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Pharmacognostic, Physicochemical and Phytochemical Analysis of Fruits of *Taliparitielatum* (Sw.) in Cuba

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Abstract

Taliparitielatum (Sw.) Fryxell is an endemic tree from Cuba, also known as "Majagua", and has traditionally been used to improve asthmatic crisis and flu, as expectorant, appetitive, emollient, sudorific and laxative. Used as ornamental in park and avenues, its wood is used to make furnitures and baseball baths. In order to offer aspects related to the quality and effectiveness of the plant, its pharmacognostic, physicochemical, and phytochemical parameters of the fruits was realized. Moisture content (13.47%), alcohol extractive value at 95% (4.07%) and water extractive value (7.36%), total ash (3.58%), water soluble ash (0.70%) and acid insoluble ash value (1.56%) were tested for. Phytochemical screening revealed the possible presence of flavonoids, alkaloids, tannins, anthocyanidins, reductants sugars and astringents and/or bitter principles, and the absence of coumarins, saponins, quinones, cardiotonic glycosides and triterpens and/or steroids.

Subject Areas

Pharmacology, Plant Science

Keywords

Taliparitielatum, Pharmacognostic, Physicochemical Parameters, Phytochemical Screening, GC-MS

1. Introduction

Taliparitielatum is a tree of ≤25 m of height, and with a trunk of ≤1 m of di-

ameter; bark is grayish in color and cracked. The plant is the national tree of Jamaica, and was introduced in a lot of parts of the Caribbean. The tree is present in almost all zones of Cuba, growing in forest between 0 - 1200 masl. *T. elatum* 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. 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 according to [1].

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 Martinica Island by maceration with methanol (24 h), and Soxhlet extraction with methanol, ethyl acetate and 1,2-dimethoxyethane as solvents as discussed in [2] [3]. The last mentioned compound was identified by Cuban and Martinicans researcher in 2016 using UV, IR, HPLC-MS and NMR spectroscopy after its isolation and purification from ethanolic extracts at 95% by Soxhlet extraction of the red petals of the flowers as referred in [4].

Figure 1 shows the macromorphological characteristics of the fruits. Fruits are dehiscent capsules, globular or ovoid, 5(-10)-lobulated. Several seeds in each locale, reform, subglasses, pubescent's or tomentoses. Capsules are ovoid, from 2 - 4.2 mm long, with simple and long trichomes, antors and small start trichomes. Seeds have kidney form, 4 - 5 mm long, and tomentoses with brown-yellowish trichomes taking into account the results in [5]. The aim of this research was to establish the pharmacognostic and phytochemical control methods for the fruits of this plant that grows in Cuba and Martinica.

2. Materials and Methods

2.1. Plant Material and Reagents

Fruits were collected in June 2018 in the gardens of the Faculty of Pharmacy and

Figure 1. Morphological details of fruits of *T. elatum*.

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 fruits were collected after their mature and dried at shadow on the ground around the trees and kept into the nylon bag at room temperature. All reagents used were of analytical grade (Merck). All solvents were degassed prior to use in an ultrasonic bath without filtration.

2.2. Pharmacognostic Analysis

2.2.1. Macroscopic and Organoleptic Studies

Twenty fruits were examined for morphological characters including size, shape, color, odor, taste, and extra features. The macro-morphological characteristic of the fruits were observed under the magnifying lens (10×). Measurements were carried out using line ruler and a Stereoscopic microscope NTB-2B with camera model HDCE-50B (China).

2.2.2. Microscopic Studies

Dried fruits were ground to coarse powder and packed, for microscopic identification, in a suitable container. The sample was powdered by hand using a meet mill. Microscopic analysis was carried out on the powdered sample using a light microscope NOVEL (China) with 10× microscope objective lens, and coupled toHDCE-50B digital camera (China) and Scope Image Dynamic Pro software. Ground powder was cleared for some minutes in sodium hypochlorite solution. It was washed in water and then coloured with saffranin at 1% and stained in glycerinated gelatin according to Gattuso M and Gattuso S, 1999 [6].

2.2.3. Physicochemical Parameters of the Fruits Powder

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 and Miranda y Cuéllar [7] [8].

