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Original Article

Pharmacognostic Characterization and Comparative TLC Fingerprinting of *Siphonodon celastrineus* Griff. and *Suregada multiflora* (A. Juss) Baill. Heartwoods: Toward Ethnomedicinal Validation and Herbal Standardization

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ABSTRACT

This study aimed to establish standards and perform a pharmacognostic analysis to compare of *Siphonodon celastrineus* Griff. (Ma Duuk) and *Suregada multiflora* (A. Juss.) Baill. (Khuan Thong Phayabaht) heartwood. Botanical characteristics, physicochemical properties, and TLC fingerprints were evaluated. Both species exhibited unique macroscopic and microscopic characteristics, with variations in bark texture and heartwood color. TLC fingerprints provide distinctive chromatographic profiles, enabling authentication. This study also established a limitation for the ethanolic-soluble extractive of both herbs at not less than 3% (w/w). The limitations of water-soluble extractive content of *S. celastrineus* Griff. and *S. multiflora* (A. Juss) Baill. were determined to be not less than 5% and 3% (w/w), respectively. The findings support the establishment of quality control criteria for the heartwood of *S. celastrineus* Griff. and *S. multiflora* (A. Juss) Baill., contributing to their potential inclusion in the Thai Herbal Pharmacopoeia and validating their ethnomedical use.

Keywords: Pharmacognostic Evaluation, *Siphonodon celastrineus* Griff, *Suregada multiflora* (A. Juss.) Baill., Physicochemical parameter, Ethnomedicinal Validation

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INTRODUCTION

Siphonodon celastrineus Griff. (*Celastraceae* family) is a medicinal plant whose common name in Thai is Ma Duuk or Duk Hin (Isan) or Bug Coke (Khmer-Surin) and Grandma Pluak (Surat Thani). It is a tree that grows up to 25 meters in height and can be found in the northern and central parts of Thailand. According to, Thai traditional medicine, its root has a narcotic taste[1,2]. It is given orally as a bone tonic and also used for the treatment of inflammation, abscesses and skin diseases. The chemical investigation of *S. celastrineus* Griff. showed that there were 24 types of triterpenes in the dichloromethane extract including the derivatives of lupane, friedelanes, oleanane, ursane, sterols, fatty acids, sesquiterpene alkaloids, and glycerol derivatives[3]. The methanolic extract from root bark was found to contain the oleanane triterpenes; 3β-acetoxy-11α-benzoyloxy-13β-hydroxyolean-12-one, and the quinone methide triterpene; pristimerin. According to reports on the biological activities of *S. celastrineus* Griff. 27 chemical compounds that were isolated from the stem showed promise as anticancer agents by suppressing cancer cells *in vitro*. The *in vitro* anticancer activity of these compounds were tested in 6 types of cancer cells isolated from humans, including human hepatocarcinoma (HepG2), human lung cancer (A549), human cholangiocarcinoma (Thai; HuCCA-1), human cervical carcinoma (HeLa), human breast cancer (MDA-MB-231), and T-lymphoblast leukemia (MOLT-3) cell 21 lines. The T-lymphoblast (MOLT-3) cell line was effectively inhibited by 3-secours-12-en-2-oic acid, with an IC_{50} of 4.5 μM, whereas the IC_{50} of etoposide, the standard anticancer drug, was 0.03 ± 0.009 μM[3,4,5].

Suregada multiflora (A. Juss.) Baill. belongs to the *Euphorbiaceae* family its common name in Thai is Khuan Thong Phayabaht or Duk Sai (Isan). Its others common names Golden Vengeance Bowl (Central Region), Rubber Sleeve Grandma Pluak Ho Saphan Kwai (Phrae Nan), Khao Tak (Kanchanaburi), Maduk Lem (North), and Khan Thong (Phitsanulok, Prachuap Khiri Khan)[6]. It is a shrub or tree with a height of 2-15 meters. The bark of *S. multiflora* (A. Juss.) Baill. also has a narcotic taste[7,8]. The heartwood and bark have been used for various skin diseases including rashes, eczema, ringworm, venereal disease, urticaria as well as vertebral cancer. The boiled bark is used for topical compress or applications to treat these skin ailments[9]. It has been reported that the major chemical constituents of *S. multiflora* (A. Juss.) Baill. are diterpene lactone[7]. The bark also contained diterpenoids; helioscopinolide A, C, I, triterpenoids; suremulol C, D, and abbeokutone[10]. The hexane and dichloromethane extracts from the stem bark of *S. multiflora* (A. Juss.) Baill. were reported have a strong inhibitory effect on nitric oxide with an IC_{50} of 8.6 μg/mL. Helioscopinolide A, showed the highest NO inhibitory activity with an IC_{50} of 9.1 μM, followed by helioscopinolide C and suremulol D with IC_{50} of 24.5 and 29.3 μM, respectively[10,11].

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Since both herbs similarly have narcotic taste, they were used either as a single herb and as part of herbal formulas. *S. celastrineus* Griff. heartwood is utilized to decrease toxicity and improve bone in traditional medicine. Moreover, these two herbs demonstrate therapeutic properties recognized in the National List of Major Medicines since 2023. They are also an ingredient in the mixtures of *Derris scandens* formula[12]. The mixtures of *D. scandens* formula is a Thai traditional medicine, which comprises four medicinal plants including *Derris scandens* (Roxb.) Benth. (vine part), *Zingiber cassumunar* Roxb. (rhizome), *S. multiflora* (A. Juss.) Baill. (heartwood), and *S. celastrineus* Griff. (heartwood). This formula is a use traditionally for relieving muscle pain. The recommended dosage is 900-1,500 mg, administered orally three times daily, immediately following a meal[13,14]. The chemical constituents of *S. celastrineus* Griff. and *S. multiflora* (A. Juss.) Baill. were reported to contain several major phytochemical groups such as triterpenes, alkaloids, and sterols.

Since 1989, the Ministry of Public Health has established the Thai Herbal Pharmacopoeia (THP), which established meets the requirements and provides quality control standard for each herbal medicine. THP, serves as the official Pharmacopoeia for Thai Herbal Medicine as published in the Government Gazette. The Thai Herbal Pharmacopoeia 2021, Supplement 2024 is the latest publication in the Thai Herbal Pharmacopoeia series, featuring 17 newly developed monographs on herbal drugs and preparations. However, traditional use, comprehensive pharmacognostic data and physiochemical parameters of the heartwood of *S. celastrineus* Griff. and *S. multiflora* (A. Juss.) Baill. are currently lacking from Thai Herbal Pharmacopoeia (THP). Previous work has primary focused on preliminary microscopic description. There is existing monograph of dried root of *S. multiflora* (A. Juss.) Baill. However, monograph of the heartwood of *S. celastrineus* Griff. and *S. multiflora* (A. Juss.) Baill. have not been established[15].

Thus, this study aims to evaluate the pharmacognostic specifications of the heartwood of *S. celastrineus* Griff. and *S. multiflora* (A. Juss.) Baill. This includes an analysis of macroscopic and microscopic characteristics, physicochemical properties, and a comparison of the TLC chromatograms of the samples from various sources using thin layer chromatography.

MATERIALS AND METHODS

Preparation of plant materials

Four authentic samples of heartwood of *Siphonodon celastrineus* Griff. (Ma Duuk) were collected in May 2021, from Amphur Kap Choeng (au_SC1), Surin province, Nong Phok (au_SC2, au_SC4), Roi Et province and Kosum Phisai (au_SC3), Maha Sarakham province. Six authentic samples of heartwood of *Suregada multiflora* (A. Juss.) Baill. (Khaun Thong Phayabaht) were collected in May 2021, from Kap Choeng (au_SM1, au_SM2), Surin province, Nong Phok (au_SM3), Roi Et province, Amphur Kosum Phisai (au_SM4, au_SM5) and Na Dun (au_SM6), Maha Sarakham province (Table 1). The authentic samples were identified by Mr. Choi Sukphinij, a folk medicine practitioner from Surin province and Asst.Prof. Sombat Appamaraka, Walai Rukhavej, Botanical Research Institute, Mahasarakham University. The voucher specimens of *S. multiflora* (A. Juss.) Baill. and *S. celastrineus* Griff. were No.MSU.PH-EUP-SM1 and No.MSU.PH-CEL-SC1, respectively. The crude drug samples were purchased from 10 different herbal drug stores in Surin, Maha Sarakham, Bangkok, Yasothon, Phichit, Phitsanulok and Nakhon Pathom province, Thailand as detailed in Table 1. The samples were cleaned and sliced into small pieces and dried in a hot air oven at 60^{O}C. The dried pieces were pulverized and passed through a sieve no.60. The dried powders were used for the study[8].

