

Discrimination of Botanical Origin of Chan-thet, Chan-hom, Chan-chamot, Chan-khao and Chan-thana Using Chemical Test and Thin-layer Chromatography

นิพนธ์ต้นฉบับ

Original Article

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บทคัดย่อ

Abstract

วัตถุประสงค์: เพื่อพัฒนาวิธีจำแนกชนิดทางพฤกษศาสตร์ของเครื่องยาจันทน์เทศ จันทน์หอม จันทน์ชะมด จันทน์ขาว และจันทนา **วิธีการศึกษา:** ใช้แก่นของพืชที่มีโอกาสใช้เป็นเครื่องยาดังกล่าวข้างต้น ได้แก่ *Santalum album*, *S. spicatum*, *S. lanceolatum*, *Tarenna hoaensis*, *Mansonia gagei*, *Diospyros decandra* และ *Myristica fragrans* ทดสอบด้วยปฏิกิริยาเคมีต่าง ๆ และศึกษาลายพิมพ์ขององค์ประกอบทางเคมีด้วยเทคนิคโครมาโทกราฟีแบบชั้นบาง (ทีแอลซี) **ผลการศึกษา:** พืชแต่ละชนิดให้ผลการทดสอบทางเคมีแตกต่างกัน และมีทีแอลซีโครมาโทแกรมเป็นเอกลักษณ์ไม่เหมือนกัน **สรุป:** สามารถจำแนกแก่นของพืชทั้ง 7 ชนิดด้วยการทดสอบกับสารละลาย vanillin/กรด sulfuric การทดสอบกับสารละลาย 10% potassium hydroxide และการทดสอบด้วยวิธี Liebermann-Burchard แล้วยืนยันผลด้วยทีแอลซีโครมาโทแกรมที่ใช้ส่วนผสมของ hexane-ethyl acetate-methanol (60:30:0.2) และส่วนผสมของ dichloromethane-methanol-formic acid (60:10:1) เป็นวัฏภาคเคลื่อนที่ แล้วตรวจสอบผลด้วยน้ำยาพ่น vanillin/กรด sulfuric

คำสำคัญ: จันทน์เทศ, จันทน์หอม, จันทน์ชะมด, จันทน์ขาว, จันทนา, การทดสอบทางเคมี, โครมาโทกราฟีแบบชั้นบาง

Objective: To develop the method to discriminate the botanical origin of crude drugs named Chan-thet, Chan-hom, Chan-chamot, Chan-khao and Chan-thana. **Methods:** Heartwoods of *Santalum album*, *S. spicatum*, *S. lanceolatum*, *Tarenna hoaensis*, *Mansonia gagei*, *Diospyros decandra* and *Myristica fragrans* which were likely used as above mentioned crude drugs, were examined with various chemical test reactions. Their chemical fingerprints using thin-layer chromatography (TLC) were also studied. **Results:** Each plant species gave different results on chemical tests and possessed unique TLC chromatograms. **Conclusion:** Heartwoods of all seven plant species could be discriminated by the reactions with vanillin/sulfuric acid, 10% potassium hydroxide solution and Liebermann-Burchard test. The results were confirmed by their TLC chromatograms developed from hexane-ethyl acetate-methanol (60:30:0.2) and dichloromethane-methanol-formic acid (60:10:1) as mobile phases and detected with vanillin/sulfuric acid spraying reagent.

Keywords: Chan-thet, Chan-hom, Chan-chamot, Chan-khao, Chan-thana, chemical test, thin-layer chromatography

Introduction

“Chan” is the name of many Thai crude drugs. This study focused on the crude drugs which are heartwoods with white, whitish or grayish color, named Chan-khao, Chan-thet and Chan-chamot. These crude drugs are important ingredients of many Thai traditional medicinal formulae. In the List of Herbal Medicinal Products A.D. 2018¹, recipes containing these crude drugs were used for two symptomatic groups, i. e. cardiovascular disorders (Yahom-thiposot, Yahom-theppajit, Yahom-navakot, Yahom-kaewingwien and Yahom-inthrajak) and fevers (Ya-khiao-hom, Ya-chantaleela, Ya-prasa-chandaeng, Ya-prasa-praohyai and Mahanin-tangthong).¹ Chan-thet is also used in two anti-flatulence recipes (Yaa Mantha-dhatu and Ya-apaisalee).

