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Research Article

NMR ^{1}H Confirmation of Gossypetin-3'-O-Glucoside in *Talipariti elatum* (Sw.) Malvaceae

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Abstract

From red petals of the flowers of *Talipariti elatum* (Sw.) a flavonol glucoside was isolated and characterized by NMR ^{1}H spectroscopy. Structure analysis of that chemical component revealed that It have the identical glucoside moiety attached to a flavonol skeleton like gossypitrin (gossypetin-7-O-ß-glucoside) but in different position for which the structure of gossypetin-3'-O-glucoside was deduced using the H and OH correlations. Therefore, our observations suggest that both samples of the purified solids contains only one flavonoid glucoside instead the two probably isomers that must be present in the petals after their extraction with etanol at 95%.

Keywords: *Talipariti elatum*; Flowers; Flavonoid; NMR Spectroscopy; Characterization

Introduction

The West Indies are considered one of the biodiversity hotspots with high priority for conservation in the World due to its biological richness and the fragility of the ecosystem [1,2]. In 2008, Acevedo- Rodríguez and Strong estimated for the first time the total percentage of endemism exhibited by the seed plants of this region to be nearly 72%. Endemism is tallied by island in the case of Cuba, Hispaniola, and Jamaica; as biotic region in the case of Puerto Rico and the Virgin Islands; or as an archipelago in the case of Bahamas and the Lesser Antilles [3].

The West Indies contain a total of 208 families of seed plants of which only 183 are indigenous to the region. There are no endemic families of seed plants in the West Indies, and the ten most species-rich families are contributing to nearly 60% of the native taxa of the region. Among the countries that have the biggest amount of plant families, number of genera, percent of generic endemism, total taxa, native taxa and percent of endemic taxa, Cuba is the most important contributor [4].

Malvaceae is one of the Ten Most Diverse Families of Seed Plants Native to the West Indies. That family have 31 native genera and among them, 2 endemic genera [5]. *Talipariti elatum* Sw. (Fryxell) is a spice considered a member of the Talipariti gender; ancient known as Hibiscus, but since 2007 was reintroduced in this new gender because of different characteristics that justified its separation from Hibiscus. Those characteristics were: arborescent habit, prominent stipules, coriaceous foliar lamina, margin majoritarian entire, capsule 10-locular and relatively higher chromosomal number (2n = ca. 80, 90, ca. 92, ca. 96 and 120) [6].

In wetter areas in the islands of Cuba, Jamaica, US Virgin Islands, Puerto Rico and Martinica there are extensive population of a tree named *Talipariti elatum* Sw. (Malvaceae) growing in a wide range of elevations, up to 1200 meters (3900 Ft.) and is often used in reforestation. The 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 [7].

The flowers of this medicinal tree are used by Cuban population as cataplasm and infusions mixed with sugar cane or honey bee [8]. The main flavonoid glucoside extracted, isolated, and purified from the petals of the flowers named gossypitrin, has been have been first isolated from cotton flowers (*Gossypium herbaceum*) [9] and later the compound was detected in different *Equisetum* species [10,11]. Gossypitrin was also identified in yellow petals of *Papaver nudicaule* and flowers of *Talipariti elatum* [12,13].

Drosera peltata (shield sundew), a species distributed in India and Southeast Asia, was found to contain both herbacitrin and gossypitrin; this plant is used as an antitussive in the phytotherapy [14]. The antibacterial and antifungal activities of gossypitrin were recently demonstrated against a series of microorganisms, and gossypitrin showed a potent intrinsic antioxidant capacity evidenced by low IC_{50} and EC_{50} values for DPPH/ABTS/malondialdehyde and ferric reducing power, respectively. Pre-treatment of PC_{12} cells with gossypitrin, significantly increased their survival against KCN, restored the levels of GSH and the SOD and CAT enzymes activities, as well as reduced the level of lipid peroxidation. Its antioxidant effects were higher than those elicited by rutin [13,15].

The purpose of this investigation was to elucidate the structure of the main constituent found of two solid samples precipitated on hydroethanolic extract from *T. elatum* by 1H NMR experiments.

Material and Methods

Plant material

Flowers were collected in January 2018 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.

Solvents

DMSO-*d6* analytical grade (Merck), analytical grade ethanol (Merck), analytical grade methanol (Merck) was 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 40ºC, during 5 days and at room temperature, during 10 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 ºC and 500 mbar. For to the purification, 1 g 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).