Macroscopic and microscopic examination

Macroscopic examinations of color, odor, taste, shape and texture were performed. Microscopic characteristics of the powdered drugs were examined by microscope (Zeiss Axioskop, Germany) with 75% chloral hydrate solution and iodine-iodide solution for starch and phloroglucinol staining for fibers and sclereids. Magnifications of the figures are indicated by scale-bars[8,15].

Physicochemical properties examination

A total of eight samples (n=8) from each species were analyzed, comprising both authentic and herbal drug stores samples sources from diverse regions. This sample size was selected to represent diverse sources while ensuring analytical feasibility. Physicochemical properties were determined following Thai Herbal Pharmacopeia 2018 guideline. This examination includes loss on drying (measurement of moisture content), ash values (determination of total ash, acid-insoluble ash) and extraction values (water-soluble and ethanol-soluble extractive values)[8,16].

Thin layer chromatography

Each dried powdered sample (5 g) was extracted with 95% ethanol by maceration for 30 minutes. The resulting crude extracts were subjected to Thin layer Chromatography analysis on silica gel GF_{254} plates. Various solvent systems such as dichloromethane: methanol (93 : 7) and toluene: ethyl acetate: formic acid in different ratios (50 : 10 : 10, 60 : 35 : 5, 70 : 40 : 10) were employed as the mobile phase[14,17]. After development, the color of the bands was recorded under ultraviolet light at UV_{254} and UV_{366}, marking the quenching bands and after spraying the plates with anisaldehyde-sulfuric acid reagent and heat at 105^{O}C for 10 minutes; purple-blue to pink bands for detection of terpenoids[8,18]. TLC fingerprints of *S. celastrineus* Griff. and *S. multiflora* (A. Juss.) Baill. heartwood ethanolic extract were performed and compared with reference standards.

Chemicals

All solutions were prepared with analytical grade chemicals. Analytical grade of dichloromethane, methanol, toluene, ethyl acetate and formic acid were obtained from Carlo Erba (Italy). The standards of stigmasterol as a phytosterol and lupeol as a triterpenoid were purchased from Sigma-Aldrich (USA) (Figure 1).

RESULTS

Macroscopic characteristics

The four authentic samples of *S. celastrineus* Griff. heartwood and six authentic samples of *S. multiflora* (A. Juss.) Baill. heartwoods were collected from Maha Sarakham, Roi Et, and Surin Provinces. The *S. celastrineus* Griff. bark is rougher than that of *S. multiflora* (A. Juss.) Baill. Furthermore, the *S. celastrineus* Griff.'s blade was serrate, margin entire and slightly undulating. The *S. celastrineus* Griff.'s fruit was round-oval, similar to a pear, while the *S. multiflora* (A. Juss.) Baill.'s fruit was a globose capsule with three lobes, as shown in Figure 2. Therefore, the botanical characteristics can be used to initially differentiate between *S. celastrineus* Griff. and *S. multiflora* (A. Juss.) Baill.

The crude drug samples of *S. celastrineus* Griff. were obtained from 10 sources, including Bangkok, Yasothon, Phichit, Phitsanulok, Surin, Nakhon Pathom, Buriram, and *S. multiflora* (A. Juss.) Baill., and 8 sources, including Bangkok, Yasothon, Phichit, Phitsanulok, Surin, and Nakhon Pathom, with a total of 28 samples. The *S. multiflora* (A. Juss.) Baill. heartwood was observed to be lighter in color and finer in texture

than that of *S. celastrineus* Griff. Furthermore, many *S. celastrineus* Griff. samples, including those obtained from herbal drug stores, often retained dark brown, rough pieces of bark (Figure 3), which was less common in the *S. multiflora* (A. Juss.) Baill (Figure 4).

Microscopical characteristics

Microscopic examination of the powdered drugs revealed that both *S. celastrineus* Griff. and *S. multiflora* (A. Juss.) Baill. contained similar elements, including xylem rays, prismatic crystals, bordered-pitted vessels, wood fibers, and sclereids. However, a key differentiating feature was identified *S. multiflora* (A. Juss.) Baill. contained an abundance of starch grains, which were absent in *S. celastrineus* Griff (Figure 5 and Figure 6).

Physicochemical evaluation

The physicochemical parameters of crude drug samples of *S. celastrineus* Griff. and *S. multiflora* (A. Juss.) Baill. were summarized in Table 2. These parameters, including foreign matter, loss on drying, total ash, acid-insoluble ash, ethanol-soluble extractive, and water-soluble extractive. The average total ash and acid-insoluble ash contents were determined; for *S. celastrineus* Griff., total ash content was measured at 4.06 ± 1.44% (w/w), while acid-insoluble ash was 0.17 ± 0.08% (w/w). In the case of *S. multiflora* (A. Juss) Baill., total ash content was measured at 2.67 ± 1.58% (w/w), with acid-insoluble ash at 0.08 ± 0.03% (w/w). The average ethanol-soluble extractive was determined to be 4.23 ± 1.63% (w/w) for *S. celastrineus* Griff. and 4.07 ± 0.90% (w/w) for *S. multiflora* (A. Juss) Baill. The average water-soluble

