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Microscopic, Molecular and Scopolamine Content Evaluations of *Datula Metel* L. Var. *Metel* and *D. Metel* L. Var. *Fastuosa* in Thailand

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ABSTRACT

The present study aims to compare the difference among *Datula metel* L. var. *metel* and *D. metel* L. var. *fastuosa* in Thailand using microscopic and molecular studied and evaluation of scopolamine content. Macroscopic and microscopic examination was performed according to WHO Geneva guideline. Genomic DNA was prepared by modified CTAB method, the PCR amplification DNA sequence of internal transcribed spacer (ITS) were analyzed. Scopolamine content was analyzed by TLC and HPLC. The results taken by the cross section of midrib and stem showed almost similar major plant components. The aniscytic stomata were present in lower epidermis more than upper epidermis. Sequence analysis of two varieties of *D.metel* L. showed 2 single nucleotide polymorphisms (SNP) within the ITS2 region. The scopolamine contents from leaves, flowers and fruits of *D. metel* L. var. *metel* using TLC image and HPLC analysis showed good correlation between two methods and scopolamine contents was high in flower part of *D. metel* L. var. *metel* while in the fruit part of *D. metel* L. var. *fastuosa.* The combination of macroscopic, microscopic and molecular method is able to authenticate these closely related plant varieties and both of them have a potency to be a source of scopolamine production.

Keywords: Microscopic; molecular evaluation; scopolamine content; *Datula metel* L.; internal transcribed spacer (ITS)

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INTRODUCTION

Datura metel L is an annual herb or perennial undershrub belonging to the family Solanaceae or Nightshade family and widely distributed in South America, Africa, tropical area especially Asia including Thailand [1, 2]. *Datura metel* L has a long history usage in Thai traditional medicine as a remedy for many ailments. Small dosage of dried seeds powder of *Datura* plant was used to treat fever and as a cerebral tonic. The dried flower was smoked by asthmatics as a bronchodilator and also curbs nausea. Decoction of the root was also used to treat asthma, as well as bronchitis and cough. Decoction of the leaves was used traditionally to treat mucous or blood in the stool and the juice of the fruit was administered in drops to treat infections of the ear [3]. All part of this plant contains chiefly tropane alkaloids**,** hyoscine (also known as scopolamine), hyoscyamine, datumetine and atropine. According to these tropane alkaloids, scopolamine is the main constituents and used worldwide in medicine as mydriatic, anticholinergic and parasympatholytic agents that act on the parasympathetic nervous system [4]. Because of its widespread occurrence throughout Thailand, it may be considered as an attractive source for the production of medicinally useful tropane alkaloids, particularly scopolamine [5]. These are several methods for analysis of tropane alkaloids including thin layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC), and capillary electrophoresis (CE). Each method has their advantage and disadvantage aspect. Thus development of rapid and simple analytical methods is interested [6-9].

There are two varieties of *Datura metel* L. in Thaland, the white variety called *Datura metel* L. var. *metel* (Thai name: Lamphong Khaao) and the purple variety called *Datura metel* L. var. *fastuosa* (Thai name: Lamphong Kaa-sa-lak) [10]. Although these two varieties of *D. metel* L. share commonly morphological characteristics, identification based on morphology is not always conclusive in case of process material. The identification using molecular marker has been widely application in medicinal plant identification because of the uniqueness of genetic information within the species.

In order to clarify whether there are any different between these two *D. metel* L. varieties collected in Thailand, combination of microscopic, molecular and scopolamine content were applied for evaluation and comparison.

MATERIALS AND METHODS

Plant Samples

Whole plant of *D. metel* L. var. *metel* and *D. metel* L. var. *fastuosa* were collected from different locations of Thailand. The plants were authenticated by the expert-Associate Prof. Dr. Nijsiri Ruangrungsi and deposited at College of Public Health Sciences, Chulalongkorn University and compared with the herbarium specimens at Forrest Herbarium Thailand (BKF). Vouchers were deposited at the College of Public Health Sciences, Chulalongkorn University.

