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Research Article

An ethnobotanical study of the genus *Smilax* in Thailand and its botanical authentication for Hua-khao-yen crude drugs

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ABSTRACT

The first ethnobotanical work of the genus *Smilax* in Thailand was carried out to investigate the medicinal use of the *Smilax* species by folk healers, using the semi-structured interview, questionnaire survey, and literature review. Three quantitative indices were calculated, i.e. the Use Value (UV), the Relative Frequency Citation (RFC), and the Informant Agreement Ratio (IAR). Eleven species were recorded. Three of which, i.e. *S. bracteata*, *S. hemsleyana*, and *S. leucophylla*, are new records as medicinal species in Thailand. The genus has the potential use for the treatment of the genitourinary illnesses (IAR=1.00) and the neoplasms (IAR=0.71). *Smilax glabra* was the most used species with UVs=0.67, RFC=0.39. The results indicate the species being threatened due to over harvesting from the natural habitat. Furthermore, this study shows that the semi-structured interview coupled with the questionnaire survey is useful to gather the ethnobotanical data of a specific plant group. Botanical authentication of the commercial crude drugs based on chromatographic fingerprinting and macroscopic characters was carried out to verify the botanical identity of the homonym Hua-khao-yen crude drugs. *Smilax glabra* and *Premna herbacea* (Lamiaceae) were authenticated as the source of Hua-khao-yen-nuea and the Hua-khao-yen tai crude drugs, respectively.

Keywords:
Smilacaceae, Folk medicine, Chemical authentication, Market survey, Thai materia medica

1. INTRODUCTION

The genus *Smilax* L. (family Smilacaceae) comprises ca. 260 species distributed in the tropical and subtropical regions, mainly in Southeast Asia and Malesia[1-3]. The members of the genus are woody, herbaceous climbers, or occasionally becoming shrubs. The leaves are petiolate with a pair of wings and tendrils. The rhizomes or roots are thickened[3]. In Thailand, 32 species of the genus *Smilax* were enumerated[1,3-5].

The underground parts of the *Smilax* species are used as medicines in traditional medicines, e.g. Traditional Chinese Medicine Ayurvedic Medicine and Thai Traditional Medicine (TTM)[6-8]. The Hua-khao-yen crude drug is well-known in TTM and Thai folk medicines, and perceived to be the rhizomes of the genus *Smilax*[9]. In TTM, the crude drug is used for lymphatic and skin diseases, inflammation, and cancers, which are major health problems around the world[10-13]. The demand of the crude drug was the third rank of Thailand[14].

Based on the folk taxonomy, the crude drug is classified into Hua-khao-yen-nuea and Hua-khao-yen-tai which caused the confusion of the botanical source. Furthermore, the rhizome of the crude drug is difficult for botanical identification when it is separated from the distinguishable aerial part. Ethnobotanical and market

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surveys in Thailand showed that the Hua-khao-yen has multiple sources with similar appearances, including *S. corbularia* Kunth, *S. glabra* Roxb., *S. verticalis* Gagnep., *Dioscorea birmanica* Prain & Burkill, *D. membranacea* Pierre (Dioscoreaceae) and *Premna herbacea* Roxb. (Lamiaceae)[15]. To assure the safety of the herbal medicine, the urgent need is to authenticate the botanical origin of the Thai materia medica.

This study aims 1) to gather traditional use data of the genus *Smilax* in Thailand by performing the ethnobotanical survey and literature review; 2) to study the botanical authentication of the Hua-khao-yen crude drugs by using chemical fingerprinting and macroscopic character.

2. MATERIALS AND METHODS

2.1. Ethnobotanical survey

In order to gather the folk healer knowledge of the Hua-khao-yen crude drugs, the questionnaires were sent to 187 folk healers, 45 of which replied (24.06% response rate)[16-19]. The folk healers who replied the questionnaire with the consent to be an informant were contacted for fieldwork. Semi-structured interviews with 15 informants (Thai nationality, 14 males and 1 female, of ages 37 to 86, who have used the *Smilax* for their medical practices) were conducted between January 2019 and January 2020 in 12 Provinces, i.e. Chiang Mai, Phetchabun, Loei, Udon Thani, Nong Khai, Bueng Kan, Sakon Nakhon, Mukdahan, Maha Sarakham, Chaiyaphum, Surin, and Songkla. During the surveys, vernacular name, part used, method of preparation, mode of administration, and indication were noted. Voucher specimens with the underground part, which also used as authentic specimens for botanical authentication, were deposited at the Herbarium, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University (PBM), see Table 1. The generic and specific identification were made using 1) taxonomic keys in Flora of Thailand volume 2(3)[3]; 2) comparisons with authentic specimens kept at Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Ministry of Agriculture and Cooperative (BKF); and 3) expert determination by the third and the last authors, and Dr. Chirdsak Thapyai, Faculty of Science, Naresuan University, Thailand.