In addition to these three crude drugs, two other related crude drugs named Chan-hom and Chan-thana, are available

in Thai traditional drugstores. Without experience, macroscopic characters of these five crude drugs are difficult to identify. Their nomenclature system was also confusing and different various botanical origins were mentioned in textbooks.

Heartwoods of six plant species were uncertainly suggested as their original sources, i.e. *Mansonia gagei* (Chan-chamot, Chan-hoom, Chan-khao, Chan-phama), *Tarenna hoaensis* (Chan-khao Chan-thana, Chan-ta-nia, Chan-bai-lek), *Santalum album* (Mai-hom-india, sandalwood), *Aglaia silvestris* (Chan-chamot), *Diospyros decandra* (Chan-khao, Chan-in, Chan-o, Chan-luk-hom) and *Myristica fragrans* (Chan-thet, Chan-ban, Nutmeg tree).²⁻¹⁰ However, our previous studies found that three latter plant species were not currently available in Thai traditional drugstores. In addition to

S. album, heartwoods of the other two *Santalum* species, i.e. *S. spicatum* and *S. lanceolatum*, were also used as Chan-thet and Chan-hom. Some unknown species were also found as Chan-khao and Chan-thana. Most of Chan-thet and Chan-hom were used as substitutes, whereas most of Chan-khao and Chan-thana were obtained from the same plant species.

All Chan crude drugs were mostly originated from more than one plant species. Most of Chan-thet and Chan-hom were heartwoods of three *Santalum* species (*S. album*, *S. spicatum* and *S. lanceolatum*). Most of Chan-khao and Chan-thana were heartwoods of *T. hoaensis*, whereas Chan-chamot was heartwood of *M. gagei*.¹¹⁻¹³ Heartwoods of these plants possessed different chemical compositions. *Santalum* had volatile oil possessing sesquiterpenes such as santalol derivatives, as the main constituents.¹⁴ *T. hoaensis* has only been reported for geniposidic acid¹¹, whereas the chemical compositions of *M. gagei* were phenolics^{15,16} and chromones.¹²

Correct identification of crude drugs is an important factor affecting the quality of medicine. Unfortunately, Chan-thet, Chan-hom, Chan-chamot, Chan-khao and Chan-thana are currently not botanically specified. Therefore, there has been a need to correctly identify botanical sources of these crude drugs for their correct use. This study aimed to develop simple methods to discriminate the possible botanical origins of these crude drugs. Chemical test and thin-layer chromatography (TLC) were the suggested techniques.

Methods

Plant materials

Two authentic samples of the heartwood of *S. album*, were obtained from two sources namely Professor Dhanushka S. Hettiarachchi, Wescorp Group of Companies, Western Australia, and Prachuap Khiri Khan Silvicultural Research Station, Royal Forest Department of Thailand; while two samples of both *S. spicatum* and *S. lanceolatum* were provided solely by Prof. Hettiarachchi. Authentic samples of the heartwood of *T. hoaensis*, *M. gagei*, *D. decandra* and *M. fragrans* were from the Medicinal Plant Research Institute, Department of Medical Sciences. All samples were ground and sieved through 60 mesh sieve.

Chemical tests

Three grams of the sample were extracted with 20 mL of water or 80% ethanol on a water bath for 5 minutes and

filtered. The test methods were modified from those described in Fransworth's work¹⁷, as follows.

1. Test with ferric chloride test solution (9% FeCl₃ TS). A few drops of ferric chloride test solution were added into 1 mL of the extract. The compounds with the phenolic functional group will produce a blue, green or brown to blackish color.

