Pharmacognostic, Physicochemical Standardization and Phytochemical Analysis of Pistils with Pollen from the Flowers of *Talipariti elatum* Sw

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**Abstract**

Towards authentication and quality assurance of medicinal plants, pharmacognostic, physicochemical and preliminary phytochemical studies of the pistils and pollen from the flowers of *Talipariti elatum* Sw. were carried out. The macroscopic and microscopic evaluation revealed characters that are of diagnostic value and useful in authentication of the plant. The Physicochemical analyses reveals values for moisture content, alcohol extractive, and ash values (total ash, water soluble ash, acid insoluble ash) which are within the World Health Organization (WHO) rules for crude drug from medicinal plants. Phyto-screening for secondary metabolites revealed the presence of alkaloids, oils and fats, reductants sugars, triterpenes and steroids, flavonoids, anthocyanidins and aminoacids/amines while coumarins, quinones, cardiotonic glycosides, bitter principles/astringents, saponins, resins/balsam and catequins were absent. Information obtained from these studies can be used as markers in the identification and standardization of this plant as a herbal remedy and also towards monograph development on the plant.

**Keywords**

Pistils; Pollen; Pharmacognostic; Physicochemical; Phyto-screening; Talipariti

**Introduction**

The pollen is the structure used in the transport of the male gamete (sperm cells) to the female part of the flower; it is made up of a fine to coarse powder which consists of micro gametophytes (pollen grains), which produce the male gametes of seed plants. Pollen grains are microscopic; usually about 15 to 100 microns and just a pinch of pollen powder contains thousands of grains [1].

Pollen is made up of an outer wall called the exine, composed of a very tough unusual substance known as sporopollenin and an inner wall called the intine which is made up of cellulose similar in construction to an ordinary plant cell wall. Pollen grains come in a wide variety of shapes (most often spherical), sizes and surface markings characteristic to the species. Furrows in the pollen grain called colpi and pores are major criteria for the identification of pollen classes [2].

The differences in these characters can be used in differentiating plants of the same genus or family. It is well known that palynological studies performed by light microscope (LM) and scanning electron microscope (SEM) have great value in plant taxonomy [3], specifically for the taxonomy of the family Malvaceae [4].

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**Talipariti elatum** 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 1,200 meters (3,900 ft) and is often used in reforestation. It is the national tree of Jamaica [5].

The **Talipariti elatum** tree is quite attractive with its straight trunk, broad green leaves and hibiscus-like flowers. It grows quite rapidly, often attaining 20 meters (66 ft) or more in height. The attractive flower changes color as it matures, going from bright yellow to orange red and finally to crimson (Figure 1). 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 [6]

**Figure 1. Flower of T. elatum Sw**

The aim of this research was to determine the pharmacognostic and phytochemical control methods for the pistils with pollen from the flowers of this plant that grows in Cuba for the development and utilization of the promising medicinal plant.

**Materials and Methods**

Flowers were collected after their fall spontaneously in January 2017 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. Pistils were separated from the rest of components by hands carefully. The isolated pistils used were dried in an oven with controlled temperature, at 40°C, during 5 days, ground into powdered form and stored in airtight containers.

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. All reagents used were of analytical grade (Merck). All solvents were degassing previously before used in an ultrasonic bath without filtration.

The pistils were examined for morphological characters including size, shape, color, odor, taste, and extra features. The macro-morphological characteristics were observed under the magnifying lens (10x). Dried pistils were ground to coarse powder and packed, for microscopic identification, in a suitable container. None clearing agents were used. Photomicrographs of the powder section were taken with the help of Biomicroscopy Primo Star (Zeiss Group, Germany) with 10x and 40x microscope objective lens (400 xs) and CX21 bio-microscopy unit (Canon-1000D EOS digital camera, Japan, coupled to PC software EOS-utility).

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, 1998. The chemical constituents were screened to ascertain their presence in the 70% ethanol extract according to [7]. The UV spectrometric experiments were carried out on a UV-VIS JASCO V-530 (Japan). The scan range was 200 to 500 nm; absorbance 0.000-3.0000, band width 2.0 nm, spectral resolution 0.1 nm and the analyzed samples were diluted in methanol, into quartz cuvettes (d = 1 cm).

**TLC conditions:** 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 eluent (v/v/v), concentrated sulfuric acid plus heat, FeCl₃, and AlCl₃ were the developer agents.
Rutin (R), quercetin (Q) and gossypitrin (G) were used as standard. The TLC were examined under ultraviolet (λ 254 nm and 365 nm) and ordinary light.

Results and Discussion

The external morphology of the pistil from the flowers of *T. elatum* showed in Figure 2 present a stamina column measuring 4-10 cm long, filaments and anthers yellow in color, styles with red branches 8-12 mm long, stigmas red purple in color [4].

**Figure 2.** External morphology of pistils from *T. elatum* Sw

Analyzed individually, fragmented drug shows the anatomical features in Figure 3. Pollen grains are yellow in color, spherical in shape, diameter of 98-105 or 150-190 μm, polipantoporate, with 20-30 apertures. Exine 3 or 4.5-8(-10) μm wide; sexing more wide than nixing; tectum scrobiculate and microgranulated, with spines of 14-20 or up to 34 μm long, conical or cylindrical, acute or obtuse [8-9].