2.2. Data Analysis

2.2.1. Data extraction

The traditional use of the *Smilax* in Thailand was extracted from scientific databases, including Web of Science, Google Scholar, ScienceDirect, National Center for Biotechnology Information (NCBI). Keywords used for searching included '*Smilax*, ethnobotanical survey, traditional uses. The non-digitized Thai bibliographical sources related to traditional and folk uses of the *Smilax* in Thailand, i.e. published journal articles, scientific reports, were surveyed for the term '*Smilax*' records. All herbarium specimens of the genus *Smilax* kept at Forest Herbarium (BKF), which is the biggest herbarium in Thailand and houses enormous collections of vascular plants, ca. 280,000 specimens, were examined for their uses on herbarium notes which ethnobotanists recorded the local use from informants[20]. The accepted scientific names and synonyms of the *Smilax* were verified using the Plants of the World Online database and the updated taxonomic revisions of the *Smilax* in Thailand[1,4-5,21]. Misidentified species were excluded from the analysis. The medicinal properties of *Smilax* gathered from the surveys and literature reviews were categorized using the WHO International Classification of Diseases version 11 (ICD-11)[22].

2.2.2. Quantitative indices

The datasets of the ethnobotanical survey and the data extraction were combined. For the data extracted from the scientific database, each article represented one informant because the information in the article was insufficient to verify the exact number of informants. The use report was counted as the species mentioned. For example, the species A used for the treatment of diabetes and hypertension, we counted two use reports. The Use Value (UV), Relative Frequency Citation (RFC), and Informant Agreement Ratio (IAR) indices were analyzed for measuring the importance of each *Smilax* species in Thailand and carried out using Microsoft Excel 2019.

The UV was calculated using the following formula: $UV = \sum U_i/N$, where U_i is the number of use reports mentioned by the informant for a use species and N is the total number of informants in the study[23]. The UV shows the relative importance of *Smilax* species.

The RFC was calculated using the following formula: $RFC = FC/N$ ($0<RFC<1$), where *FC* is the number of informants maintaining the use of a particular species and *N* is the total number of informants in the study[24]. The RFC evaluates the frequency of the use species. The value varies from 0 (when nobody cites the plant as useful) to 1 (when all informants refer it as useful).

The IAR for particular medicinal use category was assessed using the following formula: $IAR = (N_{ur}-N_t)/(N_{ur}-1)$, where N_{ur} is the total number of use reports for a particular ailment category of every species by all informants and N_t is the number of species used in that category[25]. The IAR shows the consensus of use category of certain plant groups among the informants.

Table 1. Details of authentic materials.

Species	Code	PBM no.	Geographic location and collection number	Part used
Dioscorea membranacea Pierre	DM1	PBM 5515	Bangkok, Thawi Wattana, Sanam Luang 2 Market, 29 Jan. 2019, *Pansumrit* 7 (PBM)	Rhizome
	DM2	PBM 5522	Songkla, Saba Yoi, Ku Ha, 28 m, 21 Nov. 2019, *Pansumrit 30* (PBM)	Rhizome
D. birmanica Prain & Burkill	DB1	PBM 5516	Bangkok, Khlong Sam Wa, cultivated in Eastern Sam Wa Tawan area, 7 March 2019, *Pansumrit 13* (PBM)	Rhizome
	DB2	PBM 5544	Nakhon Pathom, cultivated in Sireeruckhachati Nature Learning Park, Mahidol University, 17 Jan. 2020, *Pansumrit 95* (PBM)	Rhizome
Premna herbacea Roxb.	PH1	PBM 5518	Phetchabun, Chon Daen, Dongkhui, 18 Aug. 2019, *Pansumrit 23* (PBM)	Rhizome
	PH2	PBM 5519	Phetchabun, Chon Daen, Dongkhui, 18 Aug. 2019, *Pansumrit 24* (PBM)	Rhizome
Smilax bracteata C.Presl	SB1	PBM 5538	Sakon Nakhon, Kham Ta kla, 149 m, 15 Dec. 2019, *Pansumrit 70* (PBM)	Rhizome
	SB2	PBM 5543	Surin, Kab Choeng, Neng Mud, 185 m, 2 Jan. 2020 *Pansumrit 84* (PBM)	Rhizome
S. corbularia Kunth	SC	PBM 5530	Chiang Mai, Mae Rim, Pong Yaeng, 800 m, 17 Nov. 2019, *Pansumrit 54* (PBM)	Rhizome
S. glabra Roxb.	SG1	PBM 5527	Chaiyaphum, Mueang, Sub See Tong, 758 m, 24 Oct. 2019, *Pansumrit 40* (PBM)	Rhizome
	SG2	PBM 5534	Bueng Kan, So Phisai, Nong Pun, Lum Huai Kem, 185 m, 30 Nov. 2019, *Pansumrit 62* (PBM)	Rhizome
	SG3	PBM 5536	Udon Thani, Ban Dung, Ban Muang, Lum Huai Luek, 178 m, 1 Dec. 2019, *Pansumrit 66* (PBM)	Rhizome
S. ovalifolia Roxb. ex D.Don	SO	PBM 5535	Nong Khai, Fao Rai, Fao Rai Non-Formal and Informal Education Center, 30 Nov. 2019, *Pansumrit 6 5* (PBM)	Rhizome
S. perfoliata Lour.	SP	PBM 5526	Loei, Dan Sai, Khok Satorn, Phu Lom Lo, 19 Oct. 2019, *Pansumrit 36* (PBM)	Rhizome
S. verticalis Gagnep.	SV1	PBM 5528	Chiang Mai, Mae Rim, Saluang, Nuan Chan Traditional Medicine Learning Center, 391 m, 16 Nov. 2019, *Pansumrit 49* (PBM)	Rhizome, Root
	SV2	PBM 5542	Surin, Kab Choeng, Neng Mud, 185 m, 2 Jan. 2020, *Pansumrit 79* (PBM)	Rhizome, Root

