

# การตรวจหาชนิดของจันทน์แดงที่วางจำหน่ายในร้านขายส่งสมุนไพรในพื้นที่กรุงเทพมหานครและ ปริมณฑล โดยใช้ลายซึบจากเทคนิค HPLC

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## บทคัดย่อ

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โดยใช้ลายซึบจากเทคนิค HPLC

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สมุนไพรเป็นความหวังของคนจำนวนมากที่ต้องการพึ่งพาตนเองในการนำมาใช้บำบัดโรคทดแทนยาแผนปัจจุบัน แต่อุปสรรคในการใช้ยาสมุนไพรมีมาก อาทิ สมุนไพรที่มีความแปรปรวนของคุณภาพวัตถุดิบ ความถูกต้องของชนิดและส่วนของสมุนไพรที่นำมาใช้ ความสับสนของชื่อที่ใช้เรียกโดยเฉพาะสมุนไพรที่มีลักษณะภายนอกคล้ายคลึงกัน **วัตถุประสงค์** การพิสูจน์เอกลักษณ์เพื่อจำแนกชนิดจันทน์แดงที่มีจำหน่ายในลักษณะผงแห้งบดละเอียดสมุนไพรในชื่อแก่นจันทน์แดงในร้านขายส่งสมุนไพรในเขตกรุงเทพมหานครและปริมณฑล ว่าเป็นแก่นจันทน์แดง (*Pterocarpus santalinus* L.f.) หรือแก่นลักจั่น (*Dracaena cochinchinensis* (Lour.) S.C.Chen) **วิธีดำเนินการวิจัย** เรียกชื่อตัวอย่างสมุนไพรในชื่อ แก่นจันทน์แดงจากร้านขายส่งในเขตกรุงเทพมหานครและปริมณฑล แยกสารของตัวอย่างโดย gradient elution ด้วยเครื่อง HPLC ที่มี diode array detector นำข้อมูลของโครมาโทแกรมของตัวอย่างที่ได้มาเปรียบเทียบกับข้อมูลของโครมาโทแกรมของสมุนไพรที่ใช้เป็นมาตรฐานซึ่งทราบชนิดแน่นอนจากการผ่านการพิสูจน์เอกลักษณ์โดยวิธีทางเภสัชเวท **ผลการวิจัย** ลายซึบจากเทคนิค HPLC ของแก่น *Pterocarpus santalinus* L.f. มีความแตกต่างอย่างมากกับของแก่น *Dracaena cochinchinensis* (Lour.) S.C.Chen ในขณะที่ลายซึบจากเทคนิค HPLC ของทั้งหกตัวอย่างมีความคล้ายคลึงมากกับของแก่น *Dracaena cochinchinensis* (Lour.) S.C.Chen **สรุป** ตัวอย่างแก่นจันทน์แดงทั้งหมดที่สุ่มซื้อมาเป็นแก่นลักจั่น (*Dracaena cochinchinensis* (Lour.) S.C.Chen)

**คำสำคัญ:** จันทน์แดง, ลักจั่น, ชื่อพ้อง, ลายซึบจากเทคนิค HPLC

## Identification of “Chan-daeng” Sold in Wholesale Herbal Stores in Bangkok and Vicinity areas by using HPLC Fingerprint

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### Abstract

#### Identification of “Chan-daeng” Sold in Wholesale Herbal Stores in Bangkok and Vicinity areas by using HPLC Fingerprint