**Figure 3.** Fragmented drug (pistils and pollen) of *Talipariti elatum* Sw

(Part of the titel: Top left pollen grains after theca rupture; Top right pollen grain with spines; Bottom left Theca spelling out yellow pollen grains and pistil; Bottom right yellow pollen grains and pistil augmented).

Preliminary phytochemical screening suggested the presence of alkaloids, flavonoids, anthocyanidins, pirochateolic tannins, fat and/or volatile oils, amino acids or amines, triterpenes and/or steroids, and reductants sugars, and the absence of resins, coumarins, cathequins, cardiotonic glycosides, saponins, quinones and astringents and/or bitter principles (Table 1). It is the first time that alkaloids appear in the chemical composition of pistils with pollen, because until now, in this part of the flowers of *T. elatum* Sw. only in toluene extracts were reported organic and fatty acids and some sugars derivatives, all of them characterized by GC-MS [10] and the presence of a chemical compound with impairing number of mass (m/z 479) by HPLC-UV-ESI-MS/MS from the petals of the flowers collected in Martinica extracted by Soxhlet on same conditions and dried at shadow at room temperature during a week [11]. Further studies are needed to establish the molecules responsible for the real chemical composition.

**Table 1.** Phytochemical screening for pistils with pollen from *Talipariti elatum* Sw

<table>
<thead>
<tr>
<th>Test for constituents groups</th>
<th>Diethyl ether</th>
<th>Alcohol</th>
<th>Water</th>
</tr>
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<tbody>
<tr>
<td>Dragendorff</td>
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<td>+++</td>
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<tr>
<td>Wagner</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Sudan III</td>
<td>+</td>
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<td>Baljet</td>
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<tr>
<td>Lieberman-Burchard</td>
<td>dark)³+</td>
<td>(green)</td>
<td>+</td>
</tr>
<tr>
<td>Cathequins</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Resins</td>
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<tr>
<td>Fehling</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Foam test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>(intense green)³+</td>
<td>(intense green)++</td>
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<tr>
<td>Ninhidrine</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Börntrager</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Shinoda</td>
<td>+</td>
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<td>Kedde</td>
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<tr>
<td>Anthocyanidins</td>
<td>+</td>
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<td>Bitter principles and/or astringents</td>
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Scan of the ultraviolet spectrum showed that there were three absorption peaks at 204 nm, 252 nm and 348 nm. There may be the presence of flavonoids in 70% ethanol extracts [12] (Figure 4).

TLCP (thin-layer chromatography plate) presented four obvious spots (Figure 5). Under ordinary light only one spot was observed, with Rf = 0.58 at the same level of rutin spot, cream-yellowish in color (5A). The colorimetric detection with concentrated sulfuric acid and heat presented two spots, the first one (1) with an Rf of 0.26 and the second one (2) with identical Rf of rutin as standard (0.58), but, with different colors, pale cream, both of them representative of chemical compounds type flavonoids [13,14] and another one (3) appear near the solvent front, red violet in color that could be related with triterpenoids structures (Rf 0.80) (5B). Colorimetric detection with FeCl₃ showed two spots elongated, the first one (4) with Rf = 0.48 and another one almost at the same level of rutin (Rf = 0.63), both of them black green in color, indicating the presence of phenolic compounds derivates from catechol (5C). In colorimetric detection with AlCl₃ under ordinary light was observed only one spot at the same level of rutin, yellowish in color (5D), but changing their colors under UV 365 nm to pale yellow (2) and near to the solvent front, another one clear blue (Rf = 0.80) (5E). Under UV 254 nm, the spots change in tonality, the three firsts with reddish orange colors, typically from chemical compounds like flavonoids and another one in the solvent front clear blue intense (5F).

Moisture content (9.61 %) was inside the limited index (8-14%). Extractable matter in ethanol at 70 % (17.14 %) suggests that this solvent is appropriated to the extraction of active components from the pistils. Total ash (6.99 %) and water soluble ash (5.26 %) were far higher than the standard allowed (3-5% and <2%, respectively), the possible reason is that medicinal drug is not clean, containing soil and another inorganic impurity, while acid insoluble ash (1.10 %) was lower than the standard (<2%) [15].

**Conclusions**

The evaluation of a crude drug is an integral part of establishing the correct identification of a plant material. For this, pharmacognostic and physicochemical parameters must be determined. In this regard, the microscopic and macroscopic features of pistils with pollen have been studied. Uniquely, a chemical component, like flavonoid types, is present in the ethanolic extracts of this part of crude drug at the same level of Rutin demonstrated by CCD.

Flowers of *T. elatum* Sw. are used in the folklore medicine in our country, as an anti-inflammatory, antasthmatic and expectorant. Hence this study provide useful information for the identification of this plant.
for the future plan and also give standardization parameters for the development of flower formulation. Present study has revealed the similarities in the pistils and pollen morphology of Malvaceae family, and specifically for genus Talipariti in Cuba.

Previous studies in the family showed that this result conforms to the values observed among the species of this family. The morphology of the pollens of the specie investigated was found to have diagnostic value and however supports the previous classification of the species by Ferreira [8] and Jiménez [9]. It also supports the importance of length of pollen spines as systematic tool in plant taxonomy. This confirms that the specie investigated is member of Malvaceae.

Conflict of Interest
The authors declare no conflict of interest.

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