2. Test with 2% gelatin solution. A few drops of 2% gelatin solution were added into 1 mL of the extract. Tannins will produce white opaque precipitates.

3. Foam test. One mL of the extract was diluted with 1 mL of distilled water and then vigorously shaken. Saponins will produce persistent foam which lasts for at least 15 minutes.

4. Shinoda test. Two pieces of magnesium ribbon and 3 drops of conc. hydrochloric acid were subsequently added into 1 mL of the extract. Flavonoids will produce a reddish-orange or magenta color.

5. Test with Dragendorff's reagent. Two mL of the extract was evaporated to dryness, and re-dissolved with 2 drops of 10% sulfuric acid solution. The clear solution was transferred and tested with 1 drop of Dragendorff's reagent. Alkaloids will produce orange-red precipitates.

6. Liebermann-Burchard test. Two mL of the extract was evaporated to dryness, and re-dissolved with 2 drops of acetic anhydride. One drop of conc. sulfuric acid was added. Steroids and triterpenoids will produce a bluish-green and a purplish-red or brownish-red color, respectively.

7. Test with vanillin/sulfuric acid. One drop of 1% vanillin solution in ethanol and 1 drop of conc. sulfuric acid was subsequently added into 0.25 mL of the extract. In general, terpenoids and phenolic compounds will produce a purple and a red color, respectively.

8. Test with conc. sulfuric acid. One drop of conc. sulfuric acid was added into 0.5 mL of the extract. In general, terpenoids will produce a purple color.

9. Test with Kedde's reagents. Two drops of Kedde A reagent were added into 1 mL of the extract. The mixture was mixed well, and subsequently added with a few drops of Kedde B reagent. The compound with the unsaturated lactone ring will immediately produce a purple color.

10. Modified Borntrager test. Six mL of the extract was concentrated to 3 mL, added with 3 mL of 10% sulfuric acid solution and boiled for 2 minutes. The mixture was partitioned with 1 mL of ethyl acetate. The ethyl acetate extract was transferred into a test tube and vigorously shaken with 1 mL

of 10% potassium hydroxide solution. Anthraquinones will produce a pinkish-red color in potassium hydroxide layer.

11. Test with 10% potassium hydroxide solution. One drop of 10% potassium hydroxide solution was added into 0.5 mL of the extract. The compounds with conjugated π -bonds may change in color.

Thin layer chromatography

Five hundred milligrams of the sample were extracted with 3 mL of methanol by sonication for 30 minutes. The supernatant was used as a sample solution. Twenty μ L of the sample solution was applied as a 10 mm band by Linomat 5 (Camag, Switzerland) on the silica gel 60F 254 thin-layer chromatography plate (Merck 5554). The plate was placed in a chromatography tank saturated with mobile phase: (1) hexane- ethyl acetate- methanol (60: 30: 0.2) or (2) dichloromethane- methanol- formic acid (60: 10: 1). The developing distance was 10-cm long. After air-drying, the chromatogram was detected under UV at 254 and 366 nm, and visually observed after spraying with (1) 1% vanillin solution in ethanol, over sprayed with 5% sulfuric acid solution in ethanol and heated at 110 °C for 5 minutes, or (2) 5% potassium hydroxide solution in ethanol (immediately observed the result), or (3) 2% acetic anhydride solution (freshly prepared), over sprayed with 5% sulfuric acid solution in ethanol and heated at 110 °C for 5 minutes.