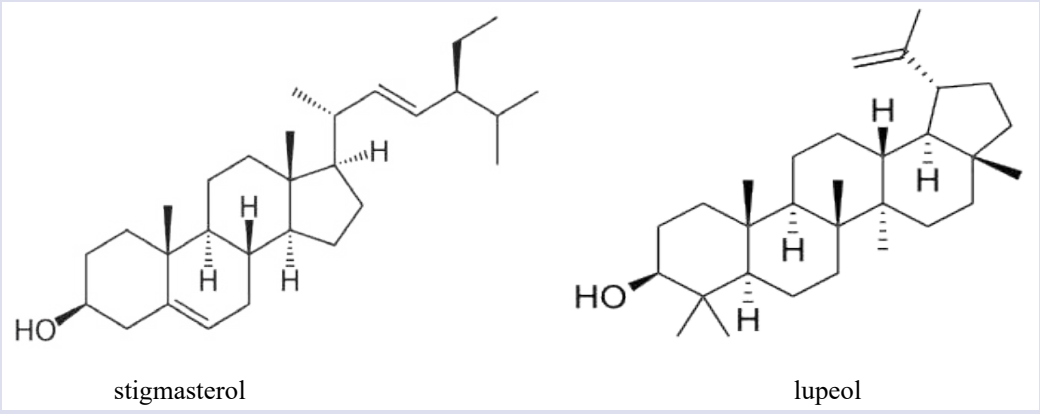

Figure 1. The chemical structures of stigmasterol and lupeol structure

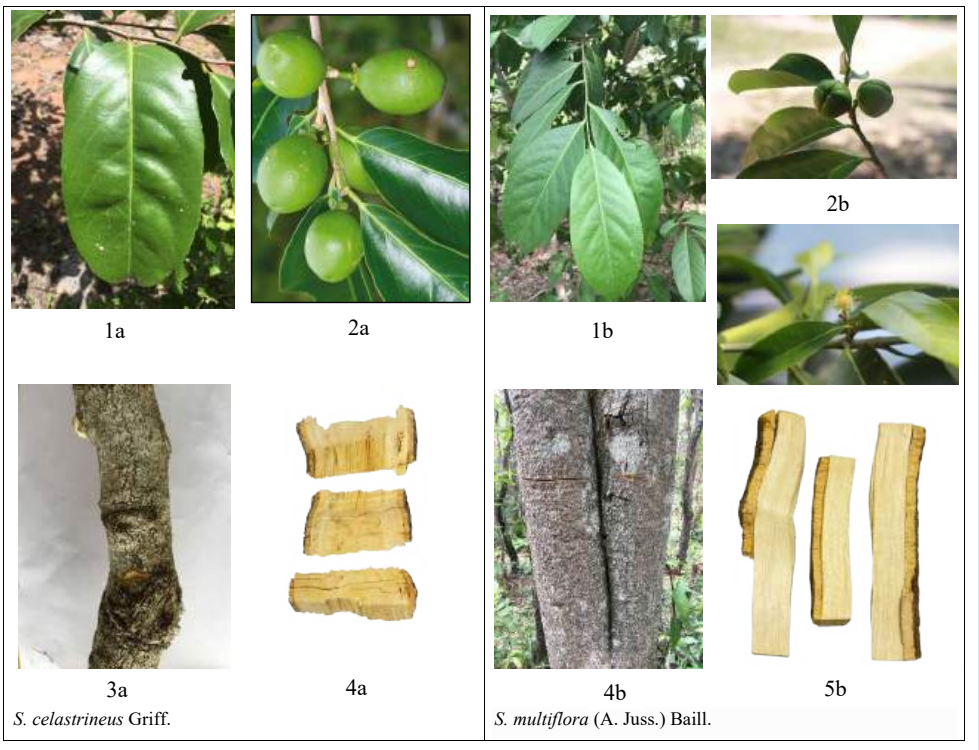

Figure 2. The botanical characteristics of *S. celastrineus* Griff. and *S. multiflora* (A. Juss.) Baill.

1a = leaf 2a = fruit 3a = bark 4a = heartwood
1b = leaf 2b = fruit 3b = flower 4b = bark 5b = heartwood

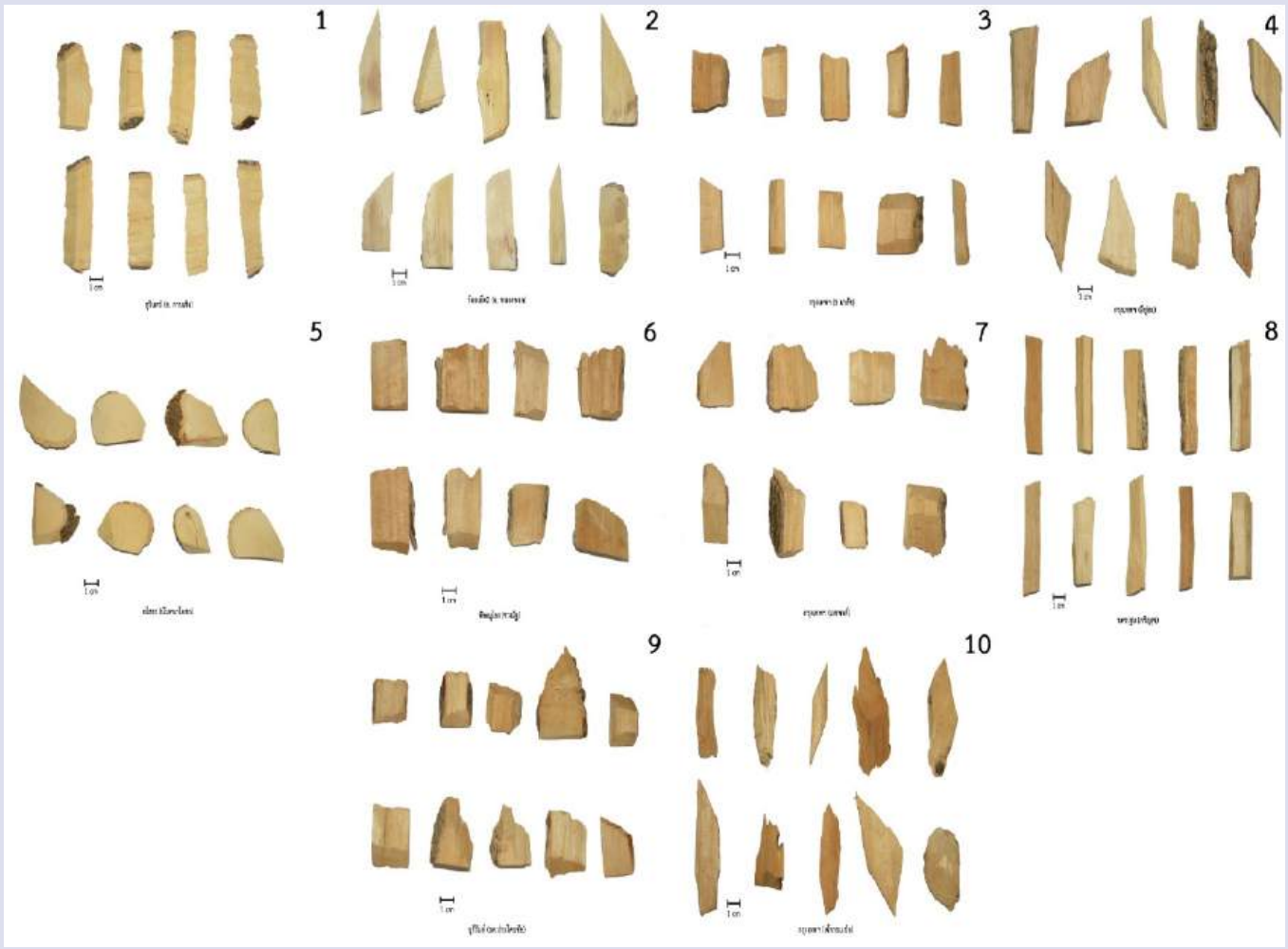

Figure 3. The crude drug samples of *S. celastrineus* Griff.

1= Surin (au_SC1) 2 = Roi Et (au_SC4) 3 = Bangkok (SC1) 4 = Bangkok (SC2). 5 = Yasothon (SC3)
6= Phitsanulok (SC5) 7 = Bangkok (SC7) 8 = Nakhon Pathom (SC8) 9 = Buriram (SC9) 10 = Bangkok (SC10)

Table 1. Sources of S. celastrineus Griff. and S. multiflora (A. Juss.) Baill. heartwoods collection

No	Sources	
	S. celastrineus Griff. (SC)	*S. multiflora* (A. Juss.) Baill. (SM)
1	au_SC1 (Surin)	au_SM1 (Surin)
2	au_SC2 (Roi Et)	au_SM2 (Surin)
3	au_SC3 (Maha Sarakham)	au_SM3 (Roi Et)
4	au_SC4 (Roi Et)	au_SM4 (Maha Sarakham)
5	SC1 (Bangkok)	au_SM5 (Maha Sarakham)
6	SC2 (Bangkok)	au_SM6 (Maha Sarakham)
7	SC3 (Yasothon	SM1 (Bangkok)
8	SC4 (Phichit)	SM2 (Bangkok)
9	SC5 (Phitsanulok)	SM3 (Yasothon))
10	SC6 (Surin)	SM4 (Phichit)
11	SC7 (Bangkok)	SM5 (Phitsanulok)
12	SC8 (Nakhon Pathom)	SM7 (Surin)
13	SC9 (Buriram)	SM8 (Bangkok)
14	SC10 (Bangkok)	SM9 (Nakhon Pathom)