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Macroscopic and Microscopic Evaluation

The fresh authentic aerial branch including flower and fruit of *D. metel* L. var. *metel* and *D. metel* L. var. *fastuosa* were collected. The description and classification were done, line drawing was also prepared. Microscopic evaluation was carried out under the appropriate magnitude using a photomicroscope (Zeiss model Axioskop, Germany) attached with digital camera (Canon Power Shot A640). The stem and midrib from mature leaf was crossed section into thin pieces and mounted onto a slide and then observed under microscope. The recorded images were illustrated in a size proportional to the original. Fresh leaf surface for stomatal number and stomatal index measurement was preparation followed the method described by Geisler [11] with some modification. The stomatal number was evaluated under microscope and then counted within a 1 mm^2 for thirty fields, and stomatal index was calculated and expressed as mean and standard deviation (SD). The palisade ratio measurement, which were followed the method described in Mukherjee [12] with some modification by putting the piece of leaf which were cut off from the middle of the leaf into the mixture of sodium hypochlorite: water (1:1). After bleaching process, placed uppermost side on the slide and then evaluated under microscope. Counted the round palisade cells in 4 continuous boundary epidermal cells then, calculated for the proportion of palisade cells in 1 epidermal cell for thirty fields and presented as mean and SD values.

Molecular Evaluation

Genomic DNA of individual young leaves of plant samples were extracted by modified CTAB method described by Doyle [13] and used as DNA template for PCR amplification. The ITS region (including ITS1-5.8s-ITS2) was amplified using the universal ITS5 forward primer (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 reverse primer (5'-TCCTCCGCTTATTGATATGC-3') [14]. PCR amplification was carried out in 20 microliter (µl) reaction mixture containing approximately 1-2 µl of genomic DNA, 1x PCR Buffer (100 mM KCl, 20 mM Tris-HCl, pH 8.0), 2.5 mM $MgCl_2$, 0.1 µM dNTPs, 0.2 µM of each primers and 0.5 units of *Taq* polymerase (Promega). The amplified was carried out under the following conditions: initial denaturation at 95^o C for 5 min, followed by 30 cycles of 95^o C denaturation for 30 sec, 55^o C annealing for 30 sec, and 72^o C extension for 30 sec, then followed by final extension at 72^o C for 5 min. Five microliter of PCR products were evaluated by 1.5% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV transilluminator. The PCR products were then purified by QIAquick PCR purification kit (QIAGEN) prior sequencing (ABI system). The ITS sequences from both sense and antisense stand were analyzed using BioEdit sequence alignment version 7.0.9 for Windows.

Scopolamine Content Evaluation

The extraction was performed as described by Gontier [15] with some modification. Each 5 g of dry powder of leaves, flowers and fruits from 3 locations of *D. metel* L. var. *metel* (LPK081001, LPK091002, and LPK011103) and *D. metel* L. var. *fastuosa* (KSL071001, KSL041104, and KSL051105) was extracted for 4-5 hours in a soxhlet apparatus with 300 ml of methanol-

chloroform- 25 % ammonium hydroxide (50-50-1.5) until it was exhausted. After filtration, the marc was washed twice with 15 ml of chloroform and the pooled filtrate was evaporated under reduced pressure in a rotary evaporator till dryness. The extract was washed with 20 ml of 0.1 N hydrochloric acid three times, and extracted twice with 15 ml of chloroform to eliminate the impurities. The acid phase was adjusted to pH 10 with 5 ml of 25% ammonium hydroxide then exhaustively extracted three times with 35 ml of chloroform. After washing the marc with 20 ml of chloroform, the chloroform extract was filtered through anhydrous sodium sulphate and evaporated under reduced pressure till dryness. This crude extract was kept in refrigerator for TLC image analysis and HPLC analysis. The crude extract was dissolved in methanol to a desire concentration of 100 mg/ml of leaves, 50 mg/ml of flowers, and 40 mg/ml of fruits and sonicated 10 minute at room temperature before used. Standard scopolamine hydrochloride (Sigma) was prepared in methanol to obtain the concentration ranges of 50-500 µg/ml for TLC image and HPLC analyses.

