PHARMACOGNOSTIC EVALUATION OF FRUITS OF Datura metel var. fastuosa (L.) Saff. AND THEIR SCOPOLAMINE CONTENTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract: Datura metel var. fastuosa (L.) Saff. or LUM-PONG-KA-SA-LAK is one of the most potential herbal in traditional Thai medicine studies. Its fruits are used for treatment of asthma and chronic bronchitis. Evaluation of the crude drug was conducted according to the World Health Organization (WHO) guidelines for herbal standardization. This study highlighted the pharmacognostic and phytochemical evaluation of dried fruits for macroscopic and microscopic characters as well as physiochemical properties. The various physico-chemical properties in percentage by weight of the mean contents were found to be: acid insoluble ash (1.38%), total ash (9.50%), ethanol soluble extracts (3.44%), water soluble extracts (19.63%), loss on drying (7.34%) and water content (12.00%). The phytochemical investigation showed the average of scopolamine content by high performance liquid chromatographic method was found to be 5.20 mg/g dried weight. In addition, a thin-layer chromatographic fingerprint of fruits of D. metel var. fastuosa was carried out. These studies help in identification and evaluation of the traditional drug in the crude form and also the finished products distributed among Thai traditional drug stores in Thailand.

Keywords: Datura metel var. fastuosa, pharmacognostic specification, scopolamine

INTRODUCTION

Datura metel var. fastuosa (L.) Saff. (Figure 1) which belongs to family Solanaceae is a tropical shrub, widely distributes in the regions of South and South-east Asia (Preissel,
In Thailand, the plant is called “LUM-PONG-KA-SA-LAK”. It is found commonly in wasteland in many parts of Thailand. The root, seeds, leaves, flowers and fruits of the plant are used in traditional Thai medicine, such as asthma, chronic bronchitis and cough (Chotchoungchatchai, 2012). All part of this plant contains chiefly tropane alkaloids, hyoscine (also known as scopolamine) is the main constituent and used worldwide in medicine as mydriatic, anticholinergic and parasympatholytic agents that act on the parasympathetic nervous system (Brown JH, 2006). Previous chemical investigation of this plant has shown that scopolamine is the major constituent and the compound was found in the fruit of *D. metel* var. *fastuosa* than the other parts (Issaravanich, 2013), the morpho-anatomical and physicochemical have been studied of dried seed also (Ratan, 2011). The standardization is an essential measurement for quality of plant drugs (Kadam, 2011). The screening of bioactive compounds from the herbal extract is also important to drug development (Liu, 2011). Hence, to control the quality of raw medicinal materials, establishment of standardization parameter is needed. The present report provided additional and more update information on the pharmacognostic properties of *D. metel* var. *fastuosa* dried fruit crude drug distributed in Thailand.

**MATERIALS AND METHODS**

**Chemicals and materials**

(-)-Scopolamine hydrochloride was purchased from Sigma Aldrich. Potassium dihydrogen phosphate, anhydrous sodium sulphate and phosphoric acid were purchased from Ajax Finechem. HPLC grade acetonitrile and methanol were obtained from RCI Labscan. Ultra-pure water was prepared by SNW ultra-pure water system (NW20VF; Heal Force, China). PTFE and nylon membrane syringe filter (13 mm x 0.45 μm, ANPEL Scientific Instrument, China) were used for non-aqueous and aqueous solvents respectively.

*D. metel* var. *fastuosa* dried fruits were collected from twelve locations (Bangkok, Bueng-Kan, Buriram, Chanthaburi, Chonburi, Lampang, Nakornpathom, Pathum-Thani, Phitsanulok, Suphan-Buri, Songkhla No.1 and 2) in four regions throughout Thailand during 2014 and authenticated by N.R. Pictures were drawn and photo by one of the authors (S.I.). The voucher of twelve specimens were deposited at College of Public Health Sciences, Chulalongkorn University. The crude drugs that free from any foreign matters were kept in closed container protected from light and moisture.

**Pharmacognostic Evaluation**

The anatomical characteristics of the crude drug were determined in cross sectional view. The powders were sifted through a 250-micron sieve before inspection. A stage micrometer etched with divisions of 0.01 mm was used for microscopic measurement.