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The use of herb products is the hope for many people who prefer self-reliance in the treatment of diseases in substitution of modern medicines. However, there are many obstacles in the use of herbal medicines in particular the variation of the quality of the raw materials, the species and the used part of the herb. The confusion is also from homonym of the herbs, especially the herbs which have similar external characteristics. **Objectives:** To distinguish the species of Chan-daeng dried wood powder from wholesale herbal stores in Bangkok and vicinity areas whether it is *Pterocarpus santalinus* L.f. or *Dracaena cochinchinensis* (Lour.) S.C.Chen (*Dracaena loureiri* Ganep.). **Method:** The samples were purchased by asking in Thai common name of the herb as “Chan-daeng” from the herbal wholesale stores in Bangkok and vicinity areas. HPLC coupled with a diode array detector was used for the separation. Mobile was set as gradient elution. Chromatograms of the samples were compared to that of the known species herbs which were previously identified by pharmacognosy method. **Results:** The HPLC fingerprint obtained from *Pterocarpus santalinus* L.f. was significantly difference from that of *Dracaena cochinchinensis* (Lour.) S.C.Chen. When compared to the standards, the HPLC fingerprints obtained from all samples were similar to the HPLC fingerprint of *Dracaena cochinchinensis* (Lour.) S.C.Chen. **Conclusion:** All samples purchased in the name of Chan-daeng were *Dracaena cochinchinensis* (Lour.) S.C.Chen.

**Keywords:** Chan-daeng, *Dracaena cochinchinensis* (Lour.) S.C.Chen, *Dracaena loureiri* Ganep., herbals homonym, HPLC fingerprint, *Pterocarpus santalinus* L.f.

## Introduction

Thai traditional herbal formula usually are composed of many species of herbs. One of the most frequently used components is Chan-daeng. Chan-daeng exhibits several indications including, antipyretic, anti-inflammatory and cardiotonic. Thai Herbal Pharmacopoeia Volume IV (2014) defines Chan-daeng as *Pterocarpus santalinus* L.f.. It is belong to the family of Leguminosae. The common names are red sandalwood, red saunders, red santal and ruby wood. It can be found naturally in the southern part of India. However, it is classified as endangered species listed by the International Union for Conservation of Nature (IUCN), and thus the price is high. A literature survey from Picheansoonthon and Vichai (2004) reported that the original identity of Chan-daeng was the imported red heartwood of *Pterocarpus santalinus* L.f.. Due to the expense and rarity, the two native substitutes of Thailand, *Dracaena loureiri* Ganep. and *Myristica iners* Blume, has been used. However, the therapeutic efficacy of these substitutes is still unknown. Regarding to the Thai common name of both species and the homonyms of their red dried powders, the question is raised to find out whether Chan-daeng in the Thai herbal markets is *Pterocarpus santalinus* L. f. or *Dracaena cochinchinensis* (Lour.) S.C.Chen. The presence of various chemical components in the heartwood of *Pterocarpus santalinus* L.f., such as carbohydrates, steroids, anthocyanins, saponins, tannins, phenols, triterpenoids, flavonoids, glycosides, and glycerides was reported. Pterocarpol, santalins A and B, pterocarptriol, ispterocarpolone, pterocarpo-diolones with  $\beta$ -eudeslols and cryptomeridol were also observed (Arunakumara *et al.*, 2011). Likhitwitayawuid *et al.*, 2002 reported the chemical constituents isolated from the stem wood of *Dracaena loureiri*. The constituents are loureiriol, eight known flavonoid and stilbenoid derivatives, including 5,7-dihydroxy-3-(4-hydroxybenzyl)-4-chromanone, 4,4'-dihydroxy-2,6-dimethoxydihydrochalcone, 2,4'-dihydroxy-4,6-dimethoxydihydrochalcone, 4'-hydroxy-2,4,6-trimethoxydihydrochalcone, 4,6,4'-trihydroxy-2-methoxydihydrochalcone, 4,3',5'-trihydroxy-stilbene, 4,3'-dihydroxy-5'-

methoxystilbene and 4-hydroxy-3',5'-dimethoxystilbene. The purpose of this study was to use HPLC gradient elution technique to separate the chemical constituents of the herbs. The HPLC fingerprints obtained from the same chromatographic condition were then compared. Chromatograms of *Pterocarpus santalinus* L. f. and *Dracaena cochinchinensis* (Lour.) S.C.Chen standards were used as a tool to distinguish the species of Chan-daeng sold in the herbal wholesale shops located in Bangkok and vicinity areas.

