Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(4):803-806

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Dioscorine content in *Dioscorea hispida* dried tubers in Thailand by TLC-densitometry and TLC image analysis

Nonglapat Sasiwatpaisit[1], Worathat Thitikornpong[2], Chanida Palanuvej[1*] and Nijsiri Ruangrungsi[1,3]

[1]*College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand*
[2]*Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand*
[3]*Faculty of Pharmacy, Rangsit University, Pathumthani, Thailand*

ABSTRACT

Dioscorea hispida Dennst. (Dioscoreaceae) is a valuable source of edible starch. Its dried tuber has been used as a crude drug in Thai remedy named Thoraneesanthakhat. It has traditionally been used to treat constipation. D. hispida contains neurotoxic dioscorine, an isoquinuclidine alkaloid causes nervous system paralyze. Even though there are traditional wisdoms to detoxify the tuber, emphasis on quality control of dioscorine content is essential for D. hispida development. This research was to establish dioscorine content of D. hispida dried tubers in Thailand by TLC - densitometry and image analysis. Dried tubers collected from 14 areas throughout Thailand were successively extracted in ethanol by soxhlet apparatus and analyzed for dioscorine by TLC. Aluminium oxide 60 GF_{254} neutral was used as stationary phase and methanol-chloroform (3:97) was used as mobile phase. Intensity of dioscorine under UV254 was analyzed using Camag TLC scanner 3 and Scion image software. Each sample was quantitated in triplicate. The grand average dioscorine content of the dried tuber was 0.72 ± 0.07 % w/w (mean ± 3 SD = 0.51 - 0.93 % w/w) by TLC-densitometry and 0.66 ± 0.07 % w/w (mean ± 3 SD = 0.44 - 0.88 % w/w) by TLC image analysis. Method validation showed that TLC image analysis could be altered to high cost densitometer for dioscorine quantitation. TLC image analysis for dioscorine quantitation was developed and dioscorine content in D. hispida dried tuber was established. This parameter could be used as a specification for quality control of D. hispida dried tuber in Thailand.

Keywords: *Dioscorea hispida* tubers, Dioscorine, TLC - densitometry, TLC image analysis

---

INTRODUCTION

Dioscorea hispida Dennst. (Dioscoreaceae) or wild yam is a staple subsistence food in some tropical regions of the world. It is one of medicinal plants in Thailand for a long time. Its dried tuber has been used as a crude drug in Thai remedy named Thoraneesanthakhat. It has traditionally been used to treat constipation. On the other hand, *D. hispida* is well known as a poisonous plant because it contains toxic substance, dioscorine, an isoquinuclidine alkaloid [1-3]. Behavioural studies have shown that dioscorine causes dizziness, nausea, vomiting, and later sleepiness in humans. Pharmacological studies have shown that dioscorine exhibits mydriatic activity, hyperthermia and central nervous system stimulation. The latter effect causes a nervous system paralysis [4,5]. Combination of TLC with densitometer or TLC scanner offer quantitative analysis by TLC [6-8]. On the other hand, the use of TLC image analysis has been applied as low cost alternative quantitative TLC [9-11]. The major advantages of TLC are due to its simplicity, a small quantity of solvents used, minimum sample preparation and high sample throughput. With a combination of simple computer technology and image analysis software for evaluation of TLC chromatogram, the quantitative TLC method based on image analysis is more convenient and less expensive than other chromatographic methods. Commercial and free web-based image softwares for TLC image analysis are available. Scion Image is public

computer software modified from the NIH Image software of National Institutes of Health, USA. The aim of this research was to establish the content of dioscorine in *D. hispida* dried tubers in Thailand using TLC - densitometry as well as TLC image analysis.

EXPERIMENTAL SECTION

Plant materials: Tubers of *D. hispida* were collected from 14 different locations throughout Thailand. All crude drugs were authenticated by Ruangrungsi N. then sliced and air-dried. Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand.