TLC Image Analysis

The TLC method was modified from Wagner & Sabine, and Sotanaphun [16, 17]. Ten microliter of crude extract and standard scopolamine (50-500 µg/ml) were spotted onto the 20x20 cm aluminum sheets silica gel plate (G60 F254, Merck) with 0.25 mm thickness and developed in the solvent system; toluene: ethyl acetate: diethylamine (70: 20: 10 v/v) for at least 1 hour. The separated spots were visualized as orange spots after spraying with dragendorff's reagent solution. The TLC chromatogram was captured by a digital scanner (Hewlett Packard Deskjet F2280) and saved as a tagged image file (TIF) format at a resolution of 600 dpi. Quantification of each band was carried out by an image analysis using image J for windows version 1.45s (http://rsbweb.nih.gov/ij/). The colour image was converted to grayscale by photoshop software. The peak corresponding to scopolamine was selected and the area under the curve was measured. The calibration curve of scopolamine was obtained by plotting the concentration of standard scopolamine range 5-50 µg/spot versus the average (triplicate) peak areas of the densitogram. The scopolamine concentrations from the crude extract samples were calculated from peak areas of the densitogram compare to the standard concentrations on the same plate.

HPLC Analysis

The scopolamine content was determined according to the method described by Hoseini [18] with some modification. Instrumentation was HPLC model LC-20A™ series using a ODS-3, C18 column, Inertsil®, sized 5 µm, 250 x 4.6 mm equipped with LC-20 AD; binary pump, SPD-M 20 A; UV-PDA detector. A ODS-3, C18 guard column, Inertsil®, sized 5 µm, 10 x 4 mm was coupled to the analytical column. Prior injection, each sample was filtered through a 0.45 µm membrane filter. Ten microliter of each extract and standard scopolamine (50-500 µg/ml) were injected in triplicate. The mobile phase, 50 mM phosphate buffer pH 3.0: acetonitrile (80: 20 v/v) was pumped at a flow rate of 1.0 ml/min and the column temperature was maintained at 40ºC. A detector was set at a maximum absorption wavelength (215 nm) for monitoring chromatographic profile. The calibration curve of scopolamine was obtained by plotting the

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concentration of standard scopolamine range 50-500 µg/spot versus the average (triplicate) peak areas of the densitogram. The scopolamine concentrations from the crude extract sample were calculated from peak areas of standard calibration curve in each day.

Method Validation

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the equations: LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve [19]. The repeatability and the intermediate precision were expressed as percent relative standard deviation (%RSD) of the concentration. The accuracy was determined as percent recovery of spiked standard scopolamine in flower extract.

RESULTS

Whole plant of *D. metel* L. var. *metel* and *D. metel* L. var. *fastuosa* were collected and recorded from eight locations of Thailand as showed in Table 1.

Table 1. List of *D. metel* L. samples and the different sites where they were collected in Thailand.

Sample no.	Plant	Habitat (Province)	Collecting date (Month, Year)	Voucher ID
1	*D. metel* L.var. *fastuosa*	Central part (Bangkok)	July, 2010	KSL071001
2	*D. metel* L.var. *fastuosa*	Central part (Nakhonpathom)	July, 2010	KSL071002
3	*D. metel* L.var. *fastuosa*	Northern part (Chiangmai)	March, 2011	KSL031103
4	*D. metel* L.var. *fastuosa*	Eastern part (Chachoengsao)	April, 2011	KSL041104
5	*D. metel* L.var. *fastuosa*	Eastern part (Chonburi)	May, 2011	KSL051105
6	*D. metel* L.var. *metel*	Eastern part (Rayong)	August, 2010	LPK081001
7	*D. metel* L.var. *metel*	Central part (Nakhonpathom)	September, 2010	LPK091002
8	*D. metel* L.var. *metel*	Central part (Singburi)	January, 2011	LPK011103

Macroscopic and Microscopic Evaluation

D.metel L. var. *metel* and *D.metel* L. var. *fastuosa* is a shrub-like herb with 1-1.5 m high. The alternate leaves have petioles 3-7 cm long. Leaves are ovate or broadly ovate. There are some characteristics that differ between these two varieties as showed in Table 2 and Figure 1.

Table 2 Comparison of *D. metel* L. var. *metel* and *D.metel* L. var. *fastuosa* characteristics by macroscopic

Part of plant	*D.metel* L. var. *metel*	*D.metel* L. var. *fastuosa*
Flower	Single corolla with white or white cream colour	Double or triple corolla with white inside, and violet outside
Stem and branch	green colour	purple or dark violet colour
Lamina	repand-dentate or angulate margins with 3-4 coarse teeth and equal or symmetrical at the base	cordate or heart-shaped margins with sinuate to irregularly toothed edges and unequal or asymmetrical at the base
Fruit or capsule	Spiny, green colour capsule	Glabrous or short spines, purple colour capsule