2.2.4. Phytochemical Studies

The chemical constituents were screened according to Chhabra [9] and Miranda y Cuéllar [8] to ascertain the presence of chemical components in diethyl ether, ethanol and water, respectively.

2.2.5. Extracts Preparation

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 according to [10].

2.2.6. Physicochemical Parameters of the Extract

The extracts were prepared with the ground material getting the physico-chemical parameters like organoleptic properties (odor and color), pH, refraction index, relative density and total solids as discussed in [8] [11].

2.2.7. TLC and Capillary Analysis

TLCP (thin-layer chromatography plate) 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 65:25:10) as developing agent (v/v/v), concentrated sulfuric acid plus heat. The TLCP were examined under ultraviolet (254 nm and 365 nm) and ordinary light. Vanillin at 1%, rutin (R), quercetin (Q) (Merck) and gossypitrin (G) were used as standard.

Capillary analysis was developed according to NRSP 312 and Miranda y Cuéllar, using 20 mL of the extract and Whatman paper #1 (4 cm × 20 cm). The experiment was carry on during 2 hours in a dark chamber with a temperature of 25˚C (±2), taking into account the height, and description of the parts and possible changes under ammonia vapors.

2.2.8. Determination of Total Phenolic and Total Flavonoid Contents

Total phenols were calculated by the Folin-Ciocalteu method, using as reference the gallic acid (Sigma-Aldrich) at concentrations of 10, 20, 30, 40 and 50 mg/mL as referred by Chlopicka *et al.*, 2012 [12]. On the other hands, the content of total flavonoids was carried out by the colorimetric method according to Pourmorad *et al.*, 2006 [13], using aluminum trichloride and quercetin (Sigma-Aldrich) as reference substance at the concentrations of 10, 15, 25, 50 and 100 µg/mL. In each case, calibration lineal curve was constructed with absorbance read in a spectrophotometer Rayleigh UV-1601 (China) at 715 nm vs. concentration of reference compound, which was then obtained respective concentration of total content of phenol or flavonoids and SD in the studied extract (mg/mL).

2.2.9. GC-MS Procedures, Instrumentation and Parameters

The samples were subjected to chromatographic analysis in equipment GC/MS, brand Shimadzu QP2010, equipped with a splitter split/splitless. With a BP5 (30 m × 0.25 mm × 0.25 microns) capillary column under the following chromatographic conditions: Helium gas carrier obtained by electron impact fragments to a power of 70 eV rate of 1.2 mL/min, 1:50 split flow and the volume of injected sample of 1 µL. Programmed oven temperature: initial temperature was 70˚C with a heating ramp of 10˚C/min to 300˚C and remained stable at this temperature for 10 minutes. Subsequently the temperature was increased at a rate of 10˚C/minute to 300˚C for a total time of 78 minutes with an injector temperature 250˚C and the interface temperature 300˚C. The compounds were analyzed using GC/MS NIST21 and NIST107 library and having into account the results obtained after phytochemical screening according with González *et al.*, 2018 [14]. Silylation agent was N,O-bis (trimethylsilyl) trifluoroacetamide (**BSTFA**), CAS 25561-30-2, Lot: 0901-1 Macherey-Nagel GmbH & C. KG.

2.2.10. Statistical Analysis

Results are presented as mean ± SD. Statistical analyses were performed by Student's t-test. The values of $p < 0.05$ were considered significant. Duncan test was used utilizing the Statgraphics® Plus, version 5.0 program. The mean effective

concentration (IC_{50}) was determined with the help of the Graphprism 5.0 statistical program.

3. Results and Discussion

Botanical Characteristics

1) Macroscopical Description

Figure 2 shows that fruits have globular or ovoid form, 5(-10)-lobulated. The color varies from light green to dark green. After measured, the wide average was 2.03 cm, 2.9 cm long and peduncle 3.39 cm long. High average was 2.03 cm. The average weight is around 2.97 g. The capsule surface was covered with smooth hairs. Its bloomed was at June-July, and fructified at July-August. It smelled slightly aromatic and tasted slightly pungent and bitter. Each lobule has inside a lot of dark brown seeds with kidney form. Bracteoles are dark green in color.

The powder drug is reddish brown in color, and displays a characteristic flavor and smell with a lot of dark brown seeds with kidney form as shown in Figure 3.