Dioscorine reference material: Dioscorine reference material was prepared from *D. hispida* dried tubers as follows. The tubers were extracted with 95% ethanol by cold maceration. The extract was concentrated under reduced pressure until dryness then dissolved in 5% acetic acid. The acid supernatant was filtered, made to alkaline by ammonia water then extracted with dichloromethane until the base was exhausted. The combined dichloromethane extract was evaporated under reduced pressure to give a syrupy mass which then was dissolved in a small amount of absolute ethanol. Saturated picric acid in water was added to form yellow dioscorine picrate. The dioscorine picrate was made alkaline by ammonia followed by extraction with dichloromethane until the base was exhausted. Dioscorine base was further purified by silica gel column chromatography using acetone: water: ammonia (90: 7: 10) as eluent. The purity of dioscorine was analyzed by ^{1}H and ^{13}C NMR spectrometer (500 MHz Varian INOVA, USA).

Plant extraction: *D. hispida* dried tubers were ground and successively extracted in 95 % ethanol by soxhlet apparatus. The solvents were completely removed *in vacuo*. The extracts were weighed and kept in the desiccator.

Chromatographic condition: TLC analysis was performed on TLC Aluminium oxide 60 F_{254} neutral (Merck, Germany). Five microliters of dioscorine standard solution (0.5 – 2.5 mg/ml) and 14 sample solutions (5 – 20 mg/ml) were spotted as 6.0 mm bands in length onto a same TLC plate using a Camag Linomate V syringe. A distance between each spot was 9.4 mm. The plate was then developed to a distance of 8.0 cm in a TLC chamber previously saturated with methanol-chloroform (3 : 97, *v/v*) for at least 30 minutes.

TLC-densitometry: After development, TLC plate was scanned by a CAMAG TLC scanner III with CAMAG winCATS software in the absorbance mode at 254 nm. The slit dimension was 4.00 mm × 0.30 mm and the scanning speed was 20 mm/s.

TLC image analysis: The developed TLC plate was visualized under UV 254 nm. The photo was taken using Canon, PowerShot A650 IS camera. Quantitation of dioscorine was carried out by Scion image software. The image file which saved as Tiff format was opened with Scion Image for Windows version Alpha 4.0.3.2 (Scion Corporation, Maryland, USA) [12]. The natural colour was converted to grayscale by Photoshop software. A profile plot along the chromatogram was generated using the macro Gelplot2. The peak corresponding to dioscorine was selected by the wand tool for measuring the area under the curve.

Method validation: Limit of detection (LOD) and limit of quantitation (LOQ) were determined from the calibration curve as 3.3SD/S and 10SD/S respectively where SD was the y-intercept standard deviation of regression line and S was the slope of regression line. The repeatability and intermediate precision were determined by analyzing six replicates of five different concentrations (2.5, 5.0, 7.5, 10.0 and 12.5 μg/spot) of dioscorine and expressed as percent relative deviation (%RSD). *D. hispida* sample was spiked with dioscorine and the accuracy was expressed as percentage of recovery.

RESULTS AND DISCUSSION

Standard dioscorine was prepared from dried tuber by ethanolic extraction, obtained as picrate crystallization, repeated extraction and purified by column chromatography. The purity of isolated dioscorine from *D. hispida* was examined by ^{1}H NMR and ^{13}C NMR and shown to be identical with previous reports [1-3]. The dried powder of *D. hispida* tubers from 14 different locations were extracted with 95 % ethanol to obtain crude ethanolic extract by soxhlet apparatus. The yield of ethanolic extract was 6.91 ± 1.03 % by weight. TLC using aluminium oxide 60 F_{254} neutral as stationary phase and chloroform: methanol (97: 3 v/v) as mobile phase was performed to achieve dioscorine band. A well define band of dioscorine at hRf 80 was obtained by visualization under UV 254 nm. It was converted to the peak area for quantitation by TLC-densitometric method and TLC image analysis (Figure 1-3).

