

PHARMACOGNOSTIC EVALUATION OF FRUITS OF *DATURA METEL* VAR. *FASTUOSA* (L.) SAFF. AND THEIR SCOPOLAMINE CONTENTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Somchai Issaravanich¹, Chanida Palanuvej^{1,*} and Nijisiri Ruangrungsi^{1, 2}

¹College of Public Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand

²Faculty of Pharmacy, Rangsit University, Patumthani 12000, Thailand

*Corresponding author: E-mail: chanida.p@chula.ac.th

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. The pharmacognostic of dried *D. metel* var. *fastuosa* fruits were performed on 12 samples collected from five different geographical areas of Thailand. 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 extractives (3.44%), water soluble extractives (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

บทคัดย่อ: ลำโพงกาสลักเป็นพืชสมุนไพรชนิดหนึ่งที่มีศักยภาพสูงมากในวงการแพทย์แผนไทย ผลของลำโพงกาสลักมักใช้ในการรักษาโรคหอบหืดและโรคหลอดลมอักเสบเรื้อรัง จากผลการศึกษาทางเภสัชวิทยาของผลลำโพงกาสลักแห้งที่เก็บรวบรวมจากพื้นที่ต่างๆ ของประเทศไทย จำนวน 12 ตัวอย่าง ซึ่งได้ดำเนินการตามวิธีมาตรฐานสมุนไพรขององค์การอนามัยโลก โดยการศึกษาครั้งนี้ได้ทำการประเมินคุณสมบัติทางด้านเภสัชวิทยาและพฤกษเคมีของผลแห้ง ด้วยค่าเปลวและกล้องจุลทรรศน์ รวมทั้งศึกษาคุณสมบัติทางกายภาพและเคมีต่างๆ พบว่า อัตราร้อยละโดยน้ำหนักของค่าเฉลี่ยของเนื้อที่ไม้ละลายกรดเท่ากับ 1.38 เถ้ารวม 9.50 สารสกัดที่ละลายเอทานอล 3.44 สารสกัดที่ละลายน้ำ 19.63 ปริมาณความชื้น 7.34 และปริมาณน้ำ 12.00 การหาปริมาณทางด้านพฤกษเคมีแสดงถึงค่าเฉลี่ยของปริมาณสารสโคปอลามีนด้วยวิธีโครมาโทกราฟีของเหลวสมรรถนะสูง โดยพบว่าปริมาณสาร 5.20 มิลลิกรัมต่อกรัมของน้ำหนักผลแห้ง นอกจากนี้ยังศึกษาถึงลักษณะทางเอกลักษณ์ของรงควัตถุผิวบางของผลลำโพงกาสลักอีกด้วย ประโยชน์ของการศึกษาครั้งนี้มีส่วนช่วยในเรื่องของการประเมินหรือระบุการปลอมปนของวัตถุดิบและผลิตภัณฑ์สำเร็จรูปซึ่งมีจำหน่ายทั่วประเทศไทยได้เป็นอย่างดี

คำสำคัญ: ลำโพงกาสลัก คุณลักษณะทางเภสัชวิทยา สโคปอลามีน

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,

2002). 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, hyoscyne (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 \pm 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 SPD20A, Shimadzu, Japan). System control and data analysis were processed with Shimadzu LC Solution software. The chromatographic separation was accomplished with an Inertsil® 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

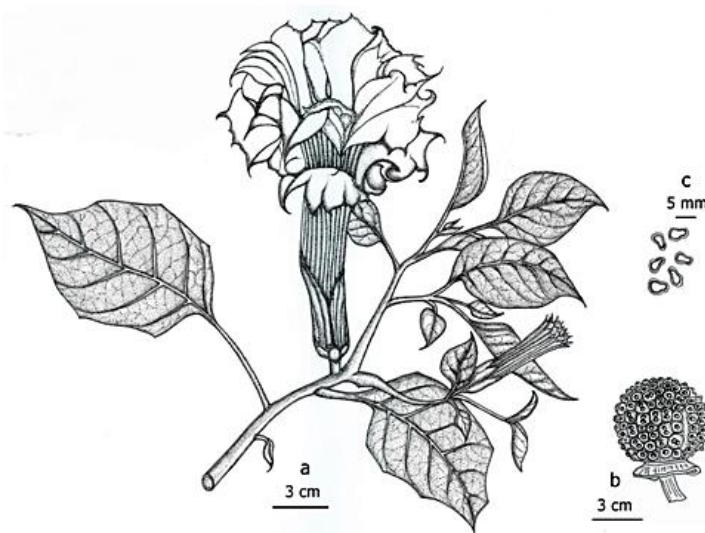


Figure 1. (a) *D. metel* var. *fastuosa* flowering branch, (b) Fruit, (c) Seeds.

