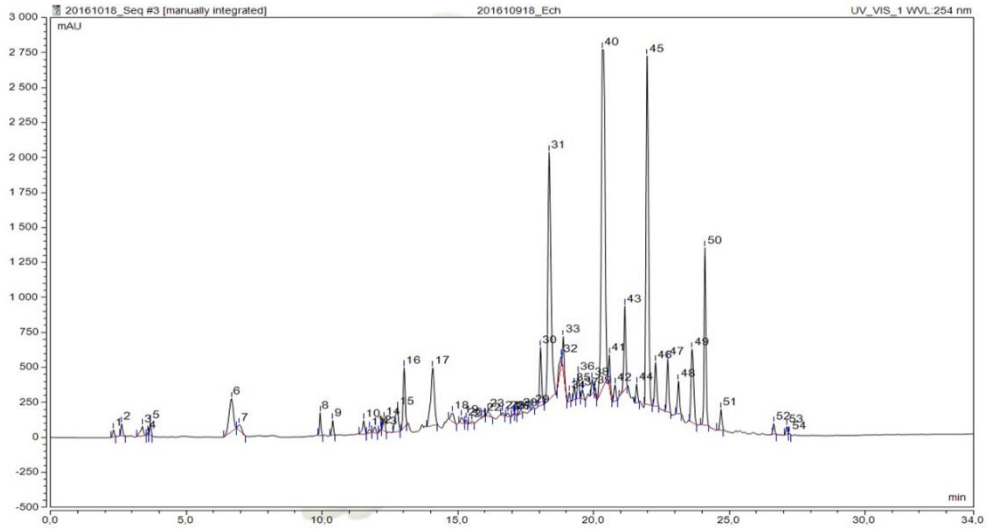

FIGURE 2: UV chromatogram of a hydroalcoholic extract of *Taliparititi elatum* (Sw.) flower recorded at 254 nm. 54 peaks were detected.

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.

The chromatographic peak "F1" was collected (Fig. A) by RP-UPLC. This fraction was harvested and reinjected (Fig. C). We note the presence of 2 peaks which correspond to 2 isomers of Dihydrobenzoic acid. The purification of this fraction therefore made it possible to obtain good purification. It is noted that Dihydrobenzoic acid is an intermediate metabolite of degradation of flavonoids according to Bhinu et al., 2002 (Figure 3).