The constant numbers due to crude drug quality were examined (World Health Organization, 2011; Yukongphan, 2013). Total ash and hydrochloric acid insoluble ash contents were performed by incineration until they were free of hydrocarbon matters. The amounts of active constituents were determined by ethanol and water extraction. Water and volatile matters were determined by loss on drying test. The azeotropic method was used for direct measurement of water in the crude drug. The crude drug from each location was tested in triplicate. The grand average ± 3 standard deviations from the grand average were calculated.

Qualitative evaluation of the crude drug was performed by maceration in methanol: chloroform: 25% ammonium hydroxide (50:50:1.5) for 6 h followed by thin-layer chromatography using silica gel 60 F254 and toluene: ethyl acetate: diethylamine (7:2:1). Visualization was done under UV 254 and 365 nm as well as Dragendorff’s reagent.
Quantitative analysis of scopolamine

The extract was performed as described by Gontier (Gontier, 1994). Each 5 g of crude drug powder was extracted with 200 ml of the mixture of methanol: chloroform: 25% ammonium hydroxide (50:50:1.5) by soxhlet apparatus. The extract was filtered through anhydrous sodium sulphate and evaporated to dryness under reduced pressure at 60°C. Twenty milligrams of each crude extract were dissolved in 1 ml of methanol and sonicated for 5 minutes. The standard scopolamine was dissolved in methanol to give concentrations of 100 - 600 μg/ml. The extract and standard solution were filtered through a 0.45 μm PTFE membrane filter before analysis.

The scopolamine content was determined according to the method described by Hoseini (Hosseini, 2011). HPLC analysis was performed using a Shimadzu DGU-20A3 HPLC (Shimadzu, Japan) equipped with a binary solvent delivery system, an auto-sampler, a column temperature controller, and a photo diode array detector (Shimadzu SPDM20A, Shimadzu, Japan). System control and data analysis were processed with Shimadzu LC Solution software. The chromatographic separation was accomplished with an Inersil® ODS-3 column (5 μm x 4.6 x 250 mm) and an Inertsil® ODS-3 HPLC guard column (5 μm x 4.0 x 10 mm). The binary mobile phase consisted of 50 mM phosphate buffer pH 3.0: acetonitrile (80:20 v/v). The mobile phases were filtrated through 0.45 μm nylon membrane filters and degassed using an ultrasonic bath before analysis. The isocratic program was set at 20% acetonitrile for 15 min with a flow rate of 1 ml/min. The column temperature was maintained at 40 °C and the injection volume was 20 μl. The detection was set at a maximum absorption wavelength (215 nm) for monitoring chromatographic profile.

Method validation

Linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, specificity, and robustness were evaluated according to the ICH guideline for validation of analytical method (Agency, 1995). LOD and LOQ were calculated based on the residual standard deviation of a regression lines and the slope of the calibration curve. The precision was evaluated in terms of repeatability and intermediate precision using 9 determinations covering the specific range (100, 300, and 600 μg/ml, 3 replicates each). The accuracy was determined by spiking and recovery method (50, 150, and 300 μg/ml scopolamine). The specificity was evaluated by peak purity test. The robustness was determined for variations in flow rates (0.995 and 1.005 ml/min) and variations in column temperature (39 and 41 °C) to evaluate whether the flow rate and temperature variations altered the results of HPLC.
RESULTS AND DISCUSSION

Figure 1. (a) D. metel var. fastuosa flowering branch, (b) Fruit, (c) Seeds.

D. metel var. fastuosa dried fruit has been used as an herbal material in traditional Thai medicine (Figure 2). Microscopic inspection is necessary for broken or powdered crude drug. The anatomical structures in transverse section view and the histological structures in powdered form of this crude drug were illustrated in figure 3 and 4. These microscopic characteristics could be useful to assure the identity and purity of D. metel var. fastuosa dried fruit material. Furthermore, TLC fingerprint, representing the chemical characteristics was also exhibited (Figure 5). Dragendorff reaction corresponded to alkaloidal compounds in this crude drug. It was found that toluene, ethyl acetate and diethylamine (7:2:1) was the optimal mobile phase for this crude drug fingerprint (Wagner, 1996).