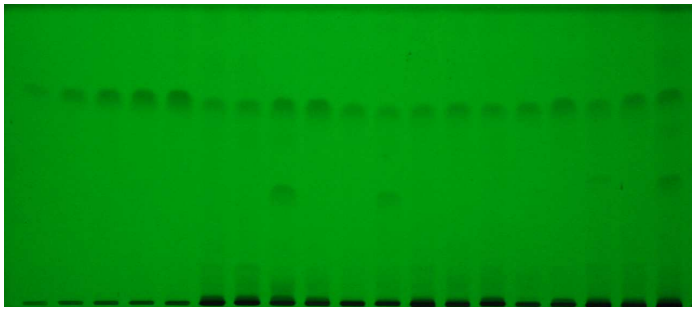

Figure 1. TLC chromatogram under UV 254 nm; (from left to right lanes) dioscorine standard 2.5, 5.0, 7.5, 10.0, 12.5 µg/spot and *D. hispida* tuber extracts from 14 different locations

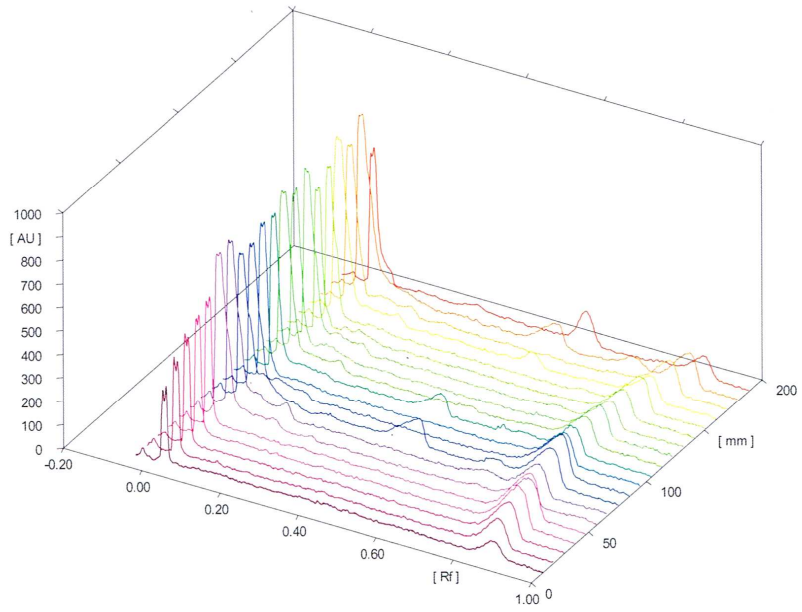

Figure 2. Three dimensional image of the scanning TLC plate by TLC-densitometry

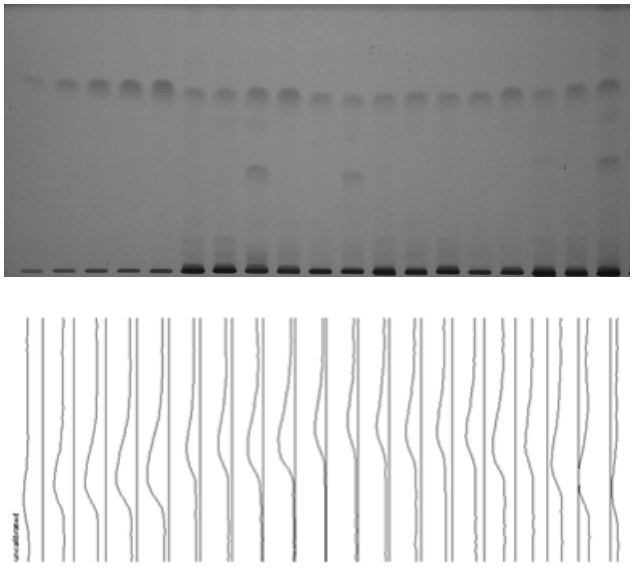

Figure 3: TLC image converted to greyscale (left) and chromatographic peak by Scion Image software (right)

By densitometry, the light reflected from a blank part of the TLC plate is measured and set as 0% absorption. When the absorbing substances are scanned, the absorption increases with increasing amount of substance. A plot of the absorption signal against the migration distance gives the absorption-position curve representing the chromatogram

[13]. By image analysis method, the acquired grayscale digital image of TLC spot is computed to obtain two-dimensional dataset (distance, accumulative gray) which can be plotted and integrated the accumulative gray value under the peak [10]. Both methods were demonstrated to be valid according to ICH guideline (Table 1). The polynomial calibration curves ranged from 2.5 – 12.5 μg/spot.