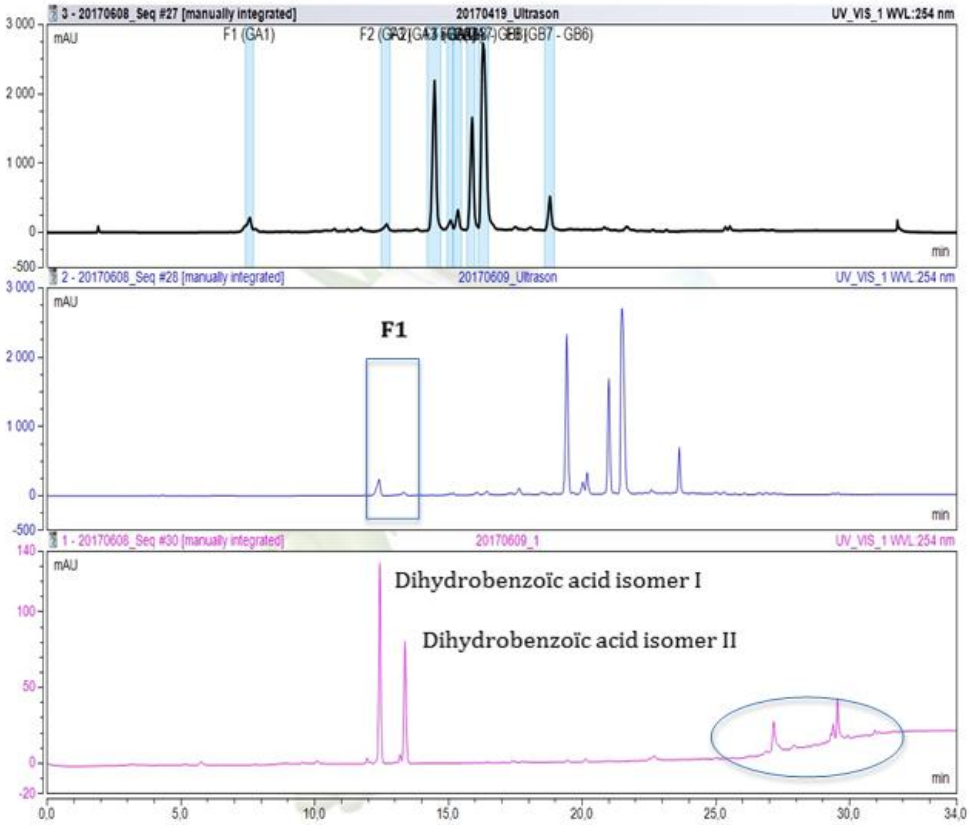

FIGURE 3: Current chromatogram of fraction "F1" isolated and reinjected by UPLC.

The chromatographic peak "F2" was collected (Fig. A) by RP-UPLC. This fraction was harvested and reinjected (Fig. C). Very surprisingly, we have no trace of the targeted chromatographic peak during the purification. Several peaks are detected and in particular the peak of the F3 fraction corresponding to the peak of quercetin-*O*-sambubioside. We also observe the presence of the isomers of dihydrobenzoic acid. Contamination by the F3 fraction and post-harvest degradation are possible (Figure 4).

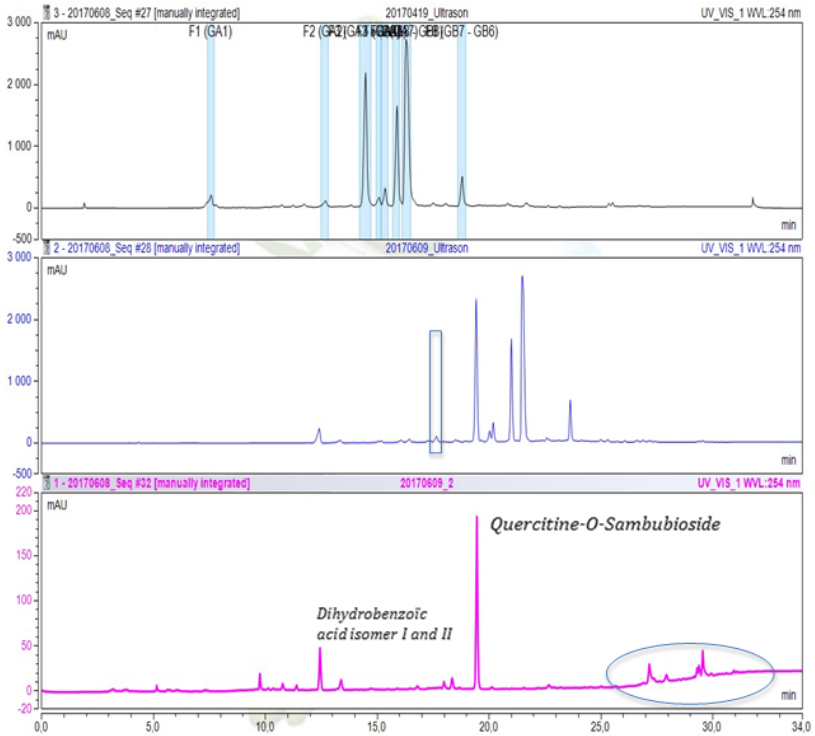

FIGURE 4: Current chromatogram of fraction "F2" isolated and reinjected by UPLC

The chromatographic peak "F3" was collected (Fig. A) by RP-UPLC. This fraction was harvested and reinjected (Fig. C). We note the presence of a single majority peak which corresponds to Quercetin-*O*-sambubioside (Mass spectrometry data not shown here). As we would expect, we mostly got the targeted chromatographic peak during the purification (Figure 5).