The physico-chemical parameters were evaluated to specify crude drug quality. The contents of total as well as acid insoluble ashes, loss on drying and water contents should be not more than 9.5, 1.4, 7.3 and 12.0 % by weight respectively. In addition, to assure the quality of active ingredients, the ethanol and water extractive matters should be not less than 3.4 and 19.6 % by weight respectively (Table 1).

Table 1. The constant numbers due to crude drug quality

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Content (% by weight)</th>
<th>Range (Mean ± 3SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-insoluble ash</td>
<td>1.38</td>
<td>0.77 – 2.0</td>
</tr>
<tr>
<td>Total ash</td>
<td>9.50</td>
<td>8.47 – 10.53</td>
</tr>
<tr>
<td>Ethanol-soluble extractive</td>
<td>3.44</td>
<td>3.04 – 3.84</td>
</tr>
<tr>
<td>Water-soluble extractive</td>
<td>19.63</td>
<td>19.44 – 19.82</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>7.34</td>
<td>6.92 – 7.76</td>
</tr>
<tr>
<td>Water</td>
<td>12.00</td>
<td>9.94 – 14.05</td>
</tr>
</tbody>
</table>
Figure 2. Dried fruits of *D. metel* var. *fastuosa*

Figure 3. Transverse section of *D. metel* var. *fastuosa* seed. (a) Seed coat (mucilage and testa), (b) Micropylar endosperm, (c) Cotyledon, (d) Endosperm

Figure 4. Powder of *D. metel* var. *fastuosa* (dried fruits). (a) Multicellular uniseriate trichomes, (b) Fragments of fibers, (c) Parenchymatous cells, (d) Fragment of bordered pitted vessel, (e) Spiral vessels, (f) Starch granules, (g) Oil globules, (h) Pollen grains, (i) Reddish masses of resin, (j) Prism crystals of calcium oxalate.
Figure 5. TLC fingerprint of D. metel var. fastuosa dried fruits developed with toluene: ethyl acetate: diethylamine (7:2:1) (I) detection under UV 365 nm, (II) detection under UV 254 nm, (III) detection with Dragendorff’s reagent.

The contents of scopolamine in D. metel var. fastuosa dried fruits performed by HPLC (Figure 6) ranged from 1.43-10.85 with the mean of 5.20 ± 2.26 mg/g of dried weight. According to ICH guideline (International Conference on Harmonization, 2005), the tests of linearity, LOD, LOQ, precision, accuracy, specificity, and robustness should be performed for the validation of analytical method. Scopolamine solutions of 6 concentrations were investigated for linearity of the HPLC method. The calibration curve was linear in the range of 100-600 μg/ml. The regression equation was y = 14910x – 63426 and the coefficient of determination ($r^2$) was 0.9978. LOD, the lowest concentration of detectable scopolamine in a sample was found to be 20.83 μg/ml and LOQ, the lowest concentration of quantitatively measurable scopolamine in a sample was 63.13 μg/ml. Both of them were calculated by residual standard deviation of regression line. The accuracy of the method was evaluated by recovery test using low, intermediate and high concentrations of scopolamine. The precision was conducted as % RSD of 9 determinations covering the specific range. RSD of repeatability and intermediate precision were found to be less than 3% (0.62-1.84% and 1.31-2.55% respectively) and the recovery was found to be in the range of 99%–102%, which revealed that the method was precise and accurate (U.S. Food and Drug Administration, 2001). The specificity performed by peak purity checking showed that peak purity index of scopolamine was more than 0.99 suggested that no impurity detected and the analyte chromatographic peak was not attributable to the other components. The robustness revealed that there was no difference (%RSD < 5) in the area of curve and retention time of scopolamine, when the flow rate of mobile phase varied from 0.995 – 1.005 ml/min and the column temperature varied from 39 – 41°C. This HPLC method was proved to be robust for scopolamine analysis in D. metel var. fastuosa dried fruit extracts, under the condition evaluated.
CONCLUSION

The cellular structures and TLC fingerprint demonstrated by this study could be useful for identification of D. metel var. fastuosa dried fruit crude drug particularly in the case of powered material. The physico-chemical constants and scopolamine content could serve to control the quality of this crude drug. These specifications provided basic requirements for further herbal monograph documentation.

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REFERENCES