Table 2. Inventory list of medicinal *Smilax* used in Thailand.

Species	Province (voucher no.)	Local name	Part used	Route of administration	Preparation	Indication	Data sources	UVs	RFC
Smilax bracteata C. Presl	Songkhla (*Pansumrit 28*)	Kam-koong	R	Oral	Decoction, pill	Skin disease	The present study	0.21	0.12
	Sakon Nakhon (*Pansumrit 70*)	Khueang-yai	R, Rh	Oral	Decoction	Nourish breast milk	The present study		
	Surin (*Pansumrit 84*)	Khueang-yai	S	Oral	Decoction	Body system imbalance, bloating, body pain, constipation	The present study		
	Maha Sarakham (*Pansumrit 77*)	Khueang-yai	R, Rh	Oral	Decoction	Hemorrhoids	The present study		
S. corbularia Kunth	Chiang Mai (*Pansumrit 54*)	Khao-yen-tai	Rh	Oral	Decoction	Breast cancer	The present study	0.27	0.21
	Songkhla (*Pansumrit 29*)	Mok-lek-thong	R, Rh	Oral	Decoction, maceration	Nourish for men	The present study		
	Chiang Mai	Ho-ka-a	Rh	Oral	Decoction	Birthing aids	Herbarium specimen, *Vidal* et al. 6136 (BKF)		

Note: A=aerial part, L=leaf, R=root, Rh=rhizome, S=stem, T=tuber, n/a=not applicable.

Table 2. Inventory list of medicinal *Smilax* used in Thailand. (cont.)

Species	Province (voucher no.)	Local name	Part used	Route of administration	Preparation	Indication	Data sources	UVs	RFC
S. corbularia Kunth	Sakon Nakhon	Hua-Khao-yen-nuea	T	n/a	n/a	Cancer	Poonthananiwatkul et al.[27]		
	Chaiyaphum	Khao-yen-nuea	Rh	n/a	n/a	Diabetic	Chuakul and Saralamp[28]		
	Pattani	n/a	Rh	Oral	Decoction	Gastrointestinal diseases	Neamsuvan et al.[29]		
	Nakhon Si Thammarat	n/a	T	Oral	Decoction	Bloating, diarrhea, gastritis	Neamsuvan et al.[30]		
S. glabra Roxb.	Chaiyaphum (*Pansumrit 40*)	Khao-yen	Rh	Oral	Decoction	Body pain, cervical cancer nourishes	The present study	0.67	0.39
	Bueng Kan (*Pansumrit 62*)	Khao-yen-nuea	Rh	Oral	Decoction	Cancer	The present study		
	Udon Thani (*Pansumrit 36*)	Khao-yen-nuea, Ya-hua-yai	Rh	Oral	Maceration	Cancer	The present study		
	Sakon Nakhon (*Pansumrit 71*)	Khao-yen-nuea	Rh	Oral	Decoction	Nourish breast milk	The present study		
	Mukdahan (*Pansumrit 72*)	Khao-yen-nuea	Rh	Oral	Decoction	Kidney disorder	The present study		
	Loei	Tao-ya-khao-yen-nuea	n/a	Oral	Decoction	Diuretic, fever	Herbarium specimen, *Nakkan* 15 (BKF)		
	Ubon Ratchathani	Khueng	T	Oral	Decoction	Diuretic	Chuakul and Soonthornchareonnon et al.[31].		
	Chaiyaphum	Ya-hua	Rh	n/a	n/a	Diabetes	Chuakul and Saralamp[28]		
	Nakhon Ratchasima	Ya-hua	Rh	Oral	Decoction	Bodily discomfort, diuretic, hematinic, oedema	Chuakul[32]		
	Surin	Ya-hua	Rh	n/a	n/a	Abscess, oedema	Chuakul and Saralamp[33]		
	Yasothorn	Khao-yen-nuea or Ya-hua	Rh	Oral	Decoction	Diabetes	Chuakul et al.[34]		
	Pattani,	n/a	R	oral	Powder	Gastrointestinal diseases	Neamsuvan et al.[29]		
	Nakhon Si Thammarat	n/a	T	Oral	Decoction	Bloating, diarrhea, gastritis	Neamsuvan et al.[30]		
S. hemsleyana Craib	Chiang Mai (*Pansumrit 56*)	Khruea-dao-sun	Rh	Oral	Decoction	Liver and lung cancer, facial cancer, lymphatic disorder	The present study	0.12	0.03
S. lanceifolia Roxb.	Nakhon Ratchasima	Tao-yang-dong	Rh	Oral	Decoction	Cancer, pruritus	Chuakul[32]	0.06	0.03
S. leucophylla Blume	Songkhla (*Pansumrit 31*)	Rok-Chang	R	Inhale	Inhalant	Nasal polyps	The present study	0.03	0.03
S. luzonensis C.Presl	Surin	n/a	Rh	n/a	n/a	Hematinic	Chuakul and Saralamp[33]	0.03	0.03

Note: A=aerial part, L=leaf, R=root, Rh=rhizome, S=stem, T=tuber, n/a=not applicable.