## Material and Methods

### Chemicals and reagents

HPLC-grade acetonitrile and methanol were purchased from Merck Co., (Merck, Darmstadt, Germany). Formic acid and tetrahydrofuran (AR grade) were obtained from Carlo Erba and Fisher Chemicals, respectively. Double distilled water was used throughout the experiment.

### Instrument

High-performance liquid chromatography analysis was carried out on a Shimadzu system, equipped with 3 high pressure pumps (LC-20AD), vacuum degasser (DGU-20A5R), autosampler (SIL 20A-80), column oven (CTO 20AC), a diode array detector (SPD-M20A) and the CBM-20A System Controller. All data acquired were proceeded by LabSolutions software (Shimadzu Japan).

### Chromatographic condition

Chromatographic separation was performed at 30°C using a Hypersil ODS column (4.0 × 250 mm, 5 µm) with a guard column supplied by Agilent Technologies. The mobile phase consisted of the mixture of 0.05% phosphoric acid in water (A), 3% tetrahydrofuran in methanol (B) and 3% tetrahydrofuran in acetonitrile (C). The gradient program was as follows; 0-15 min, 35% C; 15-23 min, 45% C; 23-35 min, 55% C; 35-37 min, 95% C; 37-40 min, 95% C; 40-45 min 35% C, when B was keep constant ratio at 5%. The flow rate was 1.0 mL/min and the detection was optimized at a wavelength of 254 nm.

### Authentic plant material

The dried wood powders of *Pterocarpus santalinus* L.f. and *Dracaena cochinchinensis* (Lour.) S.C.Chen were kindly donated from Assoc. Prof. Suppachai Tiyaoranant, Faculty of Pharmaceutical Sciences, Khonkaen University, Thailand. They have been previously identified following Thai Herbal Pharmacopoeia Volume IV (2014) page 27- 37 and page 56- 65 using macroscopic and microscopic methods, reported elsewhere.

### Sample plant material

Six samples were purchased by asking for “Chan-daeng” dried wood powder from six wholesale herbal stores in Bangkok and vicinity areas during 9-16 September, 2015. They were kept dry before analysis.

### Extraction and preparation of the crude extract

0.1 gram of the dried wood powder of authentic *Pterocarpus santalinus* L. f. , authentic *Dracaena cochinchinensis* (Lour.) S.C.Chen and all samples were accurately weighed into separate test tubes with screw caps. They were macerated in 10 mL of methanol. To increase the efficiency of the extraction, 10 minutes of ultrasonication without heat was used each day for 5 days. The extract was filtered through a 0.45 µm PTFE (polytetrafluoroethylene) syringe filter (Waters, Milford, MA, USA) before 10 µL injecting into an HPLC.