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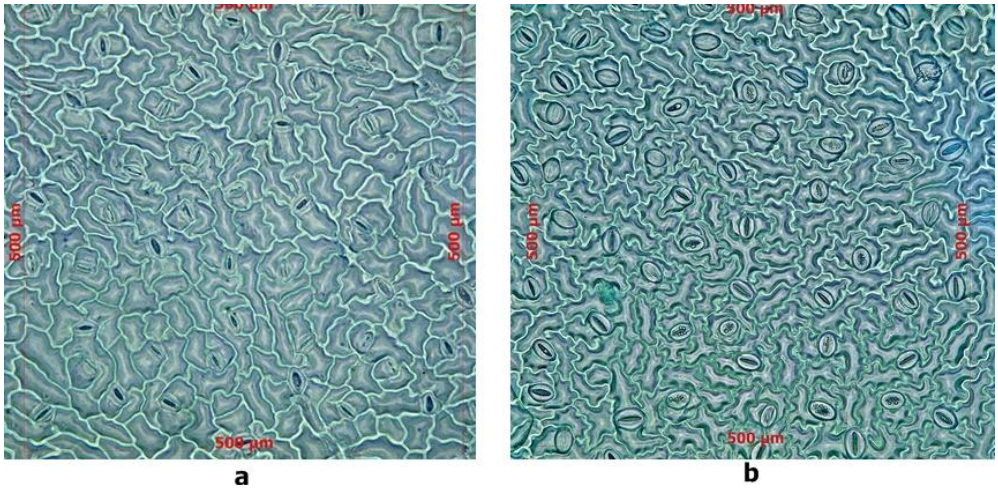
a b

Figure 1. Branch, flower, and fruit illustration of *D. metel* L. var. *metel* (a) and *metel* L. var. *fastuosa* (b)



a b

Figure 2. Stomatal number on the upper epidermis (a) and lower epidermis (b) (20x magnification)

The stomata of two varieties of *D. metel* L. observed under microscopic can be classified as anisocytic type which surrounding by three or four subsidiary cells as shown in Figure 2. A number of stomata of lower (abaxial) epidermis were more present than upper (adaxial) epidermis. The average stomatal number (upper and lower epidermis), stomatal index (upper and lower epidermis) in the area of 1 mm2 and palisade ratio of *D. metel* L. var. *metel* (a) and *metel* L. var. *fastuosa* were shown in Table 3.

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Table 3. The average leaf measurement values of *D. metel* L. var. *metel* and *D. metel* L. var. *fastuosa* from three locations. (mean ± SD, n = 90)

	D. metel L. var. *metel*	*D. metel* L. var. *fastuosa*
Stomatal number		
upper surface/ lower surface	85.58 ± 18.90/204.53 ± 23.40	190.96 ± 29.03/235.89 ± 31.81
Stomatal index		
upper surface/ lower surface	13.59 ± 1.00/19.08 ± 0.96	19.29 ± 0.98/20.82 ± 1.16
Palisade ratio	5.11 ± 0.54	6.34 ± 0.68