Table 2. Inventory list of medicinal *Smilax* used in Thailand. (cont.)

Species	Province (voucher no.)	Local name	Part used	Route of administration	Preparation	Indication	Data sources	UVs	RFC
S. ovalifolia Roxb. ex D.Don	Lamphun	Hua-khao-yen-tai	n/a	Oral	Decoction	Aphthous ulcer, cancer, hypertension, diabetes	Inta et al.[35]	0.21	0.03
	Nan	Jiam-yang-kong	A, R	Bath, oral	decoction	Scars, body pain	Srithi et al.[36]		
	Chanthaburi	Hua-yang	R	n/a	n/a	Breast cancer	Lumlerdkij et al.[37]		
S. perfoliata Lour.	Chiang Mai (*Pansumrit 55*)	Khruea-dao	Rh	Oral	Decoction	Body pain, backache, liver and lung cancer, facial cancer, lymphatic disorder	The present study	0.21	0.06
	Chaiyaphum	Khueang	Rh	n/a	n/a	Enlarged abdomen and liver	Chuakul and Saralamp[28]		
S. verticalis Gagnep.	Maha Sarakham (*Pansumrit 76*)	Khueng-lek	R, Rh	Oral	Decoction	Internal wound, ulcerative colitis	The present study	0.55	0.18
	Surin (*Pansumrit 79*)	Khueang-lek	R, Rh	Oral	Decoction	Cancer, after birthing aids	The present study		
	Sakon Nakhon (*Pansumrit 18*)	Khueang-noi	Rh	Oral	Decoction, maceration	Lymphatic disorder, inflammation	The present study		
	Chiang Mai (*Pansumrit 49*)	Khueang	R, Rh	Oral	Decoction	Liver and lung cancer, facial cancer, lymphatic disorder	The present study		
	Chiang Mai (*Pansumrit 53*)	Nam-pao, Khao-yen	R, Rh	Oral	Decoction	Liver and lung cancer, facial cancer, lymphatic disorder	The present study		
	Lamphun	Hua-khao-yen-nuea	L, S	Oral	Decoction	Aphthous ulcer, cancer, hypertension, diabetes	Inta et al.[35]		
S. sp.	Songkhla (*Pansumrit 33*)	Ku-ra-a	R	Oral	Decoction	Period fever	The present study	0.03	0.03

Note: A=aerial part, L=leaf, R=root, Rh=rhizome, S=stem, T=tuber, n/a=not applicable.

2.3. Botanical authentication

2.3.1. Macroscopic method

All authentic (Table 1) and commercial samples were studied morphological features under a stereomicroscope, including appearance, color, odor, and taste[26]. Twelve commercial samples were bought from seven herbal drug stores, which mentioned by informants in the questionnaire survey, Bangkok (n=5) and Mukdahan (n=2). The Hua-khao-yen-nuea commercial crude drugs, codes 1-7, and the Hua-khao-yen-tai commercial crude drugs, codes 8-12, were asked for purchasing from the stores.

2.3.2. Chemical fingerprinting

Thin layer chromatography (TLC) is the official specification used in Thai Herbal Pharmacopoeia, which is a simple, low-cost and rapid method for qualitative analysis. The rhizomes of authentic and commercial samples were washed up and sliced into small pieces. It was dried at 50°-60°C, then, ground and passed through a 40-mesh sieve (425 μm). The dried powder was kept in an air-tight container under 4°C until used.

The dried powder was extracted with 80% methanol using ultrasonicating bath at 45°C for 30 minutes, an extraction ratio was 1:5, except *D. birmanica* was 1:12.5. The extraction process was repeated three times. The extracts were combined and filtered through filter paper (Whatman no.1). The solvent was removed under reduced pressure using a rotary evaporator. The dried crude extracts were stored in air-tight and light-protected glass containers at -20°C until used. The dried extracts were dissolved in 80% methanol before applied for the analysis.