Results and Discussions

Chemical tests

The chemical reaction is a simple technique for screening phytochemicals in plant materials. It is one of the methods used for crude drug identification according to the Herbal Pharmacopoeia.¹⁸ This study examined the heartwoods of *S. album*, *S. spicatum*, *S. lanceolatum*, *M. gagei* and *T. hoaensis*, which are the sources of crude drugs named Chan-thet, Chan-hom, Chan-chamot, Chan-khao and Chan-thana. Water and 80% ethanol were used as the solvents for sample preparation dependent on the solubility of the tested phytochemicals. Various chemical reactions were tested and the suitable ones that could discriminate samples were selected. In addition, to obtain the most specific methods, heartwoods of *M. fragrans* and *D. decandra* were also included into the experiment. Even though they were not currently used, they were possibly

confused with the heartwoods of the above species. The results are shown in Table 1. All samples gave negative or only weak positive results to gelatin solution, conc. sulfuric acid, Dragendorff's reagent, Kedde's reagents, foam test, Shinoda test and modified Borntrager test. Tests giving clear positive results of each plant sample could be described as follows.

Heartwoods of all three *Santalum* species in this study gave an obvious magenta color with vanillin/sulfuric acid. It was expected to be the positive reaction for terpenoids¹⁹, which are the main constituents in volatile oils of *Santalum*.²⁰ All three *Santalum* samples were also positive to ferric chloride solution in accordance with their phenolic constituent.²⁰

Liebermann-Burchard test gave the distinguished results among three *Santalum* species. *S. spicatum* produced an intense greenish or bluish color mixed with a pale purplish-red color, whereas it was a pale purplish red color for *S. album* and a pale mixed color of purplish red and green for *S. lanceolatum*.

Liebermann-Burchard is the test for steroids and triterpenoids. However, there has never been a report of these compounds from the heartwoods of these plant species. To prove whether it was the false positive reaction with conc. sulfuric acid without the need for acetic anhydride, tests using only conc. sulfuric acid were confirmed. The results of *S. album* and *S. lanceolatum* were a pale purple color, whereas *S. spicatum* was negative. Therefore the test results of Liebermann-Burchard test was not false positive and could distinguish *S. spicatum* from the other two *Santalum* species.

The other confirmatory test was the reaction with 10% potassium hydroxide solution. Although all *Santalum* samples produced a reddish-brown color, the result of *S. lanceolatum* was the strongest and could differentiate *S. lanceolatum* from the others. A replicate of all *Santalum* authentic samples was tested and similar results were confirmed.

Heartwood of *T. hoaensis* gave positive results to ferric chloride solution and Liebermann-Burchard test in accordance with its previously phytochemical screening report.²⁰ There has been a lack of study on the heartwood of this plant. Geniposidic acid, which is an iridoid or monoterpene lactone, was the only report of its chemical constituent.¹¹ It was expected to give the positive clear purple color to vanillin/sulfuric acid. *T. hoaensis* also gave a bright yellow color to 10% potassium hydroxide solution, but the chemical

Table 1 Test results of chemical tests (-, +, ++, +++ and ++++ are negative, weakly positive, positive, clearly positive and strongly positive, respectively).

Test	<i>S. album</i>	<i>S. spicatum</i>	<i>S. lanceolatum</i>	<i>T. hoensis</i>	<i>M. gagei</i>	<i>M. fragrans</i>	<i>D. decandra</i>
Water extract							
FeCl ₃ TS	++ (brown)	++ (brown)	++++ (greenish-black)	+++ (brown)	+++ (brown)	+++ (brown)	+ (brown)
Gelatin solution	-	-	+	-	-	++	-
Foam test	-	+	+	-	-	+	+
80% Ethanol extract							
FeCl ₃ TS	++++ (blackish)	++++ (blackish)	++++ (blackish)	++++ (blackish)	++++ (blackish)	++++ (blackish)	+ (brown)
Vanillin/H ₂ SO ₄	++++ (magenta)	++++ (magenta)	++++ (magenta)	+++ (purple)	-	++++ (reddish-pink)	-
10% KOH	+ (reddish-brown)	+ (reddish-brown)	+++ (reddish-brown)	+++ (bright yellow)	+++ (brownish-green)	-	-
Liebermann-Burchard test	+ (purplish red)	++++ (greenish or bluish and pale purple)	+ (purplish-red and green)	+++ (green)	-	++++ (greenish-blue)	+++ (purplish-red brown)
Conc. H ₂ SO ₄	+ (purple)	-	+ (purple)	++ (green)	-	+++ (purple)	-
Shinoda test	-	-	-	-	-	+ (reddish-orange)	-
Dragendorff's reagent	-	-	-	-	-	-	-
Kedde's reagent	-	-	-	-	-	-	-
Modified Borntrager test	-	-	-	-	-	-	-