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

Parameter	Content (% by weight)		
	Mean	SD	Range (Mean \pm 3SD)
Acid-insoluble ash	1.38	0.20	0.77 – 2.0
Total ash	9.50	0.34	8.47 – 10.53
Ethanol-soluble extractive	3.44	0.13	3.04 – 3.84
Water-soluble extractive	19.63	0.06	19.44 – 19.82
Loss on drying	7.34	0.14	6.92 – 7.76
Water	12.00	0.68	9.94 – 14.05



Figure 2. Dried fruits of *D. metel* var. *fastuosa*

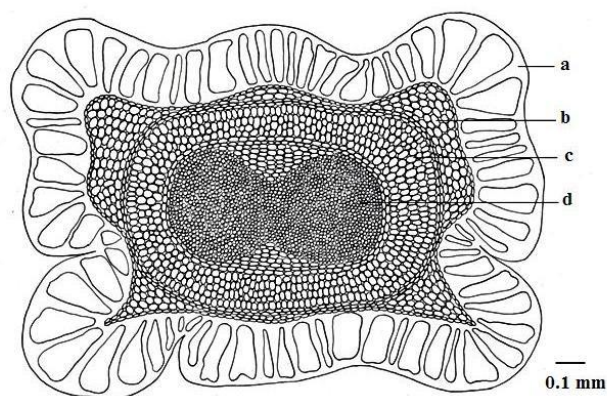


Figure 3. Transverse section of *D. metel* var. *fastuosa* seed. (a) Seed coat (mucilage and testa), (b) Micropylar endosperm, (c) Cotyledon, (d) Endosperm

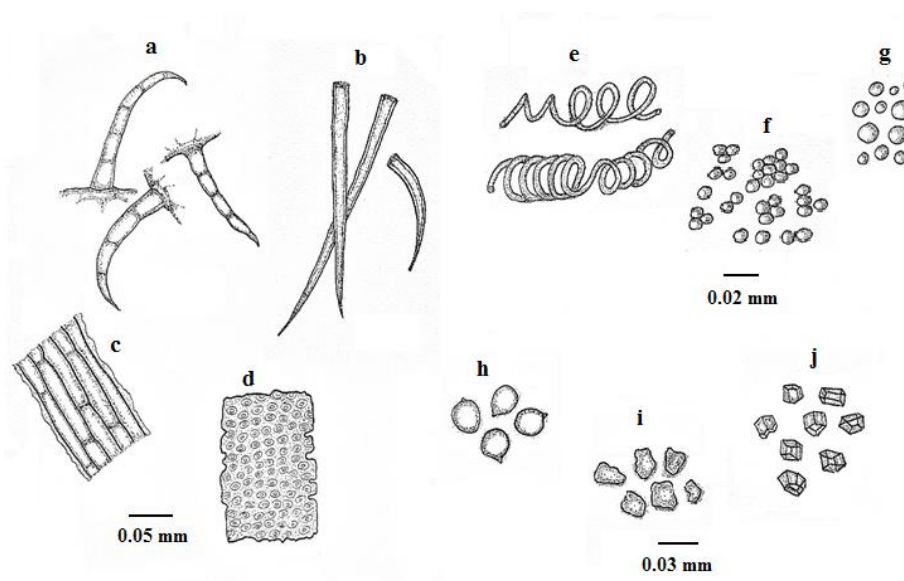


Figure 4. Powder of *D. metel* var. *fastuosa* (dried fruits). (a) Multicellular uniseriate trichomes, (b) Fragments of fibers, (c) Parenchymatous cells, (d) Fragment of bordered pitted vessel, (e) Spiral vessels, (f) Starch granules, (g) Oil globules, (h) Pollen grains, (i) Reddish masses of resin, (j) Prism crystals of calcium oxalate.

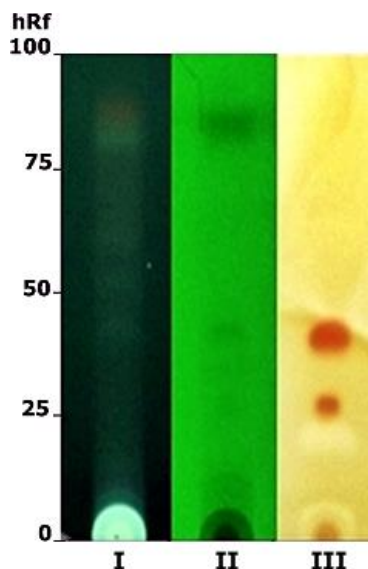


Figure 5. TLC fingerprint of *D. metel* var. *fastuosa* dried fruits developed with toluene: ethyl acetate: diethylamine (7:2:1) (I) detection under UV 365 nm, (II) detection under UV 254 nm, (III) detection with Dragendorff's reagent.

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 $\mu\text{g/ml}$. 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 $\mu\text{g/ml}$ and LOQ, the lowest concentration of quantitatively measurable scopolamine in a sample was 63.13 $\mu\text{g/ml}$. 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.