The TLC technique was used to develop the chemical protocol for identifying the Hua-khao-yen crude drugs. Stationary phase was TLC aluminum sheet silica gel $60F_{254}$ (Merck). Quercetin (PhytoLab) were used as standard marker. Applied volume of the 40 μg of authentic and commercial sample extracts and the 2 μg of the standard were applied to the plate as 5 mm band. Plates were developed with a mixture of dichloromethane: methanol: acetic acid (87:13:5) in the saturated chamber with relative 75% humidity, until the solvent front migrated up to the 70 mm. The developed plate was sprayed with natural products-polyethylene glycol spay reagent (NP/PEG) or anisaldehyde sulphuric acid spray reagent (AS) and heated with a hairdryer. The plate was observed under white light (UV 355 nm), UV 254 nm, and UV 366 nm. The TLC fingerprints of the commercial were compared to those of the authentic samples.

3. RESULTS AND DISCUSSION

3.1. Ethnobotanical and folk uses of *Smilax* in Thailand

From the ethnobotanical surveys, eight species of the genus *Smilax* were found (Figure 1). Seven species were mentioned as medicines by the folk healers (Table 2). *Smilax bracteata* C.Presl, *S. hemsleyana* Craib, and *S. leucophylla* Blume were reported as medicinal species for the first time. Seven species were reported in 11 ethnobotanical research articles (Table 2). Of the 287 herbarium specimens of the *Smilax* kept in the BKF herbarium were investigated, only two herbarium specimens were indicated for medicinal use.

This study found that the crude drugs with the homonym of Hua-khao-yen referred to four species of *Smilax*, i.e. *S. corbularia*, *S. glabra*, *S. ovalifolia* Roxb. ex D.Don, and *S. verticalis* (Table 2). *Smilax glabra* is known as Khao-yen-nuea or Ya-hua (Figure 2D-E). *Smilax verticalis* is called Khao-yen by Thai Yai folk healers in Mae Rim, Chiang Mai (Figure 2L-M) and Khueang by folk healers in northeastern Thailand. The Thai Yai folk healers used Krue-dao-sun (*S. hemsleyana*, Figure 2F) and Krue-dao (*S. perfoliata* Lour., Figure 2J-K) as the substitution of *S. verticalis*. According to the interviews, the lateral roots *S. ovalifolia* and *S. bracteata* might be removed from the rhizomes to sell as a counterfeit of the Hua-khao-yen in local markets.

The UV value ranged from 0.03 to 0.67 (Table 2). The highest UV was *Smilax glabra* (0.67), followed by *S. verticalis* (0.55). The highest RFC were reported for *S. glabra* (0.39) and *S. corbularia* (0.21) (Table 2). *Smilax glabra* showed the highest value of both indices, representing the most relative importance plants used by the majority of the informants in Thailand.

The major source of the Hua-khao-yen is from the natural habitat which is being overexploited due to lack of sustainable harvesting practice of the *Smilax*. Especially, the most commonly used species as *S. glabra* with the higher UV and RFC indices is likely to be under threat of over harvesting. According to the interview and field observation, the monoculture, e.g. para rubber, oil palm, sugarcane, is also a major threat for the status of the plants.

Diseases were classified into 12 categories (Table 3). The highest degree of agreement among informants was the genitourinary system (IAR=1.00). Only one species, *S. glabra*, was mentioned for the genitourinary treatment (diuretic and kidney disorder) that indicated the agreement of selection of a plant among informants. The second highest value was neoplasms (0.71). Seven species (22 use-reports) were used for cancers and lymphatics. The folk healer explained that the species with yellow rhizome is used for lymphatics, while the red for blood system. Therefore, *Smilax* species should also be prioritized for further anti-cancer drug discovery and *S. glabra* for genitourinary drugs.

Table 3. IAR values by categories for treating diseases.

Categories	*Nt*	*Nur*	IAR
Neoplasms	7	22	0.71
Endocrine, nutritional or metabolic diseases	5	10	0.56
Diseases of the circulatory system	4	7	0.50
Diseases of the respiratory system	1	1	n/a
Diseases of the digestive system	6	15	0.60
Diseases of the skin	4	4	0.00
Diseases of the musculoskeletal system and connective tissue	4	6	0.40
Diseases of the genitourinary system	1	4	1.00
Pregnancy, childbirth or the puerperium	2	2	0.00
Symptoms, signs or clinical findings, not elsewhere classified	3	5	0.50
Injury, poisoning and certain other consequences of external causes	1	1	n/a
External causes of morbidity and mortality	2	2	0.00