composition responsible for this reaction could not be deduced. However, both tests with vanillin/sulfuric acid and potassium hydroxide solution could distinguish the heartwood of *T. hoensis* from the other samples.

Heartwood of *M. gagei* showed a positive result to ferric chloride solution in accordance with its previous reports of phenolic compounds such as acetovanillone¹⁵, and mansoxetane.¹⁶ The reaction with 10% potassium hydroxide solution produced a unique brownish-green color mixed with a slightly brownish-red color, distinctly distinguished it from the other samples. Chromones, the main yellowish substances found in the heartwood of this plant, such as mansonone G¹², was expected to produce this positive color.

Heartwood of *M. fragrans* gave clear positive results to several tests specifically a blackish or brown color to ferric chloride solution, a greenish-blue color to Liebermann-Burchard test, a reddish-pink color to vanillin/sulfuric acid and a purple color to conc. sulfuric acid. Phenolic compounds and steroids were suggested from these results. However, a

phytochemical study of the heartwood of this plant has never been reported.

Heartwood of *D. decandra* was the only sample that did not clearly show any positive result to ferric chloride solution. This result indicated that its main chemical composition was not phenolics. However, an intense purplish-red brown color to Liebermann-Burchard test suggested the presence of triterpenoids corresponding with its previous report.²¹

Chemical fingerprints by thin-layer chromatograms

Identification of crude drugs based on the chemical test is only a preliminary method, thus confirmation using chemical fingerprint is necessary. In general, thin-layer chromatography is the technique mentioned in pharmacopoeia. Although there already have been publications of TLC fingerprints of the heartwoods of these seven plant species¹¹⁻¹³, but the detection spraying reagent used was anisaldehyde of which preparation is quite complex. Detection under ultraviolet light also could not discriminate them (Figure 1). The objective of this study was to develop a simple and more efficient detection method