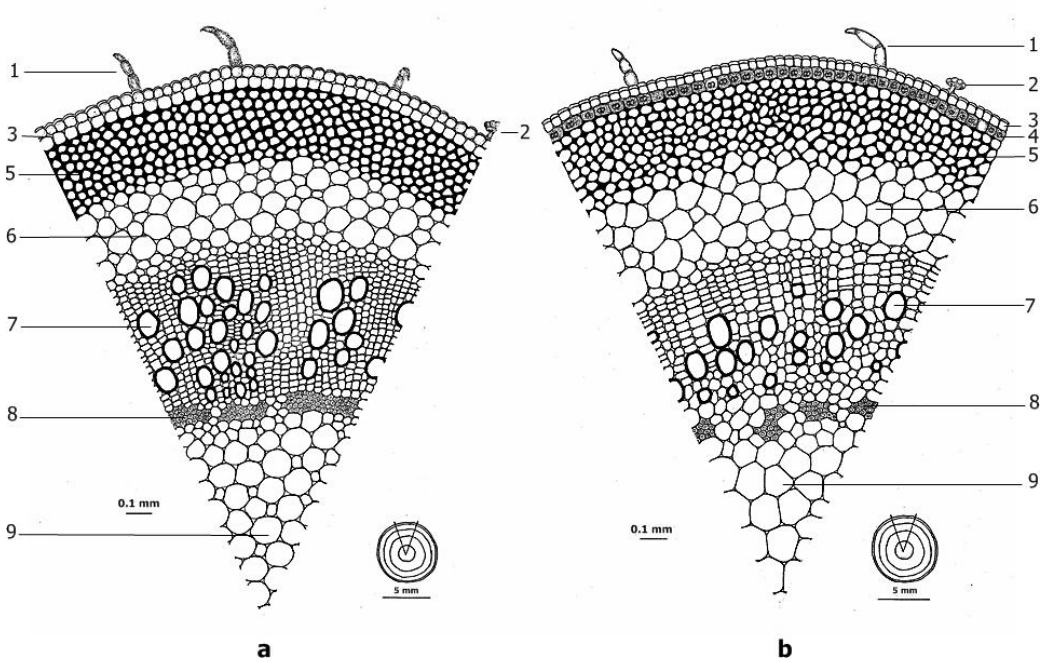

Figure 3. Stem cross section of *D. metel* L. var. *metel* (a) and *D. metel* L. var. *fastuosa* (b)
1. Multicellular uniseriated trichome, 2. Glandular trichome, 3. Epidermis, 4. Chromoplast contain anthocyanin pigment, 5. Collenchyma, 6. Chlorenchyma, 7. Xylem vessel, 8. Vascular fiber, 9. Parenchyma of pith

The stem cross section of *D. metel* L. var. *metel*, and *D. metel* L. var. *fastuosa* showed a single striated layered epidermis cells. The epidermal cells were tabular shaped and covered externally by a fairly thick cuticle, which having a few multicellular uniseriated and glandular trichomes. The chromoplast contain anthocyanin pigment was found only in *D. metel* L. var. *fastuosa*, while *D. metel* L. var. *metel* was absent. The chlorenchyma was located next to the collenchymas and the xylem vessels were aligned gather in group located next to chlorenchyma. The vascular fibers align gather in group and were interposed horizontally above parenchyma of pith (Figure 3).

The midrib cross section of *D. metel* L. var. *metel*, and *D. metel* L. var. *fastuosa* showed multicellular uniseriated trichomes on the epidermis cells, which covered externally by a fairly thick cuticle . Both of collenchyma located next to the upper and lower epidermis, while parenchyma located next to the collenchyma. The palisade mesophyll consists of a single layer of cells, which lie above spongy mesophyll. The central of midrib, situated vascular tissue is surrounded by

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parenchyma cells. The xylem vessels and vascular fibers, which align gather in group, were sparsely in vascular tissue (Figure 4).

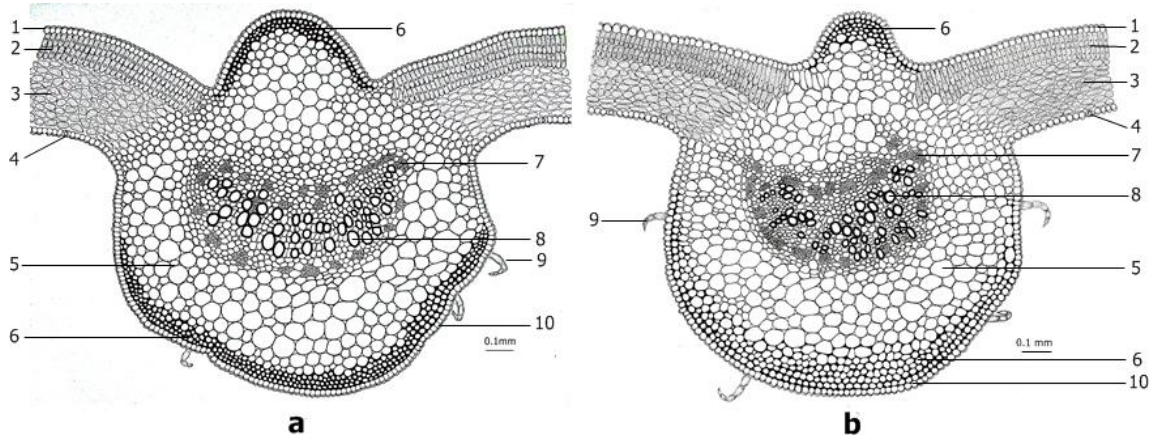

Figure 4. Midrib cross section of *D. metel* L. var. *metel* (a) and *D. metel* L. var. *fastuosa* (b)
1. Upper epidermis, 2. Palisade mesophyll, 3. Spongy mesophyll, 4. Stomata, 5. Parenchyma, 6. Collenchyma, 7. Vascular fiber, 8. Xylem vessel, 9. Multicellular uniseriated trichome, 10. Lower epidermis