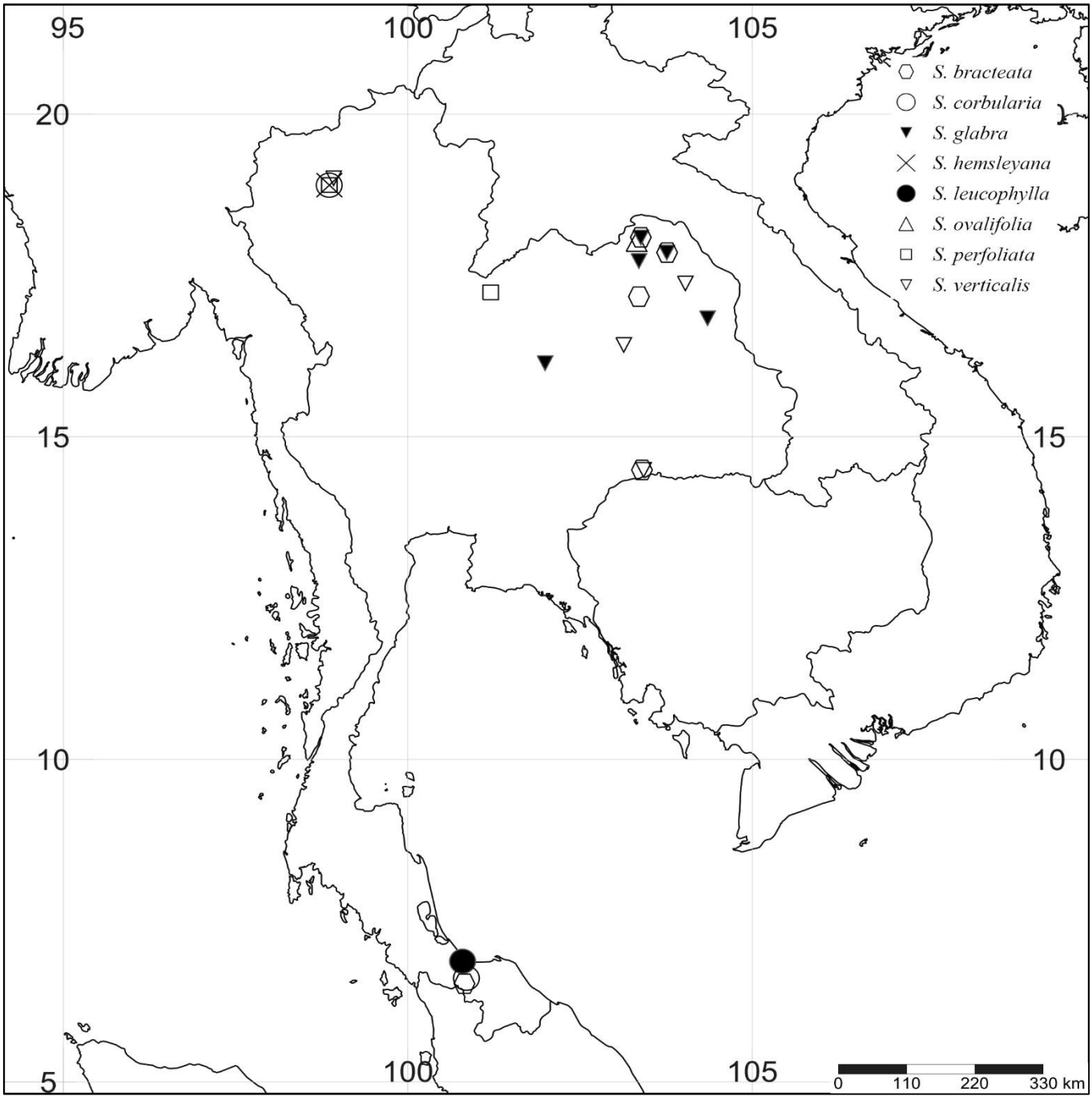

Figure 1. Studied sites and the used *Smilax* species found in Thailand.

Figure 2. *Smilax bracteata*: A, habit. *S. corbularia*: B, upper leaf surface; C, lower leaf surface. *S. glabra*: D-E, habit & inflorescence. *S. hemsleyana*: F, habit. *S. leucophylla*: G, habit. *S. ovalifolia*: H, prickly stem; I, leaf. *S. perfoliata*: J, upper leaf surface; K, tendrils. *S. verticalis*: L, habit; M, infructescence.