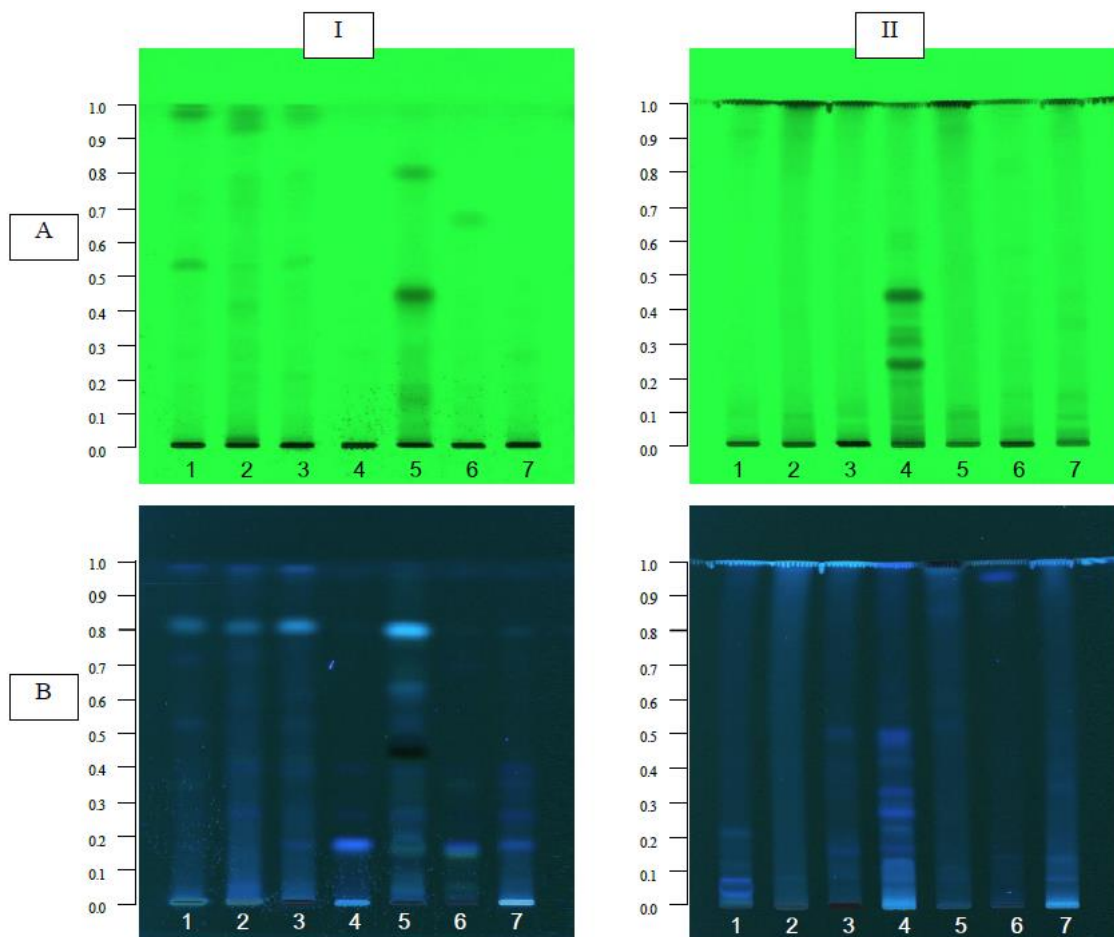


Figure 1 TLC chromatograms using mobile phase (I) hexane-ethyl acetate-methanol (60:30:0.2) and (II) dichloromethane-methanol-formic acid (60:10:1), detected under ultraviolet light at (A) 254 nm and (B) 366 nm.

Track 1 = *S. album*, 2 = *S. spicatum*, 3 = *S. lanceolatum*, 4 = *T. hoensis*, 5 = *M. gagei*, 6 = *M. fragrans*, and 7 = *D. decandra*

to discriminate all seven plant samples by modifying from the results of the chemical test. Three spraying reagents were examined, i.e. vanillin/sulfuric acid solution, 10% potassium hydroxide solution and acetic anhydride/sulfuric acid solution.

Chromatographic system was referred to previous works.¹¹⁻¹³ The mixtures of hexane-ethyl acetate-methanol (60: 30: 0.2) and dichloromethane-methanol-formic acid (60: 10: 1) were used for lower and higher polarity chemical compositions, respectively. The results indicated that potassium hydroxide solution was not a suitable spraying reagent regardless of mobile phases used (Figures 2AI and 2AII). It gave a darker color with only the substances at the base of all chromatograms. Only the yellow substance at R_f 0.42 of *M. gagei* turned greyish-green similar to the result of the direct reaction of its extract with potassium hydroxide solution. Bright yellow positive color of *T. hoensis* to potassium hydroxide solution in the chemical test was

observed as a pale yellowish mark on the chromatogram with no separation of the band.

All samples gave clear, unique chromatograms with vanillin/sulfuric acid spraying reagent. In hexane-ethyl acetate-methanol (60: 30: 0.2) mobile system (Figure 2BI), two sample sets of *Santalum* heartwoods showed the same pattern (only the result of one sample set was showed in all figures). At the lower polarity region which was the substances in volatile oils, *S. album* had two distinct bands. The most obvious one was at R_f 0.78. Four distinct bands were observed for *S. spicatum*. Two were purple bands at R_f 0.75 and 0.98, and the other two were grayish-blue bands at R_f 0.7 and 0.9. *S. lanceolatum* had only one distinct purple band at R_f 0.75. In the region of R_f less than 0.5, TLC patterns of all three *Santalum* species were similar, except that *S. spicatum* possessed an additional distinct brown band at R_f 0.3. This mobile phase system was also suitable for the identification of *M. gagei* and *M. fragrans*. The yellowish substance of *M. gagei* at R_f 0.42 was slightly