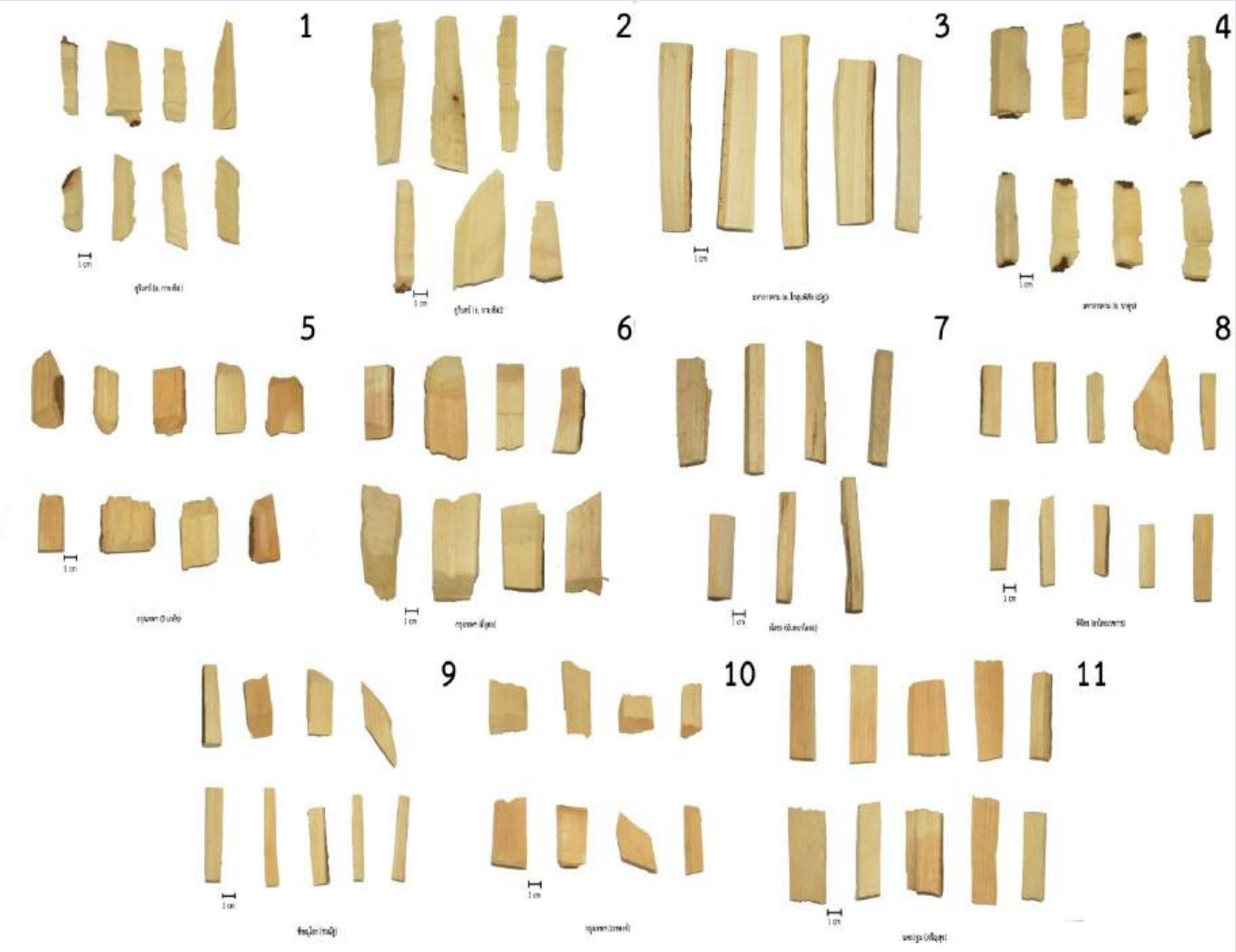

Figure 4. The crude drug samples of *S. multiflora* (A. Juss.) Baill.

1= Surin (au_SM1) 2 = Surin (au_SM2) 3 = Maha Sarakham (au_SM5) 4 = Maha Sarakham (au_SM6)
5 = Bangkok (SM1) 6= Bangkok (SM2) 7 = Yasothon (SM3) 8 = Phichit (SM4)
9 = Phitsanulok (SM5) 10 = Bangkok (SM8) 11 = Nakhon Pathom (SM9)

Table 2. Physicochemical evaluation of *S. celastrineus* Griff. and *S. multiflora* (A. Juss.) Baill. heartwood (n = 8)

Parameters	S. celastrineus Griff.		S. multiflora (A. Juss.) Baill.	
	% of dry weight (Mean±S.D.)	*Proposed Limitation (% of dry weight)	% of dry weight (Mean±S.D.)	*Proposed Limitation (% of dry weight)
Foreign matter	0.01 ± 0.01	Not more than 0.5	0.01 ± 0.02	Not more than 0.5
Loss on drying	4.22 ± 2.55	Not more than 5	3.83 ± 1.60	Not more than 5
Total ash	4.06 ± 1.44	Not more than 5	2.67 ± 1.58	Not more than 3
Acid-insoluble ash	0.17 ± 0.08	Not more than 1	0.08 ± 0.03	Not more than 1
Ethanol-soluble extractive	4.23 ± 1.63	Not less than 3	4.07 ± 0.90	Not less than 3
Water-soluble extractive	5.80 ± 1.54	Not less than 5	4.71 ±1.16	Not less than 3

*The upper limit was defined as the mean value plus 10% of the mean

extractive content in *S. celastrineus* Griff. was obtained at 5.80 ± 1.54% (w/w), while *S. multiflora* (A. Juss) Baill. was obtained at 4.71 ± 1.16% (w/w). The proposed limitations for each physicochemical parameter were established based on the mean experimental values (Mean ± S.D.) obtained in this study.

Thin layer chromatography

The solvent system of toluene: ethyl acetate: formic acid (70:40:10) was found to provide the best separation of the phytochemical bands in both *S. celastrineus* Griff. and *S. multiflora* (A. Juss.) Baill. heartwood extracts. TLC chromatogram of *S. celastrineus* Griff. extract showed six quenching bands under short UV wavelength (UV_{254} nm), three fluorescence bands (blue and green) under long wavelength (UV_{366} nm), and two colored bands (pale purple and purple) after spray with anisaldehyde-sulfuric acid reagent as shown in Figure 7 and Table 3. For *S. multiflora* (A. Juss.) Baill. extract was found seven quenching

Table 3. Rf values and detection characteristics of the ethanolic extracts of *S. celastrineus* Griff. heartwood

Rf value	Detection under UV_{254} nm	Detection under UV_{366} nm	Detection with anisaldehyde-H_2SO_4 reagent (visible light)
0.29	quenching (au_SC3, au_SC4)	-	-
0.46	quenching (SC1, SC4, SC5, SC7, SC9, SC10)	-	-
0.54	quenching (SC1, SC4, SC5, SC7, SC8, SC9, SC10)	-	-
0.59	quenching (all samples)	-	-
0.83	quenching (all samples)	-	purple (all samples including lupeol)
0.86	quenching (all samples)	-	purple (all samples)
0.48	-	blue (SC2, SC3, SC6, SC7)	-
0.79	-	green (all samples)	purple (all samples)
0.91	-	blue (all samples)	pale purple (au_SC1, au_SC4, SC1, SC2, SC4)
0.10	-	-	purple (all samples)
0.19	-	-	purple (SC2, SC3, SC7)
0.21	-	-	purple (SC2, SC3)
0.31	-	-	purple (SC1, SC2, SC3, SC7)
0.33	-	-	purple (SC7)
0.36	-	-	pale purple (au_SC1, au_SC2, au_SC3, au_SC4, SC1, SC2, SC3, SC7, SC8, SC9, SC10)
0.50	-	-	purple (SC7)
0.64	-	-	purple (au_SC1, au_SC2, au_SC3, au_SC4, SC1, SC2, SC3, SC4, SC5, SC6, SC7, SC9, SC10)
0.78	-	-	purple (all samples including stigmasterol)
0.90	-	-	pale purple (au_SC1, au_SC4, SC1, SC2, SC4)
0.95	-	-	purple (all samples)

Table 4. Rf values and detection characteristics of the ethanolic extracts of *S. multiflora* (A. Juss) Baill. heartwood