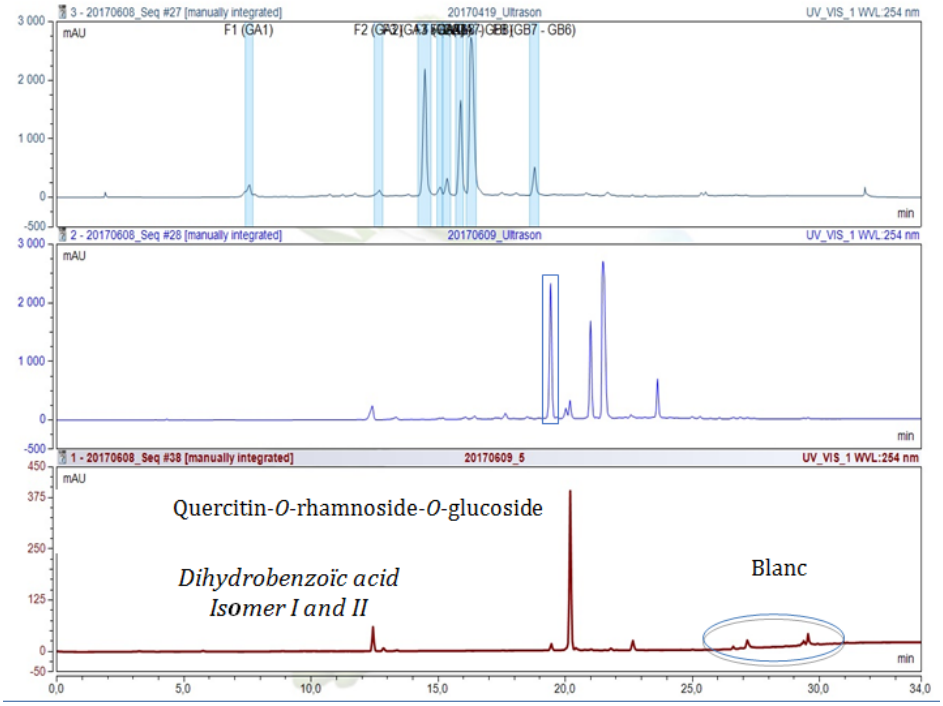

FIGURE 5. Current chromatogram of fraction "F3" isolated and reinjected by UPLC.

The chromatographic peak "F4" was collected (Fig. A) by RP-UPLC. This fraction was harvested and reinjected (Fig. C). Very surprisingly, the targeted chromatographic peak during the purification is present, but it is far from being the majority peak. Several peaks are detected and in particular the peak of the F4 fraction corresponding to the peak of quercetin-*O*-rhamnoside-*O*-glucoside.Analysis of mass spectrometry data did not allow the identification of the product that was detected before the biggest peak near the 20 min of retention time.Contamination by the F5 fraction and post-harvest degradation are possible (Figure 6).

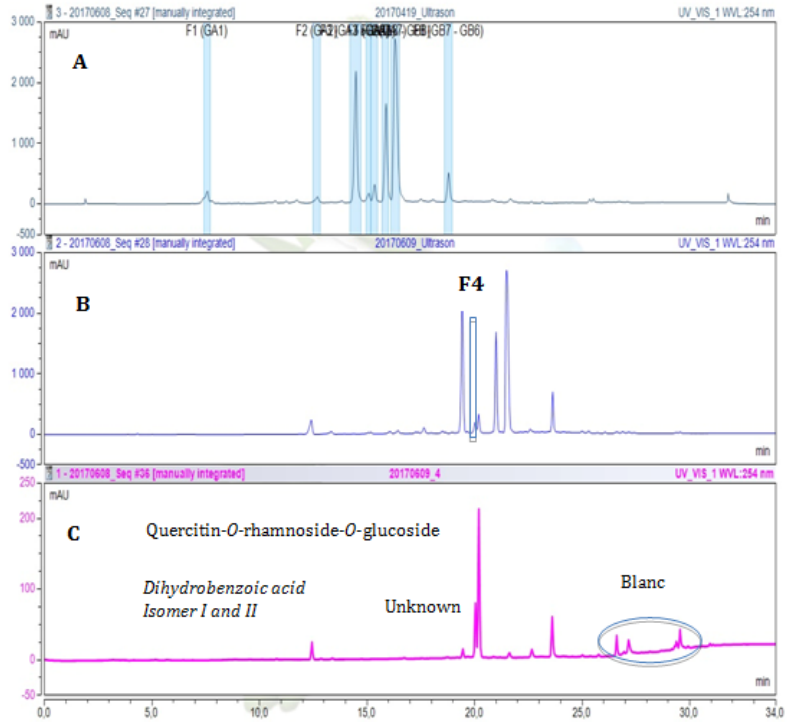

FIGURE 6: Current chromatogram of fraction "F4" isolated and reinjected by UPLC

The chromatographic peak "F5" was collected (Fig. A) by RP-UPLC. This fraction was harvested and reinjected (Fig. C). We note the presence of a single majority peak which corresponds to Quercitin-*O*-rhamnoside-*O*-glucoside (Mass spectrometry data not shown here). As we would expect, we mostly got the targeted chromatographic peak during the purification (Figure 7).