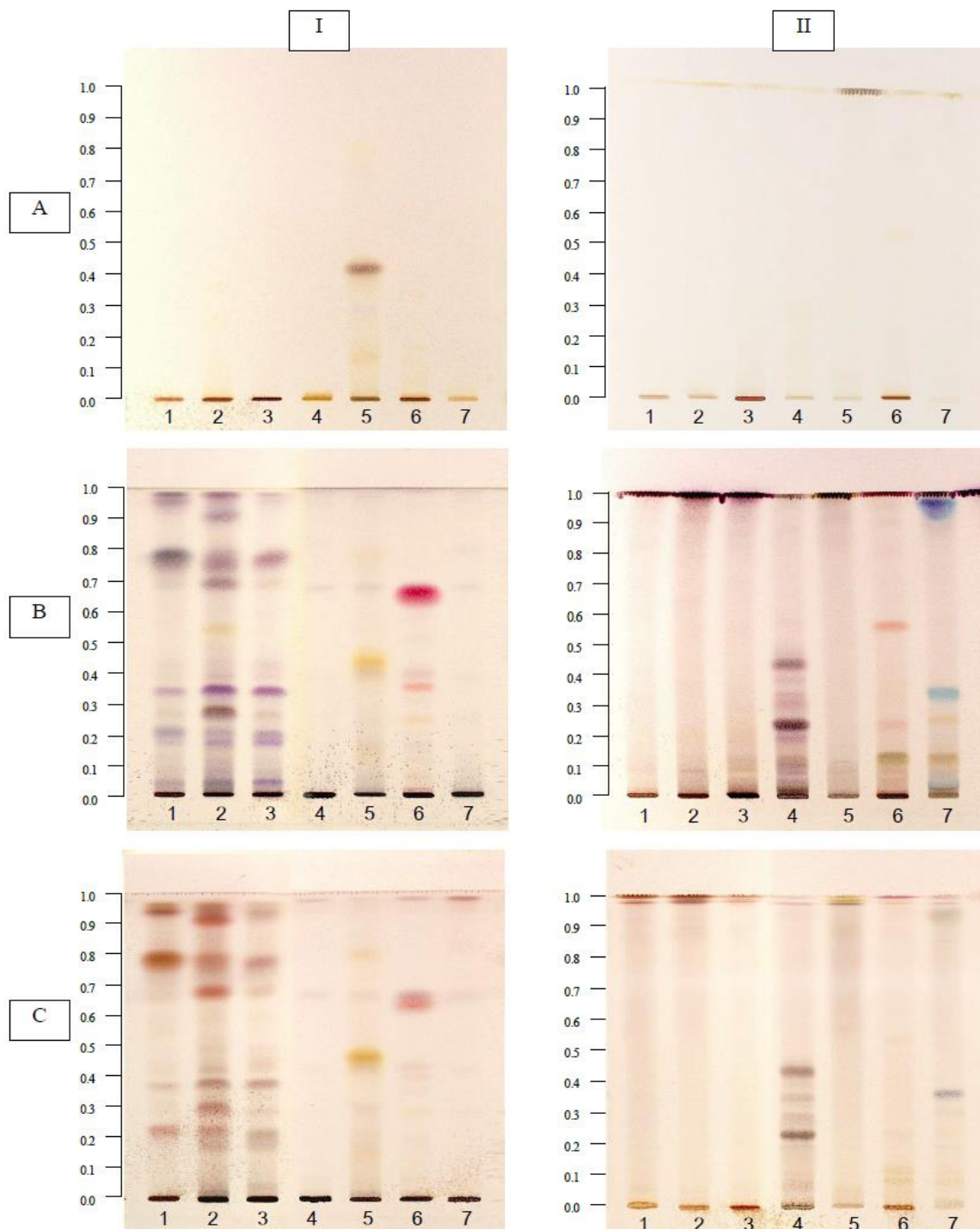


Figure 2 TLC chromatograms using mobile phase (I) hexane-ethyl acetate-methanol (60:30:0.2) and (II) dichloromethane-methanol-formic acid (60:10:1), detected with (A) 10% potassium hydroxide solution, (B) vanillin/sulfuric acid solution, and (C) acetic anhydride/sulfuric acid solution.