Rf value	Detection under UV_{254} nm	Detection under UV_{366} nm	Detection with anisaldehyde-H_2SO_4 reagent (visible light)
0.41	quenching (au_SM1, au_SM2)		
0.51	quenching (au_SM1, au_SM2, SM3, SM4, SM5, SM7, SM8)		
0.59	quenching (au_SM1, au_SM2, SM3, SM4, SM5, SM7, SM8)		
0.63	quenching (au_SM1, au_SM2, au_SM6, SM3, SM8)		
0.73	quenching (au_SM1, au_SM2, au_SM6)		
0.84	quenching (SM2, SM5)		purple (all samples including stigmasterol)
0.86	quenching (SM3)		
0.56		blue (all samples)	pale purple (all samples)
0.59		blue (SM2)	
0.68		blue (au_SM2)	
0.87		green (au_SM1, au_SM2, au_SM3, au_SM4, au_SM6, SM1, SM2, SM3, SM4, SM5, SM8, SM9)	
0.94		blue (all samples)	
0.20			pale purple (all samples)
0.29			pale purple (au_SM1, au_SM2)
0.36			pale purple (all samples except SM3)
0.40			pale purple (all samples except SM3)
0.44			pink (au_SM1, au_SM2, au_SM4, au_SM6, SM4, SM5, SM8)
0.48			purple (au_SM1, au_SM2, SM2, SM3)
0.50			pale purple (au_SM1, SM1, SM2, SM3, SM4)
0.65			purple (au_SM1, au_SM2)
0.73			pale purple (au_SM1, au_SM2, au_SM6, SM4)
0.75			pale purple (all samples)
0.90			purple (au_SM1, au_SM2, au_SM3, au_SM4, au_SM6, lupeol, SM1, SM2, SM3, SM4, SM5, SM8, SM9)
0.99			purple (au_SM1, au_SM2, au_SM3, au_SM4, au_SM6, SM2, SM3, SM4, SM5, SM7, SM8, SM9)

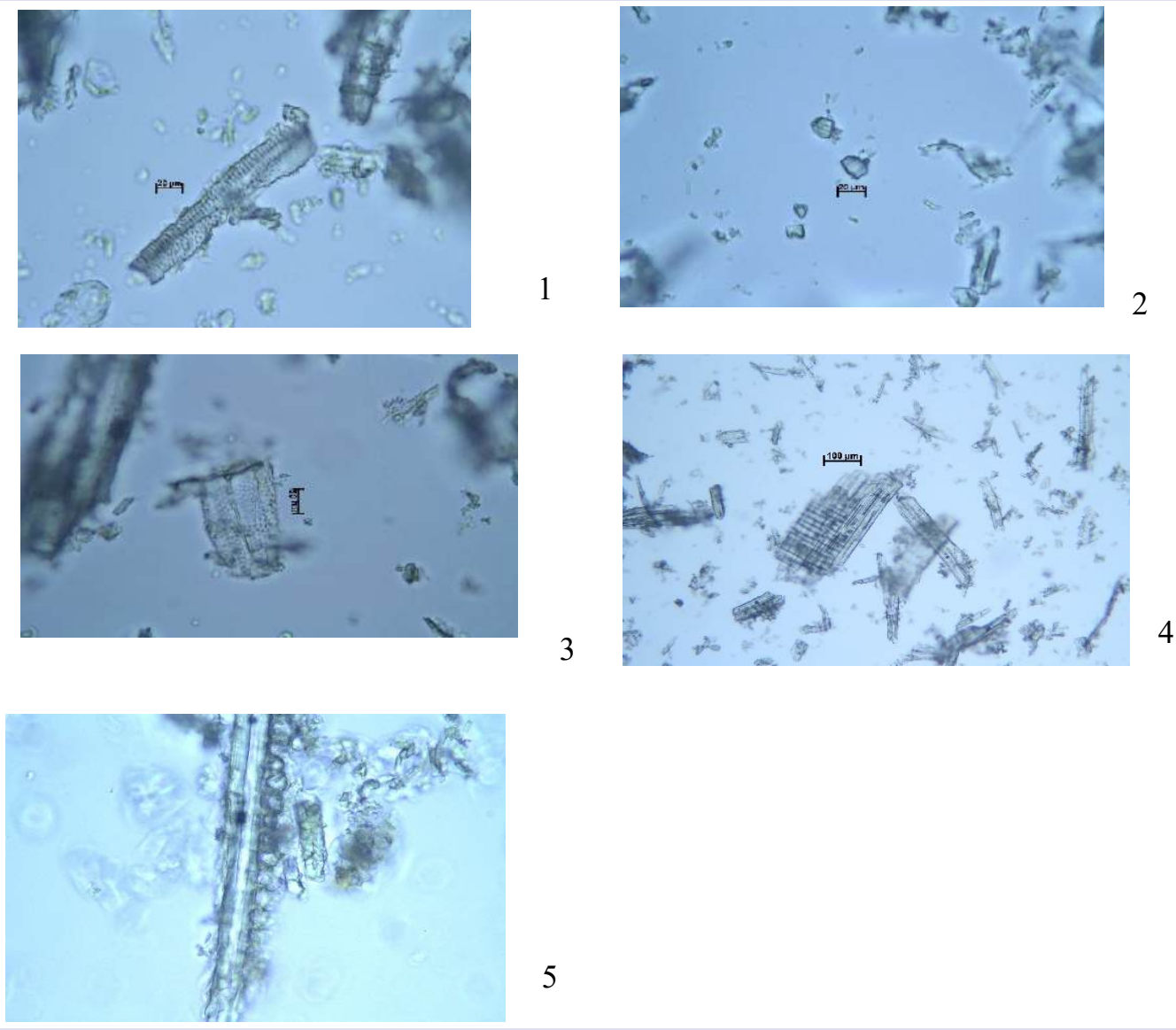

Figure 5. Microscopic characteristics of *S. celastrineus* Griff. powdered drug

1, 4 = bordered-pitted vessel
2 = calcium oxalate crystal
4 = xylem ray, with underlying fibres
3 = bordered-pitted vessel
5 = vessel with prismatic crystals and sclereids

bands (blue and green) under short UV wavelength (UV_{254} nm), five fluorescence bands under long wavelength (UV_{366} nm), and three colored bands (pale purple, purple, and pink) after spray with anisaldehyde-sulfuric acid reagent, as shown in Figure 7 and Table 4.

DISCUSSIONS

Pharmacognostic standardization is used for the identification, authentication and quality control of herbal plants[19]. By characterizing morphological, anatomical and chemical constituents, pharmacognostic evaluation facilitates the detection of adulterants and supports regulatory compliance. Pharmacognostic study involves a detailed examination of the morphological, microscopic and physicochemical characteristics of the herbal plants to support their identification and medicinal used[20].

Comparative macroscopic and microscopic characteristics for differentiation

Accurate morphological and anatomical identification is fundamental for the standardization and quality control of herbal formulations[21]. The results from this study indicated that the two herbs can be distinguished based on bark, leaves, and fruits when examining their botanical characteristics. The bark of *S. celastrineus* Griff. was substantially rougher than of *S. multiflora* (A. Juss) Baill.'s bark. However, it is difficult to identify the raw materials obtained from the samples purchased from some herbal drug stores because the macroscopic of their heartwoods is similar. The *S. multiflora* (A. Juss) Baill. heartwood is generally white and finer, appearing smooth, white, or light yellow, while *S. celastrineus* Griff. heartwood is typically white