2) Microscopical Description

Analyzed individually, fragmented drug shows the anatomical features in Figure 4. Sclerenchymatic isolated fibers (a), filliform fibers (b), abundant trichomes with star shape (c, d), xylematic spiral vessel grossed (e), calcium-oxalate in druse form (f), fibers of the sclerides type of the endocarp (g) and fundamental parenchyma with hexagonal cells (h) according to [15] [16] [17] [18]. This is the first time that the micromorphological characteristics of fruits from *T. elatum* are described, as well as the powder drug in our country.

3) Physicochemical Characteristics

Moisture content (13.47%) was inside the limited index (8% - 14%). Extractable matter in ethanol at 95% (4.07%) and in water (7.36%) suggesting that the chemical constituents are more soluble in water giving and idea that the drug

Figure 2. Macromorphological characteristics of the fruits.

Figure 3. Powder drug of fruits from *T. elatum* Sw.

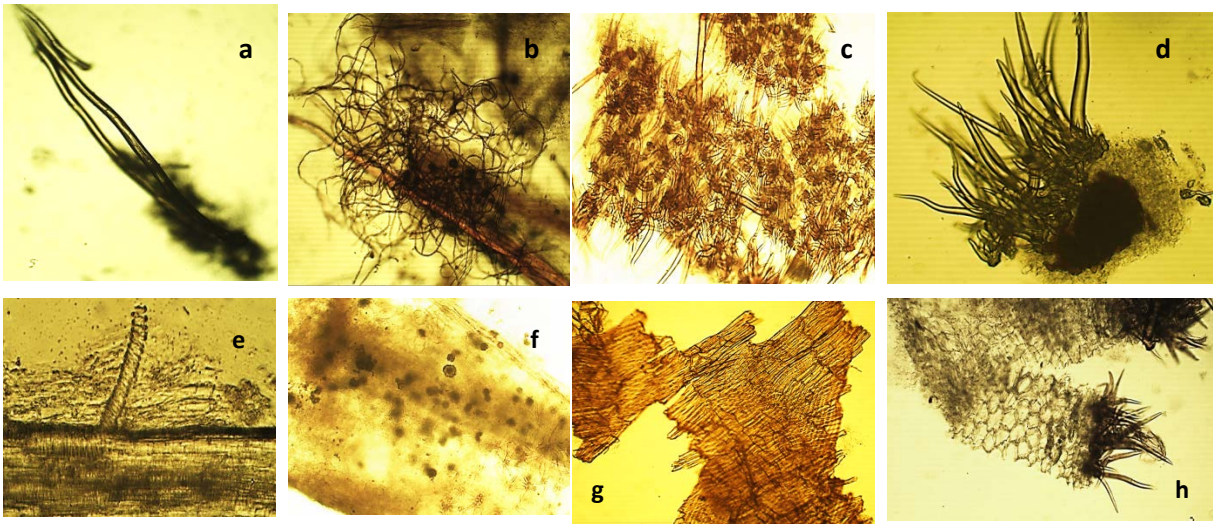

Figure 4. Micromorpholical characteristics of fruits from *T. elatum* Sw.

have more amount of polar components. Total ash (3.58%) is according to the standard allowed (3% - 5%) implying that the crude plant has low inorganic components, while water soluble ash (0.70%) and acid insoluble ash (1.56%) indicates that the crude drug not contains plenty of physiological ash and the non-physiological content and it will not affect the clinical efficacy of drug.

4) Phytochemical Screening

Table 1 summarized the preliminary phytochemical screening suggesting the presence of flavonoids, reductants sugars, fat and/or volatile oils, anthocyanidins, pyrochatecolic tannins and astringents and/or bitter principles. Triterpenes and/or steroids, alkaloids and lactones/coumarins test were found doubt and the absence of resins, cardiotonic glycosides, saponins, quinones and aminoacids or amines, this may be attributed to the false positive/negative results usually observed with preliminary phytoscreening of plants.

5) TLC

After few days in refrigeration was observed a solid that precipitated from the extract. This solid, the total extract and standard of gossypitrin, rutin and quercetin were plotted on TLCP. Under ordinary light was observed typical yellow

Table 1. Phytochemical screening of fruits from *T. elatum* Sw.