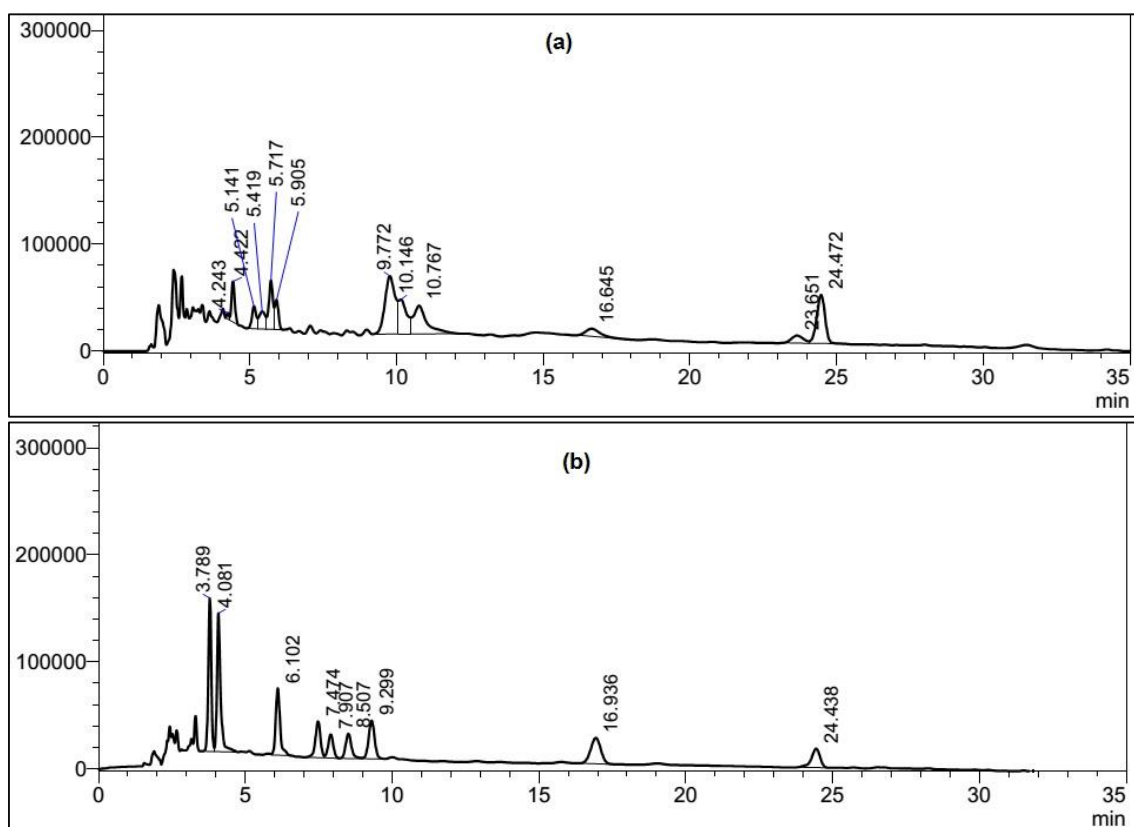
### Analysis of the HPLC fingerprint

The chromatograms obtained from the extract of authentic *Pterocarpus santalinus* L. f. and authentic *Dracaena cochinchinensis* (Lour.) S.C.Chen were compared and overlaid to investigate the difference and the similarity of the HPLC patterns. Then the HPLC fingerprints of the extract of all samples were then compared with the authentic *Pterocarpus santalinus* L. f. and authentic *Dracaena cochinchinensis* (Lour.) S.C.Chen.