Molecular Evaluation

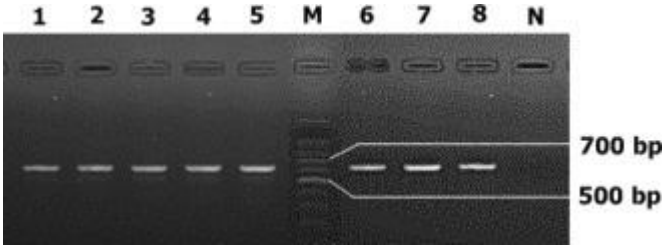

Figure 5. PCR products which dispersed in 1.5% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV transillumination.

Lane 1: KSL071001	**Lane M: 100 bp DNA ladder (Fermentas)**
Lane 2: KSL071002	**Lane 6: LPK081001**
Lane 3: KSL031103	**Lane 7: LPK091002**
Lane 4: KSL041104	**Lane 8: LPK011103**
Lane 5: KSL051105	**Lane N: negative control**

Both universal PCR primers (ITS4and ITS5) were designed from highly conserved regions flanking the ITS region and used for PCR amplification to generate the PCR product approximately 670 base pairs (bp) in size (Figure 5). Sequence analysis of *D. metel* L. var. *metel* from 3 different locations (LPK081001, LPK091002, and LPK011103) and *D. metel* L. var. *fastuosa* from 5 different locations (KSL071001, KSL071002, KSL031103, KSL041104, and KSL051105) showed 99-100% homology with 63% GC content. The multiple sequence alignment of ITS region of *D.metel* L. var. *metel*, and *D.metel* L. var. *fastuosa* was showed in Figure 6. The length of ITS1 region is 230 base pairs from position 1 to 230, 5.8S region is 169 bp from position 231 to 399, and ITS2 region is 271 bp from position 400 to 670. There were two polymorphisms within the ITS2 region and 1 indel within the ITS1 region.

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KSL071001	511	CACGTCGACG	520	611	CTTGGGCGCT	620
KSL071002	511	CACGTCGACG	520	611	CTTGGGCGCT	620
KSL031103	511	CACGTCGACG	520	611	CTTGGGCGCT	620
KSL041104	511	CACGTCGACG	520	611	CTTGGGCGCT	620
KSL051105	511	CACGTCGACG	520	611	CTTGGGCGCT	620
LPK081001	511	CCCGTCGACG	520	611	CTTAGGCGCT	620
LPK091002	511	CCCGTCGACG	520	611	CTTAGGCGCT	620
LPK011103	511	CCCGTCGACG	520	611	CTTAGGCGCT	620
		*			*	

Figure 6. Single nucleotide polymorphism (SNP) within ITS2 region (positions 512 and 614) of *D. metel* L. var. *metel* (LPK081001, LPK091002, and LPK011103) and *D. metel* L. var. *fastuosa* (KSL071001, KSL071002, KSL031103, KSL041104, and KSL051105)

