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Species diversity, usages, molecular markers and barcode of medicinal *Senna* species (Fabaceae, Caesalpinioideae) in Thailand

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The diversity of the *Senna* species found in Thailand and usages was investigated. Species identification was performed using morphological characters concurrently with DNA fragment sizes of the *trnH-psbA* spacer region. The fourteen *Senna* species in Thailand are *Senna alata*, *Senna alexandrina*, *Senna fruticosa*, *Senna garrettiana*, *Senna hirsuta*, *Senna occidentalis*, *Senna pallida*, *Senna siamea*, *Senna sophora*, *Senna spectabilis*, *Senna sulfurea*, *Senna surattensis*, *Senna timoriensis*, and *Senna tora*. Usage information in Thailand was recorded by observation, viewing, market surveys, literature reviews, and interviews with locals and traditional healers. Most of these species are widely used as foods, ornamentation, and medicine for Thai people. Using of primers from the *trnH-psbA* spacer region as barcode primer, the different DNA fragment sizes of the *trnH-psbA* spacer region were taken for a species-specific marker for further rapid, accurate and automatable species identification for a plant part namely leaf, chopped plant or powdered. Moreover, the DNA fragments were sequenced for the basis of barcode as nucleotide differences among species. These tag sequences have been submitted to the GenBank data base, with the accession numbers.

Key words: Barcodes, chloroplast DNA, medicinal plants, *Senna*.

INTRODUCTION

Senna Mill. is a genus in the family Fabaceae, subfamily Caesalpinioideae, comprising of many species, widespread and has diverse morphological characters. Formerly, *Senna* was included in the genus *Cassia* L., but it was separated out by Irwin and Barneby (1982). The number of *Senna* species is usually estimated to be about 260 species, but some authors believe that there are as many as 350 species (Randell and Barlow, 1998). About 50 species of *Senna* are known in cultivation (Huxley et al., 1992). Sennas are herb, shrub or tree. Leaves are pinnately compound. The inflorescence has long stalk, is a raceme, panicle or corymb. They have

showy flowers yellowish in color and are often asymmetric. The pedicels lack bracteoles, having five overlapping sepals and five petals which are similar to each other. There are usually ten stamens occurring in three sets. The three largest stamens are abaxial, the four medial stamens are middle sized, and the three smallest are adaxial staminodial stamens. The anthers are basifixed and the gynoecium is often enantiostylous. The fruits are often elongated, cylindrical or flattened. Sennas have played a major role in herbalism and folk medicine as purgatives as they have the active ingredients anthraquinone derivatives and glucosides called sennosides or senna glycosides. *Senna alexandrina* Mill. is used in modern medicines as a laxative. Additionally, they are components in many herbal remedies and tonics. *Senna* glycosides are listed as Anatomical Therapeutic Chemical (ATC) code

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A06AB06 on their own and A06AB56 in combined preparations (Spiller et al., 2003). *Senna alata* (L.) Roxb. root is used to regulate menstrual flow and for chronic gonorrhoea and, sap from fresh leaves is universally recognized as a remedy for parasitic skin diseases and for many eruptive and pustular skin infections (Burkill, 1995). According to Burkill (1995), the leaves of *Senna obtusifolia* (L.) Irwin and Barneby are chewed for cough and pneumonia in Nigeria and are often mixed with other drugs for fever. The seed is also used in treating conjunctivitis.

The leaf of *Senna occidentalis* (L.) Link is the most useful part of the plant and is considered to have utilitarian and therapeutic values (Soladoye et al., 2010). The leaves are added to soup as source of diet in southern parts of Nigeria. The pods of *Senna podocarpa* (Guill. and Perr.) Lock is used in the treatment of the skin diseases such as eczema, scabies and ringworm while an extract of the pods is taken as a purgative. The commercial "Senna" has been shown to be made from the leaves or pod of *Senna alexandrina*. Aside from medicines, many species of *Senna* are ornamental and shade plants such as *Senna spectabilis* (DC.) Irwin and Barneby, and foods such as *Senna siamea* (Lam.) Irwin and Barneby and *Senna sophera* (L.) Roxb.

Senna species contain medicinal compounds from the anthraquinone family, which vary from one species to the others and may be poisonous if taken in excess. These anthraquinones common in the genus lead to the apparent ethno(space) medicinal similarities between these species by as some species are used interchangeably with other *Senna* species (Soladoye et al., 2010). Therefore, an accurate, rapid and simple way to identify *Senna* species is needed to determine which of many species might occur in a medicinal plant sample that is powdered or not flowering nor fruiting, and DNA barcodes solve this problem well.