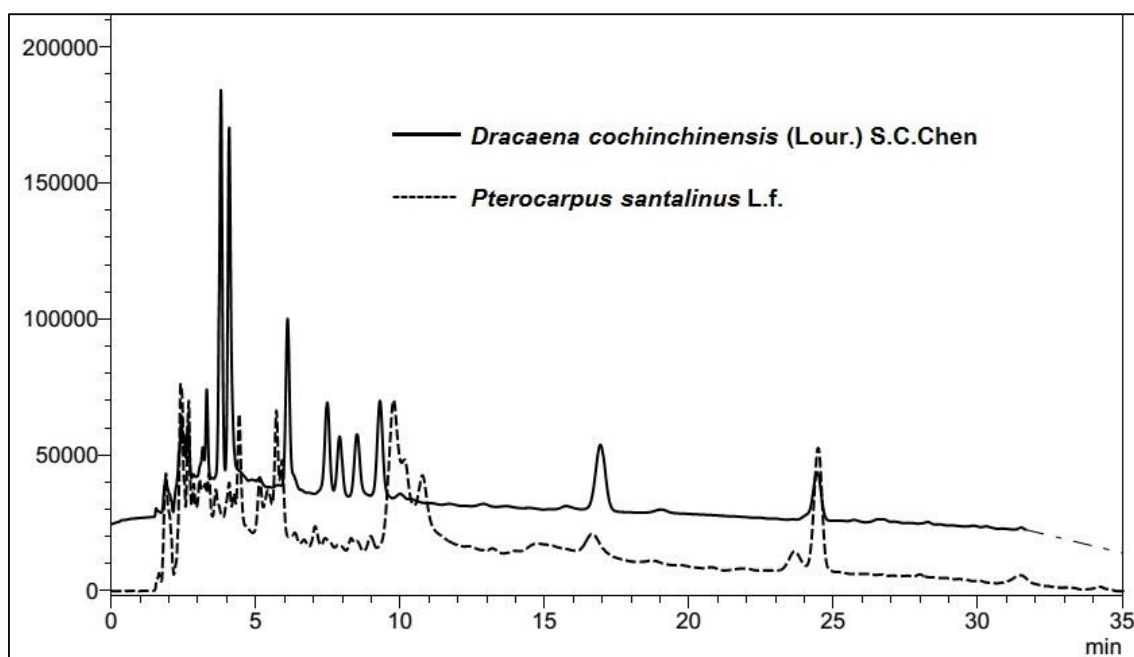
### Results

The pattern of HPLC fingerprint obtained from authentic *Pterocarpus santalinus* L. f. was significantly difference from that of authentic *Dracaena cochinchinensis* (Lour.) S.C.Chen as shown in Figure 1 (a) and Figure 1 (b), respectively. Overlaying of the chromatograms obtained from *Pterocarpus santalinus* L. f. and *Dracaena cochinchinensis* (Lour.) S.C.Chen was also shown in Figure 2. It is obviously noticed that both herbs have one peak appear at the same retention time of about 24.3-24.4 minutes with the same UV-spectrum pattern. The overlay of the UV-spectra was shown in Figure 3.

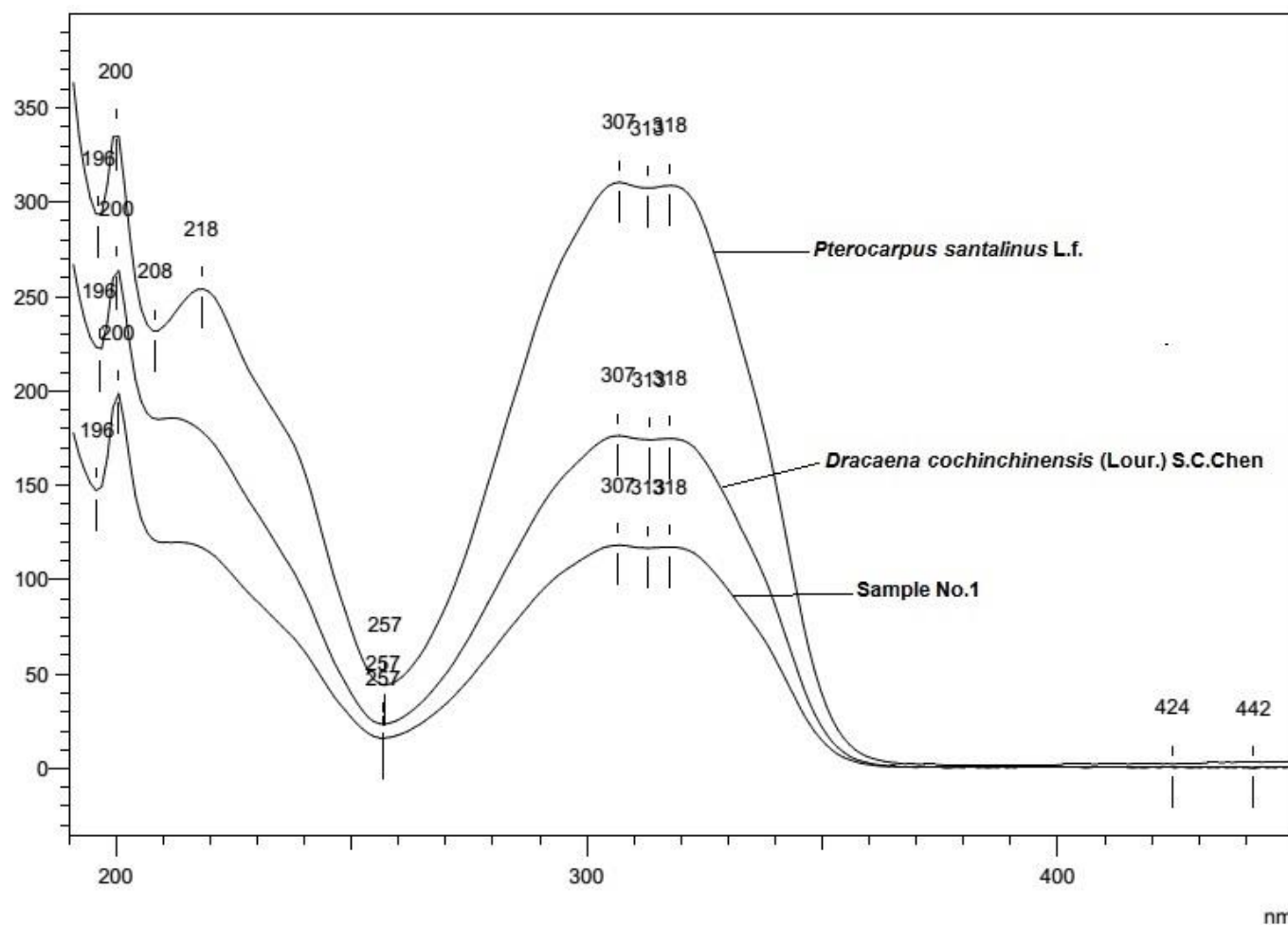
The HPLC fingerprints obtained from all samples were similar to the HPLC fingerprint of authentic *Dracaena cochinchinensis* (Lour.) S.C.Chen although the biodiversity of some components were detected in sample no.3, 5 and 6. The chromatogram of each sample compares to that obtained from authentic *Dracaena cochinchinensis* (Lour.) S.C.Chen are demonstrated in Figure 4.