*** indicate SNP**

Scopolamine Content Evaluation

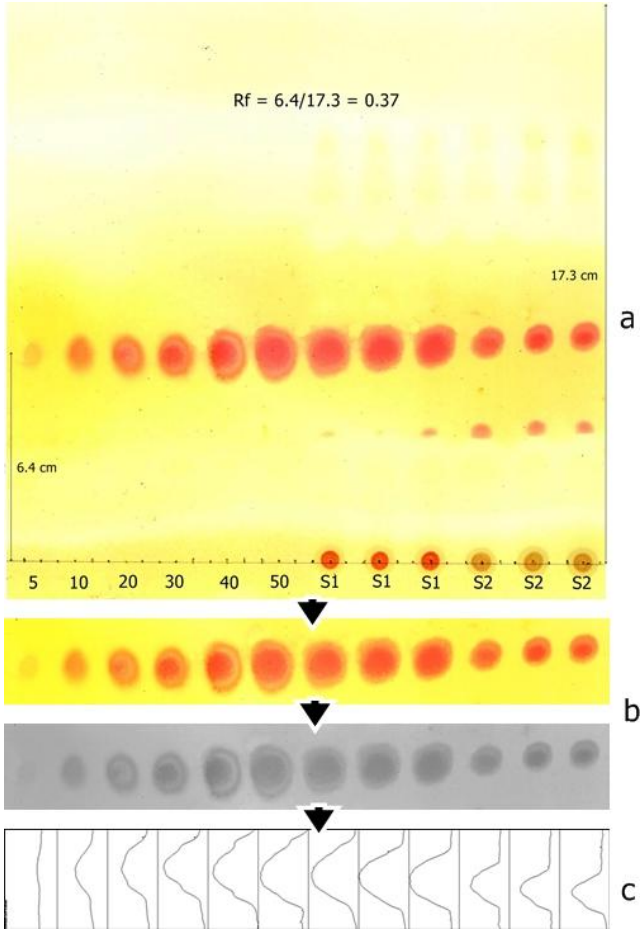

Figure 7. TLC image analysis by image J software (a); converting the image to greyscale (b); chromatogram profiles obtained from the converting image (c). (from left to right lanes: standard scopolamines at 5-50 µg/spot and triplicate of two crude extracted samples (S1,S2))

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TLC chromatogram showed the spot of scopolamine at the retention factor (Rf) of 0.37 (Figure 7) and HPLC chromatogram showed scopolamine peak at the retention time (Rt) of 5.34 min.

Table 4. Summary of validation parameters

Parameters	Method	
	TLC image	HPLC
Regression equation *	$y = -1029.5x^2 + 16405x - 5426.1$	y = 9068x + 10316
Correlation efficient	0.9994	0.9999
Detection Range	5-50 µg/spot	50 - 500 µg/ml
Limit of detection (LOD)	0.61 µg/spot	5.30 µg/ml
limit of quantitation (LOQ)	1.84 µg/spot	16.05 µg/ml
Average recovery (%)**	104.32 ± 8.87	98.64 ± 3.76
Repeatability ***	2.13 - 7.88	0.65 - 4.94
Intermediate precision ***	2.25 - 5.51	0.42 - 2.56

* y: Area, x: Concentration

** Data are mean ± SD, (n=3), with 3 concentrations level of standard

*** Relative standard deviation (% RSD, n=3), with 3 concentrations level of standard

The validation parameters of two methods was showed in Table 4. Using TLC image, the polynomial regression showed good linearity relationships (R2 > 0.99) over the range from 5-50 µg/spot for all scopolamines and the LOD and LOQ values were 0.61 and 1.84 µg/spot, respectively. For HPLC, the linear regression showed good linearity relationships (R2 > 0.99) over the range from 50-500 µg/ml for all scopolamines. The LOD and LOQ values were 5.30 and 16.05 µg/ml, respectively.

Table 5. Comparison of scopolamine content of *D.metel* L. by TLC image and HPLC methods.

	Scopolamine content of dried sample (mg/g dry weight)			
Part used	*D. metel* L. var. *metel* (n=3)		*D. metel* L.var. *fastuosa* (n=3)	
	TLC image (n=3)	HPLC (n=3)	TLC image (n=3)	HPLC (n=3)
Leaf	0.613 ± 0.554	0.560 ± 0.518	0.521 ± 0.551	0.257 ± 0.274
Flower	2.112 ± 0.738	2.036 ± 0.942	2.276 ± 0.791	2.076 ± 0.661
Fruit	1.253 ± 0.436	1.372 ± 0.590	4.067 ± 3.548	3.943 ± 3.451

Data are mean ± SD from 3 locations (triplicates)

The scopolamine contents in leaves, flowers and fruits of *D. metel* L. var. *metel* and *D. metel* L.var. *fastuosa* by using TLC and HPLC methods were shown in Table 5. Paired samples correlations showed the positively correlation between the two methods, r = 0.993 (N=18), $p = 0.05$.