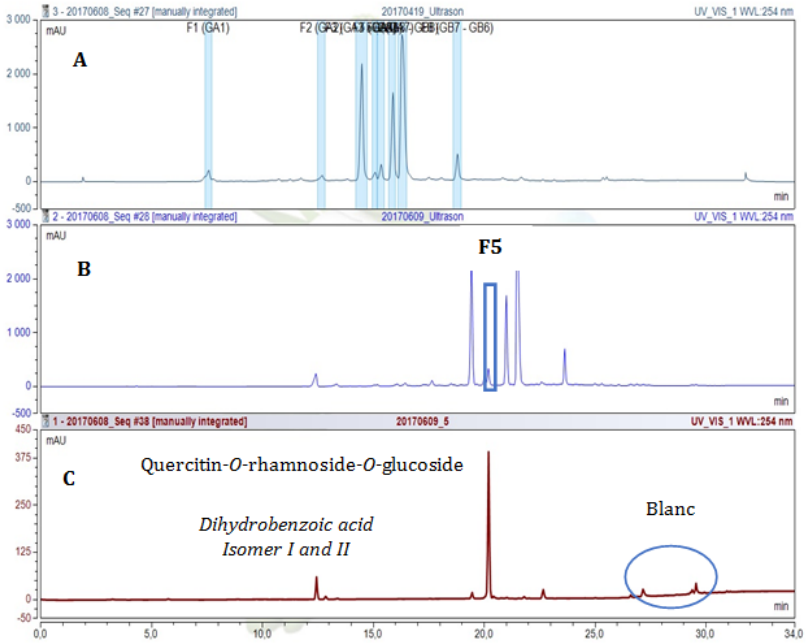

FIGURE 7: Current chromatogram of fraction "F5" isolated and reinjected by UPLC

The chromatographic peak "F6" was collected by RP-UPLC (Fig. A). This fraction was harvested and reinjected (Fig. C). We note the presence of a single major peak which corresponds to Quercitin-*O*-glucoside (Mass spectrometry data not shown here). As we would expect, we mostly got the targeted chromatographic peak during the purification (Figure 8).

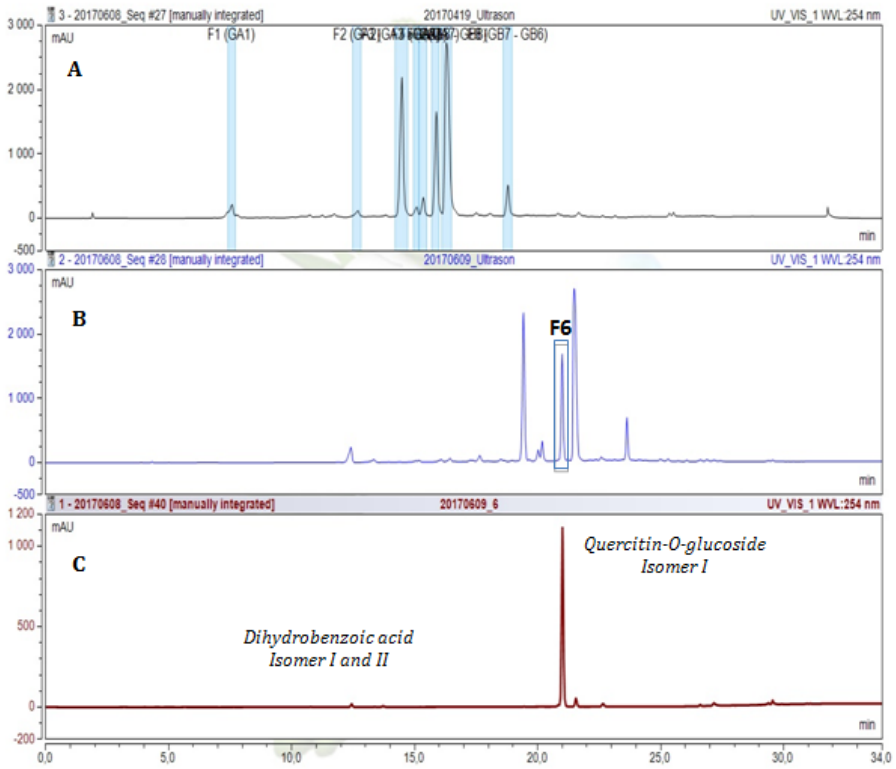

FIGURE 8: Current chromatogram of fraction "F6" isolated and reinjected by UPLC

The chromatographic peak "F7" was collected (Fig. A) by RP-UPLC. This fraction was harvested and reinjected (Fig. C). We note the presence of a single majority peak which corresponds to Gossypetin-*O*-glucoside (Mass spectrometry data not shown here). As we would expect, we mostly got the targeted chromatographic peak during the purification (Figure 9)