Table 1: Method validation of dioscorine analysis by TLC-densitometry and TLC image analysis

	TLC-densitometry	TLC image analysis
Calibration curve	$y = -12.901 x^2 + 943.53 x + 192.99$	$y = 0.3436 x^2 + 108.44 x + 25.52$
(Range 2.5 – 12.5 μg/spot)	($r^2 = 0.9997$)	($r^2 = 0.9997$)
LOD (μg/spot)	0.37	0.28
LOQ (μg/spot)	1.13	0.84
Repeatability (% RSD)	0.38 – 1.49	0.40 – 2.21
Intermediate precision (% RSD)	0.59 – 1.57	0.74 – 4.06
% Recovery	91.83 ± 7.21	90.31 ± 9.96

The dioscorine content of *D. hispida* dried tuber determined by TLC-densitometry and TLC image analysis were 0.72 ± 0.07 % w/w and 0.66 ± 0.07 % w/w respectively. The content of dioscorine in *D. hispida* dried tuber in this study was much higher than previously reported in fresh tuber (0.017, 0.060 and 0.12 % w/w) [1,5,14]. According to dioscorine toxicity, quality control of dioscorine content in *D. hispida* tuber is beneficial to the consumer both in medicinal and nutritional aspects. Analytical technique using conventional inexpensive TLC with a digital camera and image analysis free software is advantageous.

CONCLUSION

TLC image analysis for dioscorine quantitation was developed and dioscorine content in *D. hispida* dried tuber was established. This parameter could be used as a specification for quality control of *D. hispida* dried tuber in Thailand.

Acknowledgements
The authors are grateful to College of Public Health Sciences, Chulalongkorn University for providing research facilities. N.S. wishes to thank the Herbal Remedies and Alternative Medicine Task Force of STAR: Special Task Force for Activating Research under 100 Years Chulalongkorn University Fund and Research Fund; the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) for financial support.

REFERENCES

[1] E Leete, AR Pinder. *Phytochem*., **1972,** 11(11), 3219-24.
[2] E Leete. *Phytochem*., **1977,** 16(11), 1705-9.
[3] E Leete, RH Michelson. *Phytochem*., **1988**, 27(12), 3793-8.
[4] JL Broadbent, H Schieden. *Br. J. Pharmacol*., **1958**, 13(3), 213-5.
[5] J Webster, W Beck, B Ternai. *J. Agric. Food Chem*., **1984**, 32(5), 1087-90.
[6] M. Kachroo, SS Agrawal. *J. Chem. Pharm. Res*., **2010**, 2(1), 155-161.
[7] DA Shanbhag, AN Khandagale. *J. Chem. Pharm. Res*., **2011**, 3(1), 395-401.
[8] A Kumar, MK Bhardwaj, AK Upadhyay, A Tiwari, BD Ohdar. *J. Chem. Pharm. Res*., **2011**, 3(2), 907-910.
[9] AVI Hess. *J. Chem. Educ*. **2007**, 84, 842-847.
[10] T Tie-xin, W Hong. *J. Chromatogr. Sci*., **2008,** 46, 560-4.
[11] P Phattanawasin, U Sotanaphun, L Sriphong, I Kanchanaphibool, N Piyapolrungroj. *Silpakorn U. Science & Tech. J*. **2011**, 5 (1), 7-13.
[12] Scion Corporation. Scion image for windows version Alpha 4.0.3.2. Maryland, USA. **2000–2001.**
[13] E Hahn-Deinstrop, RG Leach (translator). Applied Thin-Layer Chromatography: Best Practice and Avoidance of Mistakes, 2nd Edition, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, **2007**; 116.
[14] J Webster, W Beck, B Ternai. *J. Agric. Food Chem*., **1984**, 32(5), 1087-90.