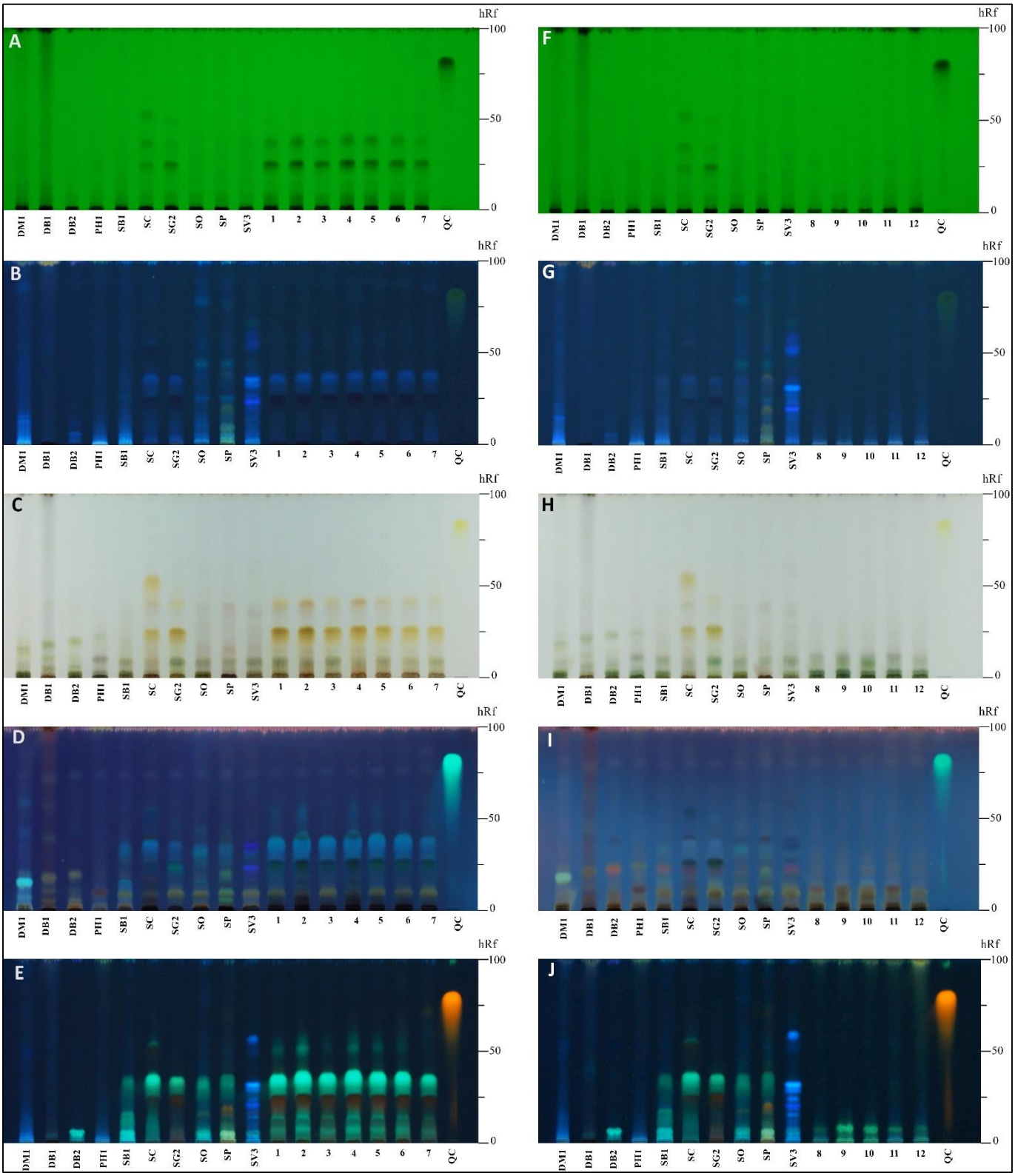

Figure 3. TLC chromatograms of methanolic extracts of authentic specimens and commercial samples A & F, observed under UV 254 nm; B & G, observed under UV 366 nm; C & H, observed under the White light; D & I, sprayed with natural products-polyethylene glycol reagent and observed under UV 366 nm; E & J, sprayed with anisaldehyde-sulphuric acid reagent. (DM1, *Dioscorea membranacea*; DB1-2, *D. birmanica*; PH1, *Premna herbacea*; SB1, *Smilax bracteata*; SC, *S. corbularia*; SG, *S. glabra*; SO, *S. ovalifolia*; SP, *S. perfoliata*; SV3, *S. verticalis*; 1-7, Hua-khao-yen-nuea commercial samples; 8-12, Hua-khao-yen-tai commercial samples; QC, *quercetin*).