Test for constituents groups	Diethyl ether	Alcohol	Water
Dragendorff	+++	+++	-
Wagner	+++	+++	-
Sudan III	+		
Baljet	+	_	
Liebermann-Burchard	+	_	
Fehling		+	+
Foam test		_	_
$FeCl_3$		+ (dark green)	+ (dark green)
Ninhydrin		_	
Börntrager		_	
Shinoda		+	+
Kedde		_	
Anthocyanidins		+	
Bitter principles and/or astringents			+

spot with a long tale that corresponding to flavonoid compounds, that increasing color under ammonia vapor, changing its tonalities under UV light at 356 nm to pale yellow. After revelation with H_2SO_4 and heat the spots changed their colors and were observed at least three different spots, the big one with an R_f of 0.89 (yellow-orange), the second one with R_f of 0.36 (green purple) and the third one with R_f of 0.21 (green). Last two spots are almost circular, typically of terpenoids structures. The tale of the big one was surrounding by a clear blue or purple color typically of alkaloids. Not evidence of presence of gossypitrin, quercetin or rutin was found according their respective R_f. The main spot after revelation with anisaldehyde showed an orange yellow color as is shown in Figure 5.

6) Physicochemical parameters of the extract

Figure 6 shows the organoleptic properties of the extract which is a translucid liquid with yellow color and characteristic odor. Table 2 summarizes the results of physicochemical parameters. The pH of the extract was noted to be 5.55 ± 0.02 (lightly acid), total solids value is low but is in correspondence with the results obtained in ethanol soluble extractive, indicating that their chemical components are less polar or middle polar.

Capillary analysis showed an image few colored, which classifies as middle high (5.0 - 8.0 cm). The fringe (superior limit of ascension) is between lineal and festinated; the sub-fringe is yellow in color, the band showed tonalities between clear yellow and cream, the sub-band sowed clear cream color. Under ammonia vapors the image intensified its color as is shown in Figure 7.

7) Phenol and flavonoid contents

Table 3 demonstrates that both results showed low concentration, suggesting

Table 2. Physicochemical parameters of ethanolic extract of fruits from *T. ealtum*.

Parameters	Results ± SD
pH	5.55 ± 0.02
Total Solids (%)	1.07 ± 0.03
Refraction Index	1.3605 ± 0.0002
Relative density	0.8197 ± 0.0024
Capillary analysis (high in cm)	5.3 ± 0.14

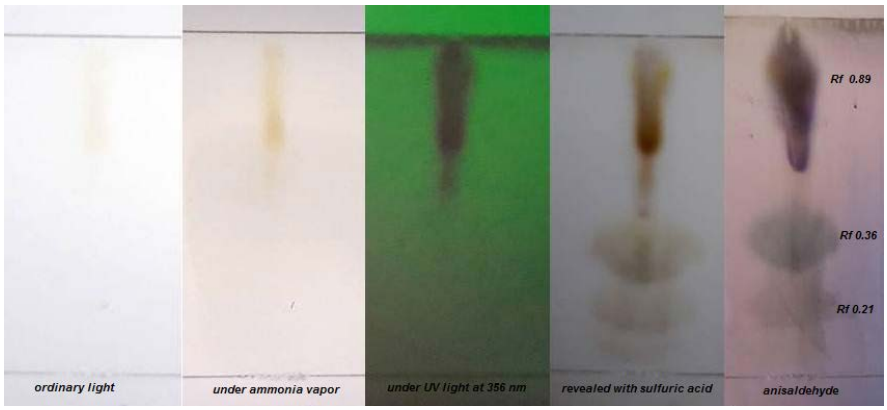

Figure 5. TLC of the total extract of ethanolic extract of fruits from *T. elatum*.

Figure 6. Ethanolic extract of the fruits.

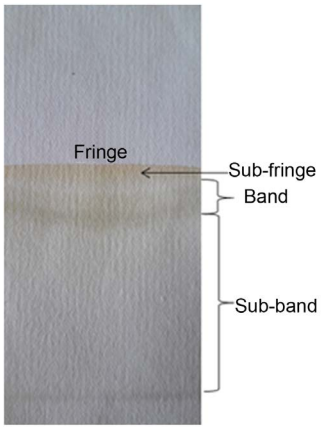

Figure 7. Capillary analysis of the ethanolic extract of fruits.