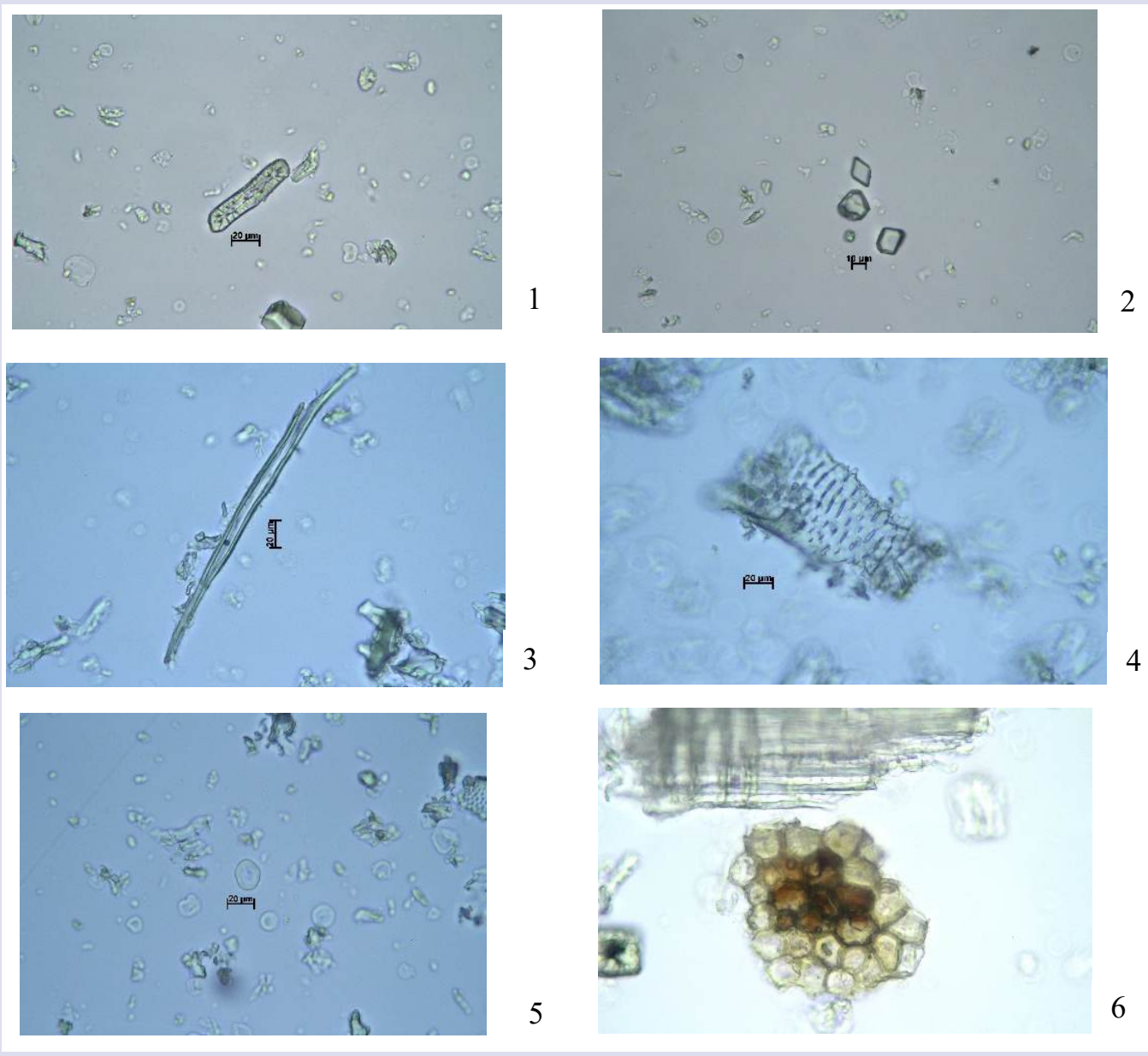

Figure 6. Microscopic characteristics of *S. multiflora* (A. Juss.) Baill. powdered drug
1 = sclereid 2 = prismatic crystals 3 = fiber
4 = bordered-pitted vessel 5 = starch grains 6 = cork in surface view and sclereids

to light yellow and may have a minor rough appearance. Thus, *S. multiflora* (A. Juss) Baill. heartwood may be traded in some herbal drug stores instead of *S. celastrineus* Griff. Microscopic characteristics of *S. celastrineus* Griff. and *S. multiflora* (A. Juss) Baill. heartwoods reported in this study would lead to the correct identification of their raw materials[20]. The microscopic examination revealed that *S. multiflora* (A. Juss) Baill. contains a significant quantity of starch granules, as shown in Figure 4 and 6. The abundant occurrence of starch grains in *S. multiflora* (A. Juss) Baill. is absent in *S. celastrineus* Griff. According to the microscopic marker, the observation emphasizes that specific samples of *S. celastrineus* Griff. (SC2, 3, 6, and 7) obtained from herbal drug stores exhibited microscopic characteristics consistent with *S. multiflora* (A. Juss) Baill., indicating possible adulteration. Macroscopic and microscopic methods are essential preliminary screening methods. These morphological and microscopic characteristics are crucial to the authentication of plants before any pharmacological use. However, their limitations, particularly for extensively processed raw materials, necessitate the use of chemical validation techniques, such as thin layer chromatography[21].

Physicochemical parameters for quality control and purity assessment

The evaluation of physicochemical properties is a fundamental approach for assessing the basic quality of herbal raw materials including the purity of herbal raw materials, detection of contamination, adulteration[22]. Consequently, it supports pharmacognostic identification and guarantees the quality, safety, and consistency of herbal materials. The physicochemical properties were also investigated for *S. celastrineus* Griff. and *S. multiflora* (A. Juss) Baill. heartwood including foreign matter, loss on drying (moisture content), total ash, acid-insoluble ash,

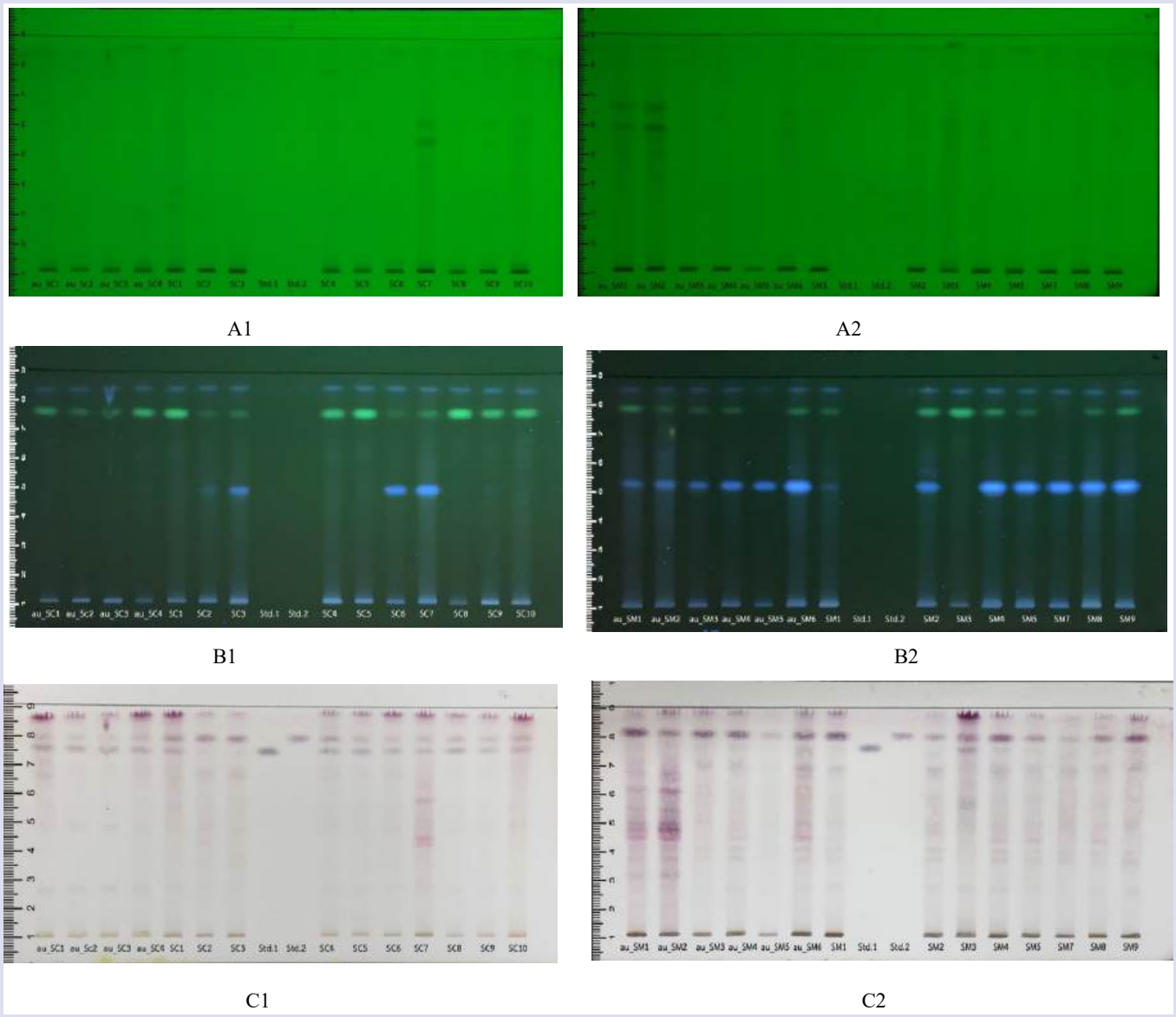

Figure 7. TLC Chromatogram of *S. celastrineus* Griff. (SC) and *S. multiflora* (A. Juss.) Baill. (SM) samples
Solvent system : Toluene: Ethyl acetate: Formic acid (70 : 40: 10)
A1 and A2 = Detection under UV_{254} nm B1and B2 = Detection under UV_{366} nm
C1 and C2 = Detection with anisaldehyde-sulfuric acid reagent
Std1 = Stigmasterol Std2 = Lupeol

ethanol-soluble extractive, and water-soluble extractive. The procedure of physicochemical analysis of the heartwood sample was carried out in accordance with WHO and Thai Herbal Pharmacopoeia 2021 *Supplement 2024* procedures to guarantee quality and homogeneity[14,22]. These parameters serve distinct purposes. Foreign matter serves as an initial assessment of cleanliness. Moisture content directly indicates the adequacy of drying and storage conditions, which affects stability. Ash content measures inorganic impurities, often signaling soil contamination. Finally, extractive values quantify the concentration of soluble chemical constituents, reflecting the potency or richness of the drug[21,23].