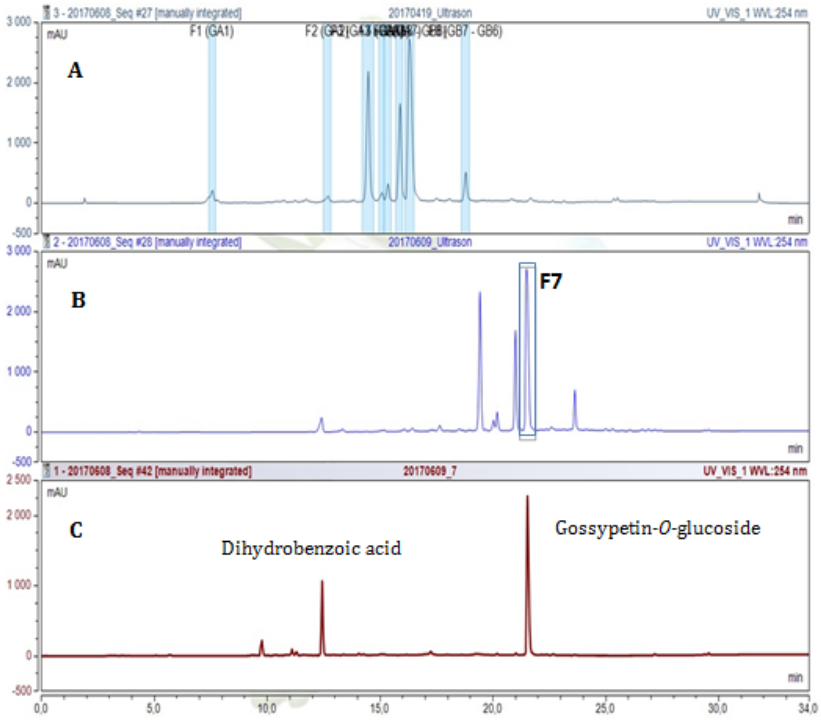

FIGURE 9: Current chromatogram of fraction "F7" isolated and reinjected by UPLC

The chromatographic peak "F8" was collected (Fig. 10 A) by RP-UPLC. This fraction was harvested and reinjected (Fig. 10 C). We note the presence of three majority peaks: Quercitin-*O*-glucoside, dihydrobenzoic acid and dihydroxyphenyl propionic acid (Mass spectrometry data not shown here). A surprising result knowing that we could expect a single majority peak. The most likely hypothesis here is post-harvest degradation since dihydroxybenzoic acid and dihydroxyphenyl propionic acid are intermediates in the degradation of Flavonoids (Bhinu et al., 2002).