Table 3. Total Phenol and Flavonoid contents.

Extract	Total Phenol Content mg/mL $\bar{x} \pm$ DS	Total Flavonoid mg/mL $\bar{x} \pm$ DS
	0.92 ± 0.01	0.33 ± 0.005

that the increments of the value are possible with the use of hydroethanolic solvent and heating the extract. The total flavonoid contents represent around the 35.8% of the total phenol content.

Figure 8 shows the curve of the average absorbance versus concentrations is a straight line, indicating the good correlation between the concentration and absorbances of reference substances tested (gallic acid and quercetin, respectively). The correlation coefficient was ≥0.99.

8) GC-MS analysis

To have a better observation, the chromatogram was clarified eliminating the Peak Integrate for TIC (All Group). Figure 9 shows the current chromatogram of ethanolic extract of fruits from *T. elatum*. The retention time of the constituents star at 14.492 min and finish at 48.278 min in the fruits and in the case of precipitate start at the same time and finished at 76.769 because before this time only artifacts or silylated compounds appear. The first compound that is not present in precipitate analysis is Trimethylsilyl ether of glycerol (14.492) and the last one is alpha.-D-Glucopyranoside (48.278).

According to our results in phytochemical screening in precipitate analysis reductants sugars (Saccharose, D-Fructose, D-Glucose), carboxylic acids (saturated and unsaturated), alcohol or derivatives (aliphatic and cyclic), steroids (Cholesterol, Stigmasterol, Stigmast-4-en-3-one), DNA derivatives (purines like Thymine), monoterpene derivatives (Thymol-.beta.-d-glucopyranoside, alpha.-Pinene) and alkanes (hexane) using the NIST Library.

Fruits chromatogram showed the presence of 156 different chemical compounds and precipitate chromatogram show the presence of 163 different chemical compounds. The differences between both determinations are only 7 chemical structures as is shown in Figure 10.

Surprisingly, at 41.335 min was reported the presence of two chemical compounds relating with alkaloids derivatives with 502 m/z. There is Bis-trimethylsilyl-.delta.-9-tetrahydrocannabinol acid and Tetrahydrocannabinolic acid 2TMS, respectively as is showed in Figure 11.

4. Conclusions

The pharmacognostic evaluation which comprises of macromorphology and microscopic characters, the estimation of physicochemical parameters and the phytochemical and TLC profile are constant features of a plant which are highly essential for raw drugs or plant parts used for preparation of phytomedicine. Therefore, the result generated from this study would be useful in identification and standardization of the plant material towards quality assurance and also for

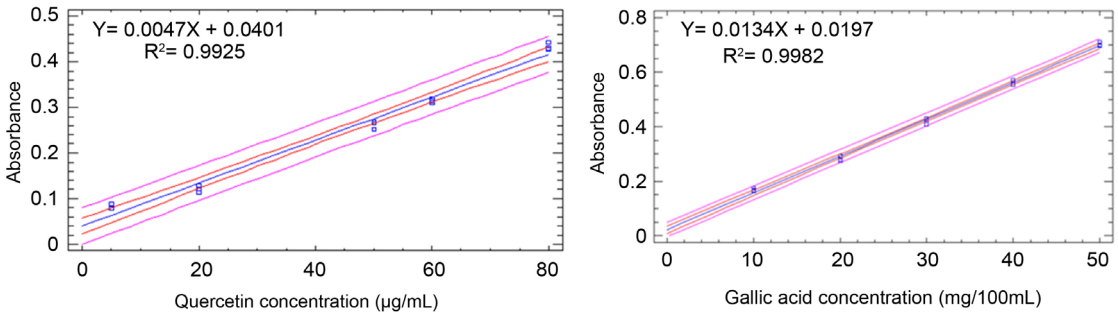

Figure 8. Curve of determination of total phenol and flavonoid contents.