DISCUSSION AND CONCLUSION

The worldwide demand of herbal medicines for human wellbeing and healthcare stimulate the needs for quality control. Correct identification of plant is an essential prerequisite to ensure the highest efficacy. There are a number of analytical tools available for medicinal plant authentication. Most of the regulatory guidelines and pharmacopoeias suggest

macroscopic and microscopic evaluation and chemical profiling of herbal materials for quality control and standardization [20, 21]. Morphological assessment is an effective tool for determining the identity of plant material as it fast and inexpensive. Based on the morphological characteristics, macroscopic observations of the entire plant require highly skilled or well trained individuals whereas microscopic observations require smaller sections of the plant such as its leaf measurement index which require only simple sample preparation and standard laboratory instruments. Leaf measurements consisting of the palisade ratio, stomatal number, stomatal index, veinislet number, and vein-islet termination number have been used for differentiation of herbal plants [22]. Therefore, the recent analytical tools based on molecular identification of DNA level have been applied for medicinal plant authentication without affected by the physiological condition and can be used at any phase of an organism's development [23]. Combinations of various analytical methods have been employed for quality assurance, control and authentication of medicinal plant species in herbal drug technology development. This present study deals with the characteristic evaluation between two varieties of *D.metel* L. by using macroscopic, microscopic and molecular methods. According to the results, macroscopic and microscopic analysis of these plants revealed the different morphology but contained almost similar cell components. Leave measurement index (stomatal number, stomata index and palisade ratio), the important property for species identification, showed different constant numbers espectially stomatal number in upper epidermis of *D. metel* L.var. *fastuosa* that found twofold higher than *D. metel* L. var. *metel*. In addition, molecular techniques have been also introduced for DNA level authentication. DNA extracted from leaves, stems or roots of plants all carry the same genetic information without being affected by physiological conditions and environmental factors. The ITS region is now perhaps the most widely sequenced DNA region in fungi, gymnosperm and angiosperm which has typically been most useful for molecular systematics at the species level, and even within species**.** This region has been widely used in plant molecular phylogenic studies due to their relatively small size (<700 bp) and high copy number of the rDNA gene which enable easy amplification even from small quantities of DNA or from herbarium materials and, due to a high degree of variation, even between closely related species which makes the ITS region an interesting site for phylogenetic investigations [24]. Comparison of ITS region sequences from eight *D. metel* L. collected from six locations were examined in this study. According to the results, two varieties of *D. metel* L. from various locations showed highly conserve sequence, especially in ITS1 and 5.8S region showed 100% similarity. There are two single nucleotide polymorphism (SNP) at positions 512 and 614 of ITS2 region could be classified these two closely related plants. According to the SNP at position 512, Restriction enzyme HpyCH41V (ACGT) can be used for differntiation these two closely related plant when performed the PCR-RFLP.

Chromatographic fingerprint is an analytical method for establishing a characteristic chemical pattern for a plant material fraction or extracts. TLC and HPLC are routinely used as valuable tools for qualitative determination of small amounts of impurities. TLC fingerprint analysis is an easy operating and time-saving method with low cost while HPLC has been used for analysis of a wide range of compounds and become the most widely applied effective separation and analysis tool for herbal products but require high cost of instrument. HPLC profiling has been used to distinguish of Panax ginseng roots produced in two different Korean

cultivation areas. [25]. In order to evaluate its accuracy and reproducibility, TLC image and HPLC method were employed in this study for comparison. Therefore, development of an economic, accurate, reproducible and convenient TLC image analysis method was also applied for rapid determination and quantitative analysis of scopolamine content of *D. metel* L. The correlation coefficient was found to be 0.999. Amount of scopolamine from leaves, flowers and fruits in *D. metel* L. var. *metel* and *D. metel* L.var. *fastuosa* from six different locations were analyzed for their scopolamine content using these two methods and the results showed good correlation ($r > 0.99$). The results of this study indicated that amount of scopolamine from flower part of *D. metel* L. var. *metel* contained high scopolamine content whereas the fruit part of *D. metel* L.var. *fastuosa* showed high scopolamine content. Because of many environmental factors can influence the scopolamine content including soil composition, soil fertilization, salinity, climate and altitude, application of plant growth regulators and hormones, insect herbivory, and plant health [26, 27].

In conclusion, authentication of herbal medicines involves many parameters including morphology, microscopy, chemical pofile and DNA fingerpringting. The combination of macroscopic, microscopic and molecular method are able to authenticate the closely related plants; *D. metel* L. var. *metel* and *D. metel* L.var. *fastuosa*. Both of them have a potency to be a source of scopolamine production.

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Author Disclosure Statement

The authors declear that there are no conflicts of interest.

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