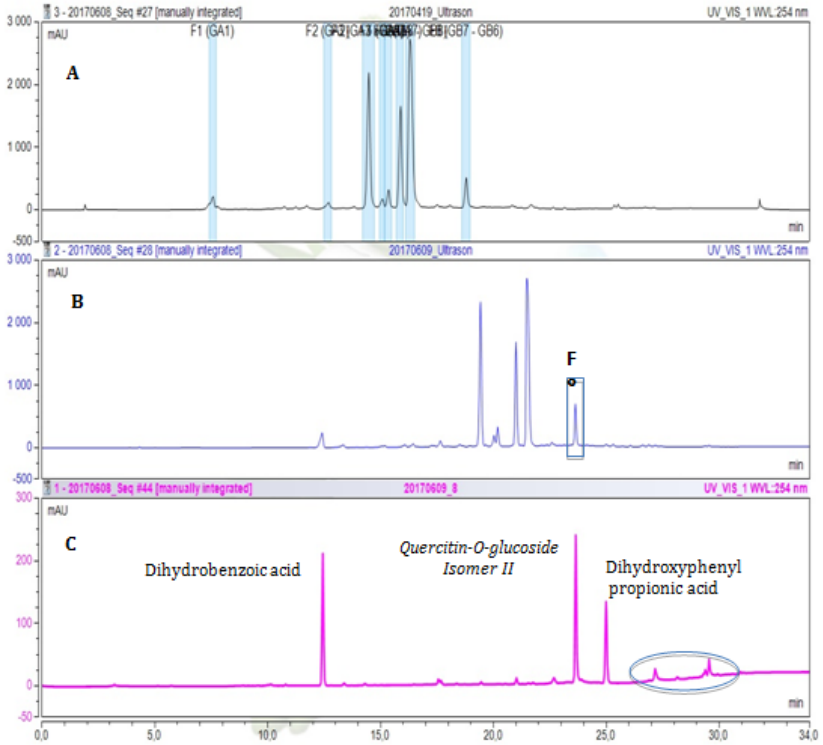

FIGURE 10: Current chromatogram of fraction "F8" isolated and reinjected by UPLC

Summarizing, the presence of three different kinds of organic acids were detected in ethanolic extracts of the petals from the flowers of *T. elatum* (Sw.) in Martinica, taking into account that dihydrobenzoic acid was detected presumably in two isomeric forms from fraction 1 to 6, while in fractions 7 and 8 this chemical component was found as a single peak. Another one was the dihydroxyphenyl propionic acid. Five different kinds of flavonoids were detected in the fractions, such as, quercitin-*O*-sambubioside, quercetin-*O*-rhamnoside-*O*-glucoside, quercetin-*O*-glucoside (in two isomeric forms), and gossypetin-O-glucoside.

Until now, we have not evidence of the presence of those chemical components in the petals of the flower of *T. elatum* (Sw.) in Martinica, for that reason our research team is proposing for the first time the mentioned chemical compounds into ethanolic extracts of this flower using UPLC-MS/MS.

Is notably that perhaps we are in the presence of two new flavonoids as phytochemical components of the petals in this specie that belongs to gossypetin derivatives: gossypetin-8-O-glucoside or gossypin, previously reported in the flowers of *Hibiscus sabdariffa* (Visweswara and Seshadri, 1946) and *Hibiscus vitifolius* (Subramanian and Nair, 1972) and gossypetin-3-*O*-glucoside or gossytrin, reported in the flowers of *Hibiscus sabdariffa* (Seshadri and Thakur, 1961) and *Hibiscus tiliaceum* (Nair et al., 1961). Both chemical compounds have the same chemical formula ($C_{21}H_{20}O_{13}$) and the same molecular mass (480u). They differ only in the position of the glucose moiety.

Quercetin-*O*-glucoside, quercetin-*O*-sambubioside, and quercetin-*O*-rhamnoside-*O*-glucoside were reported for the first time by our research team in 2017, but in the extracts of the flowers of *T. elatum* in Cuba (González et al., 2017). Those chemical compounds were previously reported in the petals and calyces of *H. sabdariffa* (Inmaculada et al., 2009; Beltran-Debon et al., 2010).

Both isomers of quercetin-*O*-glucoside could be attributed to isoquercitrin (quercetin-3-*O*-glucose) and hiperoside (quercetin-3-O-galactose) according to its molecular masses (464u) previously reported in the petals of the flowers of *T.*

elatum in Cuba by our research team in 2016 (Yaque, J.G. et al., 2016) and reported in two *Peumo* trees in Chile (Simirgiotis, 2013).

IV. CONCLUSIONS

A simple and versatile analytical method, the "UPLC-DAD-ESI-MS / MS" was implemented to allow direct identification of the constituents inhydroalcoholic extract of the flower petals of *Taliparitielatum* (Sw.). The analysis was carried out by RP HPLC coupled to a DAD detector and to a tandem ion trap mass spectrometer in order to obtain a UV profile and a spectrum of fragmentations in negative mode making it possible to achieve provisional identification. This study was also accompanied by the analysis of the extraction precipitate and the first attempts to purify the majority constituents of the extract. Eights compounds have thus been tentatively identified, all of them reported for the first time in the ethanolic extracts of this part of the plant. Thus, it has been demonstrated that gossypetin-*O*-glucoside does not precipitate alone and that the use of UPLC-DAD for preparatory purposes is promising. We were able to isolate four major products including gossypetin-*O*-glucoside.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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