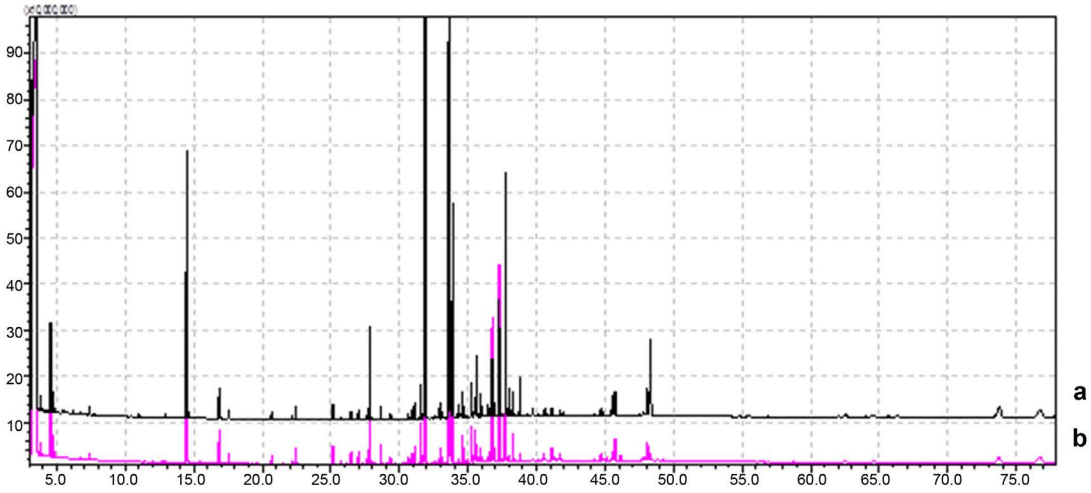

Figure 9. Current chromatograms of ethanolic extract of fruits (b) and precipitate (a) from *T. elatum* Sw. without Peak Integrate for TIC (All Group).

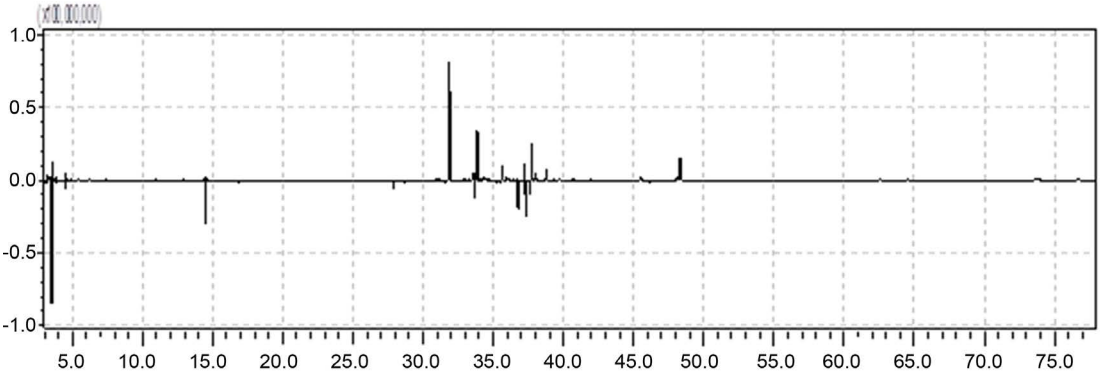

Figure 10. Current chromatograms of both samples showing the differences between each other.

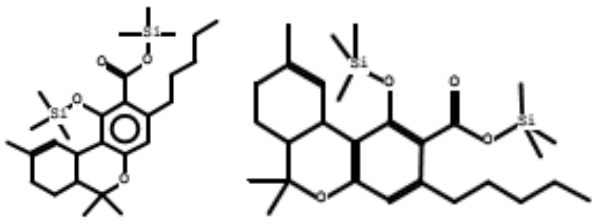

Figure 11. Chemical structures of both alkaloids derivatives.

preparation of a monograph on the plant. This research showed that, *T. elatum* Sw. can be identified by structural features or characteristics of fruits can be regarded as distinctive identification character.

Efforts have been made by the authors to bring out every detail on the macroscopical and microscopical characters of *T. elatum* Sw. The study of pharmacognostical features had shown the standards, which will be useful for the detection of its identity and authenticity.

Conflicts of Interest

The authors declare no conflict of interest.

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