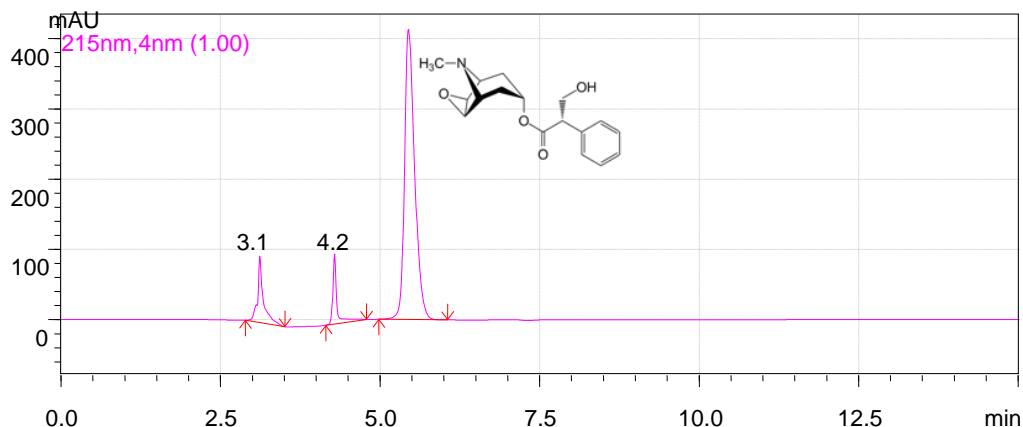


Figure 6. HPLC chromatogram of (-)-scopolamine in *D. metel* var. *fastuosa* dried fruit extract.

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 powdered 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.

ACKNOWLEDGMENTS

The authors would like to thank to the Department for Development of Thai Traditional and Alternative Medicine, Ministry of Public Health for partial financial support, College of Public Health Sciences, Chulalongkorn University for providing facilities and Mr. Niran Vipunngun, a lecturer of Faculty of Pharmacy, Rangsit University, Patumthani, Thailand for his guidance in the anatomical and histological characters of the herbal material.

REFERENCES

- Brown JH, Taylor P. 2001. Muscarinic receptor agonists and antagonists. In: Hardman JG, Limbird LE, editors. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 10th ed, New York: McGraw-Hill; pp. 155-174.
- Chotchoungchatchai S, Saralamp P, Jenjittikul T, Pornsiripongse S, Prathanaturug S. 2012. Medicinal plants used with Thai traditional medicine in modern healthcare services: A case study in Kabchoeng hospital, Surin province, Thailand. *Journal of Ethnopharmacology* 141: 193-205.
- Gontier E, Sangwan BS, Barbotin JN. 1994. Effects of calcium, alginate and calcium-alginate immobilization on growth and tropane alkaloid levels of a stable suspension cell line of *Datura innoxia* Mill. *Cell Reports* 13: 533-36.
- Hoseini N, Ebrahimi SN, Saleh P, Asghari B, Ahmadi M. Simultaneous determination of atropine and scopolamine in different parts of *Hyoscyamus arachnoideus* Pojark plants by high-performance liquid chromatography (HPLC). *Journal of Medicinal Plants Research* 5(15): 3552-3557.
- International Conference on Harmonization. 2005. *Validation of Analytical Procedures: Text and Methodology Q2 (R1)*. Geneva: ICH Secretariat. (Accessed on Mar. 31, 2016 at [http://www.gmp-compliance.org/guidemgr/files/Q2\(R1\).PDF](http://www.gmp-compliance.org/guidemgr/files/Q2(R1).PDF))
- Issaravanich S, Ruangrungsia N, Palanuvej C, Vipunngun N, Rungsihirunrat K. 2013. Microscopic, molecular and scopolamine content evaluations of *Datura metel* L. var. *metel* and *D. metel* L. var. *fastuosa* in Thailand. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 4: 1009-1021.
- Kadam PV, Yadav KN, Narappanawar NS, Shivatare RS, Bhusnar HU, Patil MJ. 2011. Development of quality standards of *Terminalia catalpa* leaves. *Pharmacognosy Journal* 3:19-24.

- Liu WJ, editor. 2011. *Traditional Herbal Medicine Research Methods: Identification, Analysis, Bioassay, and Pharmaceutical and Clinical Studies*. New Jersey: John Wiley and Sons.
- Preissel U, Preissel HG. 2002. *Brugmansia and Datura: Angel's Trumpets and Thorn Apples*. New York: Firefly Books; pp. 120-123.
- Ratan L, Meenu B, Anju D, Arun N. 2011. Morpho-anatomical and physicochemical studies of dried seeds of *Datura fastuosa* Linn. *International Research Journal of Pharmacy* 2 (3): 208-212.
- U.S. Food and Drug Administration. 2001. *Guidance for Industry: Bioanalytical Method Validation*. (Accessed on Mar. 31, 2016 at <http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf>)
- Wagner H, Bladt S. 2009. *Plant Drug Analysis: A Thin Layer Chromatography Atlas*. 2nd ed. Berlin Heidelberg: Springer-Verlag, pp. 19-23.
- World Health Organization. 2011. *Quality Control Methods for Medicinal Plant Materials*. Geneva: WHO Press.
- Yukongphan P, Thitikornpong W, Palanuvej C, Ruangrunsi R. 2013. The pharmacognostic specification of *Ardisia elliptica* fruits and their embelin contents by TLC image analysis compared to TLC densitometry. *Bulletin of Health, Science and Technology* 11(2): 21-28.