Track 1 = *S. album*, 2 = *S. spicatum*, 3 = *S. lanceolatum*, 4 = *T. hoensis*, 5 = *M. gagei*, 6 = *M. fragrans*, and 7 = *D. decandra*

darker and other bands were also appeared in light color, whereas *M. fragrans* possessed an obvious reddish-pink band at R_f 0.65 consistent with its chemical test result. The mobile phase system of dichloromethane-methanol-formic acid (60:10:1) (Fig. 2BII) was suitable for the identification of *T. hoensis* and *D. decandra*, suggesting high polarity of their chemical constituents. *T. hoensis* had some brownish-purple

bands in accordance with its chemical test result. *D. decandra* possessed two distinctly bright blue bands at R_f 0.35 and 0.95. However, this positive color was not detected when its extract was directly tested. Other chemical constituents of *D. decandra* might interfere with the reaction.

The use of acetic anhydride/sulfuric acid solution as a spraying reagent gave the similar TLC patterns to those of

vanillin/sulfuric acid solution, but less clear. Nearly all of the bands were brown (Figures 2CI and 2CII). These results were not in correspondence with the chemical test of which Liebermann-Burchard test gave clear different positive colors to each sample. An ethanol dilution for spray preparing possibly interfered with the reaction. To confirm by testing the sample extracts diluted in a small quantity of ethanol with Liebermann-Burchard test, the results were also not clear. Therefore, acetic anhydride/sulfuric acid solution was not the suitable detection spraying reagent.

Conclusion

Heartwoods of seven plant species that are likely to be used as crude drugs named Chan-thet, Chan-hom, Chan-chamot, Chan-khao and Chan-thana, were discriminated from each other by three chemical tests, i. e. the test with vanillin/ sulfuric acid, 10% potassium hydroxide test and Liebermann- Burchard test. Heartwoods of three *Santalum* species and *M. fragrans* were distinguished from the others by testing with vanillin/sulfuric acid. Their positive colors were magenta and reddish- pink, respectively. Among *Santalum* species, *S. spicatum* was further identified by its intense greenish or bluish positive to Liebermann- Burchard test, whereas *S. lanceolatum* gave reddish- brown color to 10% potassium hydroxide solution, but *S. album* gave unclear results to both reactions. Identification of *M. fragrans* was confirmed by its greenish- blue positive to Liebermann- Burchard test. Heartwoods of *T. hoaensis* and *M. gagei* were discriminated from other samples by the test with 10% potassium hydroxide solution, giving unique bright yellow and brownish- green colors, respectively. Identification of *T. hoaensis* was further confirmed by its positive green color to Liebermann- Burchard test. Among all samples, heartwood of *D. decandra* was the only one that gave a purplish- red brown color to Liebermann- Burchard test. TLC method was also developed to confirm the identification of all plant species. The mixture of hexane-ethyl acetate-methanol (60: 30: 0.2) was the mobile phase for three *Santalum* species, *M. gagei* and *M. Fragrans* while the mixture of dichloromethane- methanol- formic acid (60: 10: 1) was used for *T. hoaensis* and *D. decandra*. The suitable detection was vanillin/ sulfuric acid spraying reagent. Each plant species possessed a unique chemical chromatogram. Both chemical test and TLC methods

were suggested to use together for the identification of the crude drugs named “Chan” according to their botanical origins.

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Editorial note

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