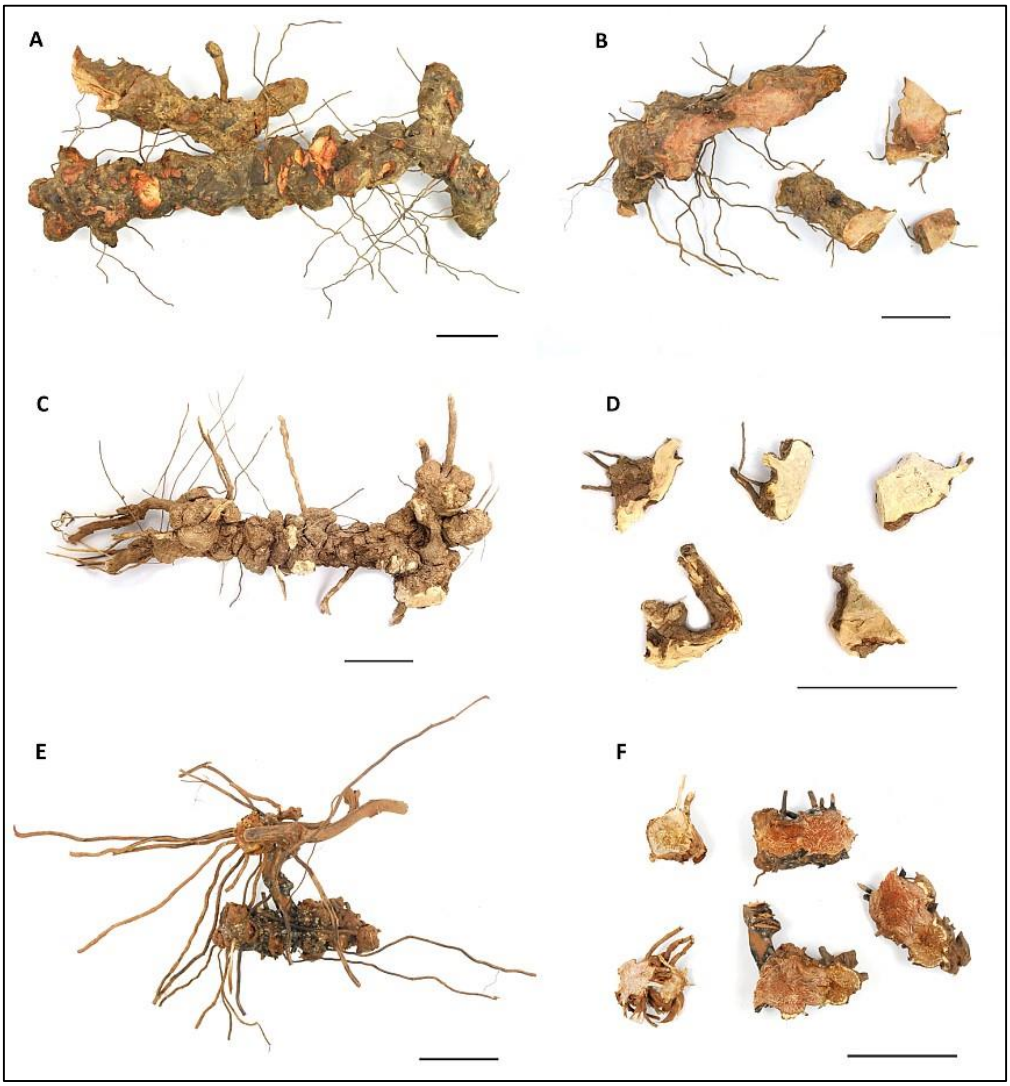

Figure 4. *Smilax glabra*: A, rhizome; B, crude drugs. *Premna herbacea*: C, rhizome; D, crude drugs. *Smilax corbularia*: E, rhizome; F, crude drugs. All scale bars=5 cm. [A, B from *Pansumrit* 40 (PBM5527); C, D from *Pansumrit* 23 (PBM5518); E, F from *Pansumrit* 54 (PBM5530)].

3.2. Botanical origin of the Hua-khao-yen crude drugs

The TLC chromatograms and macroscopic characters showed that the commercial samples no. 1-7, of which called the Hua-khao-yen-nuea, were identical to *Smilax glabra* and the samples no. 8-11, called the Hua-khao-yen-tai, to *Premna herbacea* (Figures 3-4). The chromatograms of methanolic extracts of authentic and commercial samples were presented in Figure 3.

The Hua-khao-yen-nuea crude drug was the dried rhizomes of *S. glabra* (Figure 4A) with irregular-cylindrical shape, 10-30 cm long and 2-4 cm in diameter, robust, greyish brown to dark brown, pale pink to reddish-brown when transversely cut (Figure 4B). The Hua-khao-yen-tai crude drug was the dried rhizomes of *P. herbacea* (Figure 4C) with the nodular-cylindrical shape, 10-30 cm long and 2-5 cm in diameter, stout, greyish-brown to yellowish-brown, white to pale yellow when transversely cut (Figure 4D).

The TLC chromatograms of *S. corbularia* are similar to *S. glabra*. However, the macroscopic character was informative to distinguish the crude drug. The rhizome of *S. glabra* was robust texture with powdery and smooth on cut surfaces, while *S. corbularia* was slender with fibrous and rough surfaces (Figure 4E-F). Microscopic data of *S. glabra* and *S. corbularia* were available[38].

The authentication of sample no. 12 was inconclusive with incongruent evidence between macroscopic and chemical data. The chemical fingerprint was similar to *S. bracteata*, but morphological characters were similar to *P. herbacea*. The major concern of using chemical evidence is chemical degradation during the post harvesting. Storage condition, especially temperature and humidity, could impact on crude drug quality[39]. The TLC fingerprinting effectively identified the generic level of the crude drugs. Although the TLC method is recommended for the authentication, the more sophisticated analytical instrument, e.g. High-Performance Liquid Chromatography (HPLC), should be used in the

further study.

Some herbal drug stores, which are major suppliers of herbal crude drugs in Thailand, were not included to the survey due to the limitation of the number of replied questionnaires. In the future, the purposive sampling method may be worth to perform in order to include more herbal drug stores[40].

4. CONCLUSION

Our research is the first ethnobotanical study of the genus *Smilax* in Thailand. In total, 10 species were recorded based on the field surveys and literature review (Table 2). The Hua-khao-yen is the homonym crude drugs which referred to at least four species. *Smilax glabra* is the most used species which is under threat due to the overexploitation. The use of the *Smilax* for the treatment of genitourinary illnesses has the highest IAR index. The use for the neoplasms is also confirmed by the index. This study found that the commercial samples of the Hua-khao-yen-nuea and Hua-khao-yen-tai are originally from *S. glabra* and *P. herbacea* (Figures 3-4), respectively, which the combination of chemical and macroscopic evidence is informative for the botanical verification.

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Conflict of interest

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