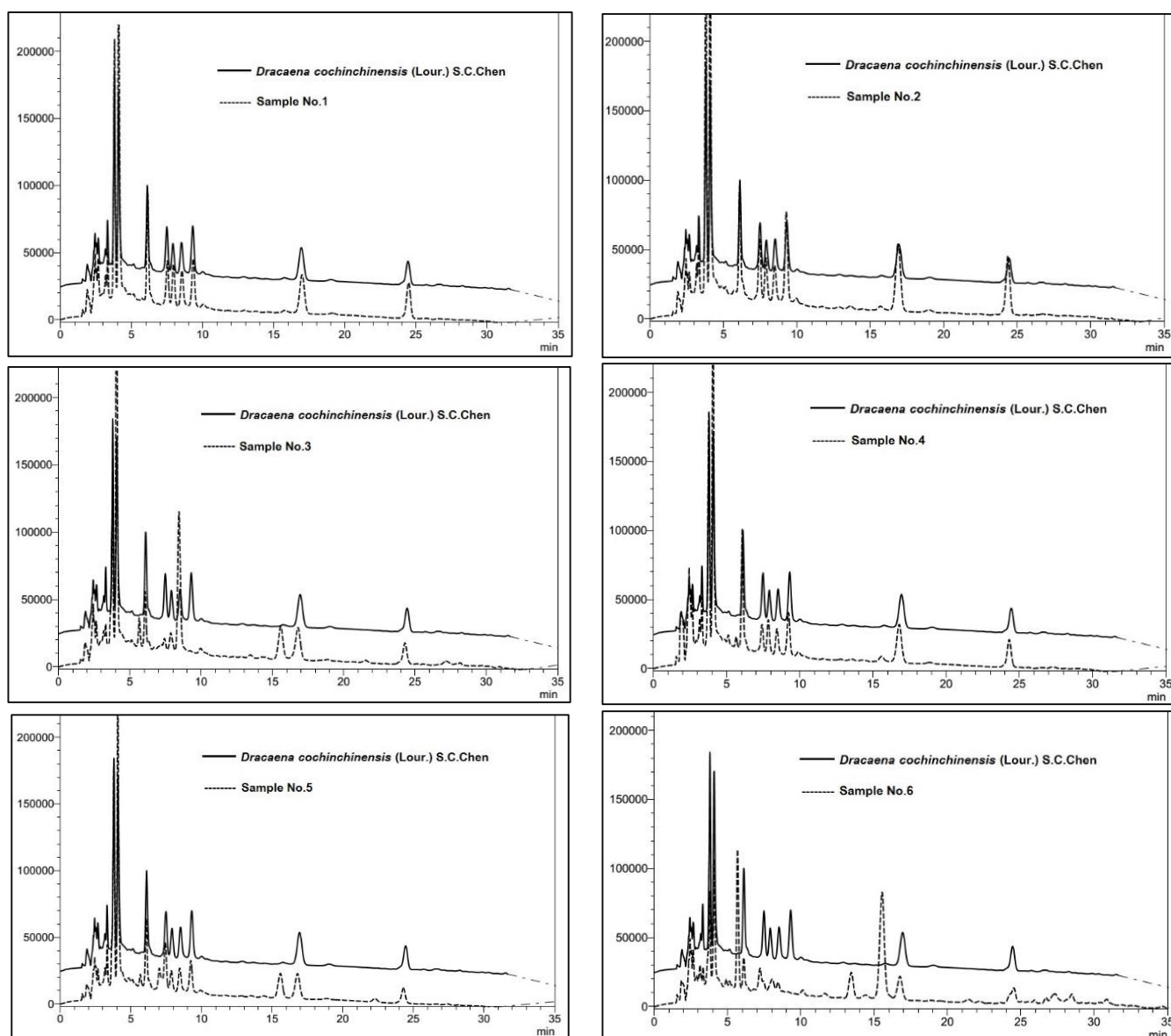
**Figure 1.** The patterns of HPLC fingerprints obtained from authentic *Pterocarpus santalinus* L.f. (a) and authentic *Dracaena cochinchinensis* (Lour.) S.C.Chen (b).