The quality of specification of herbal materials according to the Thai Herbal Pharmacopoeia, foreign matter was established at not more than 2% (w/w).[24] The study revealed low average concentrations of foreign matter for both *S. celastrineus* Griff. (0.01 ± 0.01% (w/w)) and *S. multiflora* (A. Juss) Baill. heartwood (0.01 ± 0.02% (w/w)). The study indicated a proposed limitation of foreign matter in heartwoods, specifying that it should not more than 0.5% (w/w) for based on the findings presented. This low proposed limit is justified, as the raw materials of the herbs are largely free from foreign matter since they are derived from heartwood. This study determined that the average moisture content of the heartwood for both herbs did not exceed 5% (w/w). This is in accordance with the Thai Herbal Pharmacopoeia, which specifies that the moisture content must not be more than 8% (w/w). The importance of moisture content is critical; elevated moisture levels can significantly impact the quality and shelf life of herbal medicines by promoting microbial growth including molds that may produce

aflatoxins and accelerating the degradation of active compounds[15,26]. The ash remaining following ignition of herbal materials is determined by two different methods which measure total ash and acid-insoluble ash in this study[22]. The total ash and acid-insoluble ash contents were established at not more than 2% (w/w) according to the Thai Herbal Pharmacopoeia[24]. The ash or residue yielded by an organic chemical compound is, as a rule, a measure of the amount of inorganic matter present as impurity. The total ash is particularly important in the evaluation of purity of drugs such as the presence or absence of foreign inorganic matter[21]. The acid-insoluble ash is the part of the total ash that is insoluble in diluted hydrochloric acid.[26] Based on these findings, the following limits are proposed: total ash content for *S. celastrineus* Griff. and *S. multiflora* (A. Juss) Baill. were established at not more than 5% (w/w) and 3% (w/w), respectively. For acid-insoluble ash, a limit of not more that 1% (w/w) is proposed for both herbs. The result indicated that the average of total ash content from crude drugs of *S. celastrineus* Griff. was higher than *S. multiflora* (A. Juss) Baill. This may be caused by foreign inorganic matters including sand and soil. According to the acid-insoluble ash content, especially as sand and siliceous earth. The comparison of the average acid-insoluble ash content between authentic and herbal drug store samples was consistent, indicating that the heartwood content few non-volatile inorganic impurities. The values of alcohol and water-soluble extracts were determined to evaluate the efficacy of active phytochemical extraction based on polarity, aiming to identify suitable solvents for future pharmacological investigations[21]. In this study, a proposed limitation of the ethanol-soluble content of both herbs is established at not less than 3% (w/w) according to these results. The heartwood of *S. celastrineus* Griff. and *S. multiflora* (A. Juss) Baill. contains phytochemicals, including terpenoids, diterpenoids, sesquiterpene alkaloids, and sterols[27,28,29]. This was consistent with the findings that several phytochemical groups, including alkaloids, flavonoids, terpenoids, phytosterols, and some essential oil, can be extracted in the ethanol-soluble fraction[30]. The proposed limitation of water-soluble extractive content of *S. celastrineus* Griff. and *S. multiflora* (A. Juss) Baill. were established at not less than 5% and 3% (w/w), respectively. However, the water-soluble extractive of *S. celastrineus* Griff. was higher than that of *S. multiflora* (A. Juss) Baill. However, Although, seasonal stratification was not employed, all samples were collected during the same dry season to minimize environmental variability. Future studies are encouraged to evaluate seasonal and geographical influences, as these factors may alter secondary metabolite profiles and affect the robustness of the proposed pharmacognostic parameters. The proposed limitations of the physicochemical parameters of *S. celastrineus* Griff. and *S. multiflora* (A. Juss) Baill. heartwood are presented in Table 2.

Thin layer fingerprinting and authentication

Thin layer chromatography is particularly valuable for the qualitative determination of small amounts of impurities[22]. The TLC fingerprint determined in this study also provides the characteristic chromatogram (fingerprint) of *S. celastrineus* Griff. and *S. multiflora* (A. Juss) Baill. heartwood ethanolic extract, which can be beneficially used for the identification, quality control, and control of adulteration. The TLC fingerprints profiles of both species demonstrated clearly distinguishable patterns, particularly under UV_{254} nm and after spray with anisaldehyde-sulfuric acid reagent. In *S. multiflora* (A. Juss) Baill., a prominent band was consistently observed at an Rf values of 0.56 (blue fluorescence) under UV_{254} nm and pale purple at Rf 0.44-0.50 after spray with anisaldehyde-sulfuric acid reagent. These TLC chromatogram regions may therefore be proposed as reliable chemical markers for quality control and species differentiation. In this study, stigmasterol (a phytosterol) and lupeol (a triterpenoid) were used as standard references. The resulting TLC fingerprint profiles clearly differentiated the extracts of *S. celastrineus* Griff. and *S. multiflora* (A. Juss) Baill., corroborating the macroscopic and microscopic characteristics observed for the crude drugs. Notably, several samples as *S. celastrineus* Griff. obtained from herbal drug stores (SC2, 3, and 7) exhibit TLC fingerprints closely resembling those of *S. multiflora* (A. Juss) Baill. (Figure 7, Table 3 and Table 4). This suggests the possibility of adulteration of *S. celastrineus* Griff. with *S. multiflora* (A. Juss) Baill. heartwood.

Furthermore, evidence of adulteration was observed in several herbal drug stores samples of *S. multiflora* (A. Juss) Baill. which showed partial similarity to *S. celastrineus* Griff. in the Rf 0.06-0.66. However, microscopic examination revealed distinct anatomical differences, particularly in starch grain distribution, supporting the likelihood of substitution or admixture. The combined application of TLC fingerprinting and anatomical characterization therefore provides a robust and reliable approach for detecting adulteration and strengthening quality surveillance within the herbal supply chain.

Currently, the production of herbal medicines often relies on dried crude drugs rather than fresh plant materials, making it necessary to procure herbal raw materials from herbal drug stores. The inadvertent use of incorrect or adulterated herbs may significantly affect the pharmacological efficacy and safety of herbal preparations[31]. Therefore, accurate identification and authentication of crude drugs are essential steps in quality control[26]. The findings demonstrate that preliminary selection and authentication of appropriate herbal materials can be effectively achieved through microscopic examination. In particular, thin layer chromatography proved to be a valuable analytical tool for validating the identity of crude drug samples derived from both species. Although both plants belong to similar phytochemical groups, TLC fingerprint analysis revealed the presence of distinct chemical constituents specific to *S. multiflora* (A. Juss.) Baill. These results indicate that TLC fingerprinting can discriminate between morphologically similar crude drugs and serve as a reliable method for quality assurance. Furthermore, chemical compounds isolated from *S. multiflora* (A. Juss.) Baill. should be further investigated and proposed as chemical markers, particularly those exhibiting pharmacological activities consistent with traditional ethnobotanical uses.