NMR ^{1}H Procedures, Instrumentation and Parameters

NMR spectra were recorded in DMSO-d6 on a Bruker Avance JCAMP-DX 500 spectrometer at 400 MHz (^{1}H); the signals of the deuterated solvent were taken as reference. The assignment of signals values that corresponding to each proton or hydroxyl chemical shift was established using the MestReNova LITE Program.

Results and Discussion

After isolation and purification the samples were submitted to an NMR analysis using the described conditions previously. Only the NMR ^{1}H was taken in this case. Both samples showed the same signals in general, with different intensities, being higher in the first sample (dried at 400C in an oven) than the second one (dried at room temperature) (Figure 1).

Figure 1: (a, b). NMR 1H of both solid samples analyzed.

The first one (a) showed a supplementary signal at 3.18 ppm, but this signal at 3.182 ppm belongs to the internal standard of reference (DMSO-d6) together with the intense signal at 2.52-2.53 ppm when the DMSO is partially deuterated or not deuterated, according to Markham and Andersen in 2001 [16] (Figure 2).

Figure 2: Magnified zone between 2.31 and 3.34 ppm related with MSO.

Figure 3 shows the magnified zone related with the part of spectrum where the protons resonated in the characteristic zone of glycosylated flavonoid compounds indicates a glucose moiety between 3.34 and 3.84 ppm resonances were in accordance with β-glucopyranoside confirmed by the presence of 6 carbons sp3 (5 CH and 1 CH2) where the protons at δ = 4.83 ppm (d, 1H, J = 7.32 Hz, 1''-H), δ = 3.76 ppm (d, 1H, J = 11.96 Hz, 6''-H), δ = 3.59 ppm (dd, 1H, J = 11.96 Hz, J = 4.10 Hz, 6''-H) δ = 3.44-3.34 ppm (m, 4H, 2'', 3'', 4'', 5''-H) resonated in the characteristic zone of glycosylated flavonoid compounds [17].

Figure 3: Magnified zone between 3.34 and 3.84 related with glucose moiety.

Carbon 1'' resonated unequivocally at 4.84 ppm, while the superposition of both spectrums showed the resonance of carbon 7 was between 10.39 and 10.40, respectively as is shown in Figure 4.

Figure 4: Magnified zone around 10.40 ppm that corresponding to C7.

This result confirmed that the ring B substitution is in 3' position. Therefore, the substance is consequently determined to be gossypetin-3'-O-β-glucopyranoside and not its isomeric form gossypitrin, due to the last mentioned compound not shows a signal at that position. Carbon 6 resonated here at 6.27 ppm in both spectrums, while our research team found out in previous investigation the same signal resonance at 6.26 and 6.25 ppm, respectively [18,19].

Finally, the observed resonance of the chemical shift value of proton 6-H (δ = 6.27 ppm) confirmed the presence of Hydroxyquinol (ring A) [16,20]. The corresponding OH group that belongs to C3 and C4' resonated at 9.36 ppm, but when this peak is magnified, both signal appear separated, resonating at 9.378 and 9.365, respectively (Figure 5).

Figure 5: NMR ^{1}H spectrum of gossypetin-3'-O-glucoside from *T. elatum* Sw.

Signal at 10.39 ppm (C-7) disappear in the spectrum 1H NMR of gossypitrin, while in 1H NMR spectrum of gossypetin-3'-O-glucoside the corresponding signal at 3'-C (9.34 ppm) disappears too [14,21]. Gossypitrin showed the H-C3' signal at 6.93 ppm according with Hunyadi., *et al.* 2019 when they used this flavonoid glucoside (purchased from Atomax Chemicals Co., Ltd. (Shenzhen, Guangdong, China) purity>90%) to investigate its antiretroviral activity in comparison with quercetin, a well-studied abundant flavonol. After running that sample using ^{13}CNMR the C3' signal value was 146.3 ppm, while the same carbon atom in gossypetin and gossypitrin were 144.85, 145.0, and 145.4 ppm, respectively [14,20,22].

Conclusions

To the best of our knowledge, this report is the confirmation of the presence of gossypetin-3'-O-glucoside in the ethanolic extracts of the petals of the flowers of this spice. As such determination was done using the solid samples isolated from the extracts only one of the isomers was characterized by this method. The result reveal that it was possible, probably, due to the total separation of both molecules in the purification process after their recrystallization at least twice time by porous filter # 3. Perhaps this methodology leads to separate fully both chemical components. Further LC-NMR or X-Ray Crystallography should be necessary to get better information in this matter.

Conflict of Interest

The authors declare that they have no conficts of interest.

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