**Figure 2.** Chromatogram of authentic *Pterocarpus santalinus* L.f. overlaid with the chromatogram of authentic *Dracaena cochinchinensis* (Lour.) S.C.Chen.



**Figure 3.** UV spectra of the peaks at the retention time of 24.3-24.4 minutes, obtained from sample no.1, authentic *Pterocarpus santalinus* L.f. and authentic *Dracaena cochinchinensis* (Lour.) S.C.Chen, respectively.



**Figure 4.** Chromatograms of authentic *Dracaena cochinchinensis* (Lour.) S.C.Chen compares to the chromatogram of individual sample (no.1-6).

## Discussion

Both *Dracaena cochinchinensis* (Lour.) S.C.Chen and *Pterocarpus santalinus* L. f. may contain one same compound which showed the same retention time and the same UV- spectrum pattern. This compound should be confirmed by other methods which is out of the scope of this study. The efficacy of Thai traditional herbal formula contained *Dracaena cochinchinensis* (Lour.) S.C.Chen may be different from that contained *Pterocarpus santalinus* L. f. although both plants exhibit the same action as antipyretic

and anti- inflammatory as stated in the Thai Herbal Pharmacopoeia Volume IV ( 2014) . In addition, the chromatogram of samples showed biodiversity either different of some components or the level of the components which is the commonly found in the natural products.

The HPLC coupled with diode array detector in this study was proven to be adequate for identification of the herb species without using the expensive equipment, LC-MS/MS. In addition, this method can also be applied in

several aspects such as identifying the species of the herbs contain in the recipe of traditional medicine, quality control of the herbal medicines and differentiating of the components in herbs. The limitation of this method is inability to determine the molecular weight of the components contained in the herbs. Furthermore, the authentic species of the herb for comparison are required.

## Conclusion

Regarding to the results, it can be concluded that Chan-daeng dried powder marketed in the wholesale herbal shops located in Bangkok and vicinity areas are *Dracaena cochinchinensis* (Lour.) S.C.Chen which is agreeable to the assumption of previous review by Picheansoonthon and Vichai, 2004.

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