Contribution to Thai Herbal Pharmacopoeia and Ethnomedicinal Validation

The pharmacognostic investigation conducted in this study essential baseline data for the quality control and standardization of the heartwood of *S. celastrineus* Griff. and *S. multiflora* (A. Juss) Baill., which are traditionally used in ethnobotanical formulations. The Pharmacognostic evaluation including macroscopic, microscopic of raw material and physicochemical analyses in fundamental not only for the authentication of raw material but also for assessing their purity and efficacy prior to pharmacological use[32,33]. The selection of heartwood for this study is based on ethnomedicinal practice, particularly its use in traditional formulations such as the *D. scandens* mixture for musculoskeletal ailments. The pharmacognostic parameters proposed such as moisture content, extractive values, ash values, and TLC fingerprints are directly linked to ensuring consistent quality and safety. For instance, high moisture content or ash values may indicate microbial contamination, inorganic impurities, or adulteration, which can ultimately compromise the safety, quality, and therapeutic efficacy of herbal preparations. These physicochemical limits serve not only as indicators of raw material quality but also as critical safeguards to ensure consistency, efficacy, and safety in alignment with ethnomedicinal practices. The findings from this study should be recommended to establish the quality control and evaluation of the crude drug of *S. celastrineus* Griff. and *S. multiflora* (A. Juss) Baill. heartwood in the Thai Herbal Pharmacopoeia.

CONCLUSION

This study presents a novel pharmacognostic profile of *S. celastrineus* Griff. and *S. multiflora* (A. Juss.) Baill. heartwood, incorporating macroscopic, microscopic, physicochemical, and TLC fingerprint analyses. By establishing validated diagnostic parameters were established for both authentic and market samples, addressing key gaps in crude drug standardization. These results support the formal inclusion of both species in the Thai Herbal Pharmacopoeia and advance the safe, effective integration of traditional herbal materials into evidence-based healthcare systems.

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REFERENCES

1. Tiengburanatham W. (1999). Dictionary of Thai Medicinal Plants. Bangkok, 5th Ed. Thailand: Prachachon Publishing; 1999 612-613.
2. Faculty of Pharmaceutical Sciences, Ubon Ratchathani University. Maduk *(Siphonodon celastrineus)* [Online database]. Thai Crude Drug Database.
3. Kaweetripob W, Mahidol C, Prawat H, Ruchirawat S. Lupane, friedelane, oleanane, and ursane triterpenes from the stem of *Siphonodon celastrineus* Griff. Phytochemistry. 2013;96:404-417.
4. Kaweetripob W, Mahidol C, Thongnest S, Prawat H, Ruchirawat S. Polyoxygenated ursane and oleanane triterpenes from *Siphonodon celastrineus*. Phytochemistry. 2016;129:58-67.
5. Niampoka C, Suttisri R, Bavovada R, Takayama H, Aimi N. Potentially cytotoxic triterpenoids from the root bark of *Siphonodon celastrineus* Griff. Arch Pharm Res. 2005;28(5):546-6.
6. Faculty of Pharmaceutical Sciences, Ubon Ratchathani University. Khuan Thong Phayabaht (*Suregada multiflora*) [Online database]. Thai Crude Drug Database. Available from:
7. Department of National Parks, Wildlife and Plant Conservation. *Suregada multiflora* (A.Juss.) Baill. [Online database]. e-Flora of Thailand.
8. Department of Medical Sciences. Thai Herbal Pharmacopoeia 2018. Nonthaburi, Thailand: Ministry of Public Health. 2018.
9. BGO Plant Databases, The Botanical Garden Oraganization. *Suregada multiflora* (A.Juss) Baill. [Online database].
10. Tewtrakul S, Subhadhirasakul S, Cheenpracha S, Yodsaoue O, Ponglimanont C, Karalai C. Anti-inflammatory principles of *Suregada multiflora* against nitric oxide and prostaglandin E_2 releases. J Ethnopharmacology. 2011;133:63-6.
11. Cheenpracha S, Yodsaoue O, Karalai C. Potential anti-allergic ent-karuene diterpenes from the bark of *Suregada multiflora*. Phytochemistry. 2006;67(24):2630-4.
12. Medicine Regular Division; Ministry of Public Health. National List of Essential Medicines; NLEM 2013. Bangkok: Ministry of Public Health; 2013.
13. Ayameang O, Rattarom R, Mekjaruskul C, Caichompoo W. Anti-Inflammatory activity and quantitative analysis of major compounds of the mixtures of *Derris scandens* (DZSS) formula. Pharmcogn J. 2020;12(4):828-34.
14. Department of Medical Sciences. Thai Herbal Pharmacopoeia 2021: Supplement 2024, Suregadae Multiflorae Radix:29-34. Nonthaburi, Thailand: Ministry of Public Health.2024.
15. Folashade1 KO, Omoregie1 EH, Ochogu AP. Standardization of herbal medicines - A review. Int. J. Biodvers. Conserv. 2012;4(3):101-112.
16. Mandal M, Misra D, Ghosh NN, Mandal V. Physicochemical and element studies of *Hydrotyle javanica* Thunb. for standardization as herbal drug. Asian Pac J Trop Biomed. 2017;7(11):979-86.
17. Laothamyingyong K, Kaewsuwan N, Caichompoo W. The Physico-Chemical standardization of *Siphonodon celastrineus* Griff. and *Suregada multiflora* (A.Juss) Baill. PharmD. Independent study, Faculty of Pharmacy, Mahasarakham University, Thialand, 2020.
18. Wagner H, Bladt S. Plant drug analysis: A thin layer chromatography atlas. 2nd rev. and expanded ed. Berlin: Springer-Verlag;1996.
19. Kaundal R, Kumar D. Current demands for standardization of Indian medicinal plants: A critical review. Medicine in Drug Discovery. 2025;27.
20. Limpabandhu T, Suwatronnakorn M, Widoyanti AAE, Issaravanich S, Zongrum O, Prasansuklab A. Pharmacognostic standardization and phytochemical evaluation of *Ficus rumphii* blume leaves in Thailand. Phytomedicine Plus. 2024;4.
21. Parganiha R. Comparative pharmacognostic evaluation and standardization of root extracts of selected Ayurvedic plants. IJCPS. 2025;01(1):18-35.
22. World Health Organization. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. Geneva: World Health Organization; 2000.
23. World Health Organization. Quality control methods for herbal materials (Updated edition of Quality control methods for medicinal plant materials, 1998). Malta: World Health Organization; 2011.
24. Chokevivat V. Quality of crude drug. J Thai Trad Alt Med. 2004;2(2):84-91.
25. Liang YZ, Xia P, Chan K. Quality control of herbal medicine. J Chromatogr B Analyt Techno Biomed Life Sci. 2004;821(1-2):53-70.
26. Mukherjee PK. Quality control and evaluation of herbal drug. Evaluation of natural products and traditional medicine. 2019. Elsevier: Netherlands.
27. Seephonkai P, Srisit S. Terpenoids isolated from *Siphonodon celastrineus* Griff. Burapha Science Journal. 2023;28(3).
28. Jahan IA, Nahar N, Mosihuzzaman M, Shaheen F, Choudhary MI, Parween Z. Novel diterpene lactones from *Suregada multiflora*. J Nat Prod. 2002;65(6):932-4.
29. Jahan IA, Nahar N, Mosihuzzaman M, Shaheen F, Choudhary MI. Six new diterpenoids from *Suregada multiflora*. J Nat Prod. 2004;67(11):1789-95.
30. Lee JE, Jayakody JTM, Kim JI, Jeong JW, Choi KM, Kim, TS, et al. The influence of solvent choice on the extraction of bioactive compounds from Asteraceae: A comparative review. Foods. 2024;13(19):3151.
31. Samarth S, Raut V, Patil M, Kumbhar. Standardization Techniques of Herbal Medicines. IJPRA Journal. 2024;9(3):869-82.
32. Alam F and Saqib QN. Pharmacognostic study and development of quality control parameters for fruit, bark and leaf of *Zanthoxylum armatum* (Rutaceae). Anc Sci Life. 2015;34(3):147-155.
33. Anjum F, Touqeer S, Khan MY, Jamil Q, Rida A, Shirazi JH, et al. Pharmacognostic evaluation, chemical characterization, and antibacterial activity of *Bassia indica* (Wight) A.J. Scott. Plants. 2024;13(13):1753.

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