DNA markers are essential for plant parts as leaf, short trunk, pod, chopped plant or powdered especially traditional medicines. The plants always take to medicinal industries, traditional healers or medicinal producers by dried pieces mentioned in the foregoing. Therefore, species specific marker is authentic for plant verifying. Molecular method called DNA barcoding can be served as a species specific marker for overcoming the problems.

DNA barcoding is a common method of biological species identification and can be used in samples which have only a short reliable DNA region or DNA that is highly degraded, as found in processed food, in fossil remains, or herbarium specimens. There is much extensive research on DNA barcoding in plants beginning in 2003 by Dr. Paul D.N. Hebert, a population geneticist at the University of Guelph in Ontario (Hebert et al., 2003). Since then, there have been many studies testing standard regions in plant groups aiming to provide rapid,

accurate and automatable species identification by using a standardized DNA region as a tag (Hebert et al., 2005). Chase et al. (2007) proposed to use two barcoding region options as a standard protocol for barcoding all land plants: the three combined regions of the *rpoC1*, *matK*, and *trnH-psbA* intergenic spacers, or the *rpoB*, *matK* and *trnH-psbA* regions. Newmaster et al. (2008) proposed using *matK* and *trnH-psbA* to identify plants in Myristicaceae.

Finally, Hollingsworth et al. (2009), a plant working group at the Consortium for the Barcode of Life (CBOL) recommended *rbcl+matK* as the core DNA barcode regions for land plants. Towards our aim to revise species diversity and usage information for *Senna*, we selected the short *trnH-psbA* spacer regions from the chloroplast DNA as effective and accurate standard sequences. These regions were sequenced for the *Senna* species under study and the sequences are stored in the NCBI data base for others to use for further rapid, accurate and automatable species identification.

MATERIALS AND METHODS

Species diversity of *Senna* were investigated over a two year period (2008 to 2010) across the provinces of Thailand as via participant observation, market surveys, and interviews with local people and traditional healers. All *Senna* species can grow throughout Thailand; the collections were done randomly, on some provinces namely Chiang Mai, Northern Thailand; Khonkaen, Udon thani, Nong Khai, and Loei provinces, Northeastern, Thailand; Bangkok, Saraburi, and Prachin Buri provinces, Central Thailand. Identification was conducted following Larsen and Larsen (1984) and Wu et al. (2010). Usage information in Thailand was recorded by observation, viewing, market surveys, literature reviews, and interviews with locals and traditional healers. Samples were collected from species growing in the wild and preserved by silica gel for DNA extraction while herbarium samples were pressed for deposit at BK. All collected samples underwent DNA extraction, DNA barcode amplification and DNA barcode sequence analysis with the use of the *trnH-psbA* spacer region.

DNA extraction

Genomic DNA was extracted from all collected samples using Plant Genomic DNA Extraction kit (RBC Bioscience). Extracted DNA was examined by subjecting it to 0.8% agarose gel electrophoresis stained with ethidium bromide. The quality and quantity of DNA were determined by a gel documenting instrument. Then, DNA samples were diluted to a final concentration 20 ng/μl, and these dilutions were used as a DNA template in the PCR reaction.

DNA barcoding region amplification

Amplifications were performed again for DNA barcoding development using forward and reverse primers, 5'-GTTATGCATGAACGTAATGCTC-3' and 5'-CGCGCATGGTGGATTACAATCC-3' of the *trnH-psbA* spacer region (<http://www.kew.org/barcoding/update.html>). The reaction mixing for PCR was carried out in 25 μl consisting of GoTaq

Table 1. Scientific names of studied plants and GenBank accession numbers of *trnH-psbA* barcoding region.

Species	GenBank accession number for <i>trnH-psbA</i>
<i>Senna alata</i>	GU969278
<i>S. siamea</i>	GU969279
<i>S. occidentalis</i>	GU969280
<i>S. surattensis</i>	GU969281
<i>S. garrettiana</i>	GU969282
<i>S. tora</i>	GU969277
<i>S. spectabilis</i>	JF838360
<i>S. sulfurea</i>	JF838361
<i>S. hirsuta</i>	JF838362
<i>S. sophera</i>	JF838363
<i>S. pallida</i>	JF838364
<i>S. alexandrina</i>	JF838365
<i>S. timoriensis</i>	JF838366
<i>S. fruticosa</i>	JF838367

Green Master mix (Promega), 0.25 μ M each primer and 10 ng DNA template. The reaction mixture was incubated at 94°C for 1 min and amplification was performed with the following thermal cycles: 35 cycles of denaturation for 30 s at 94°C, 40 sec annealing temperature at 53°C, 40 s extension at 72°C, and 5 min final extension at 72°C using a Swift™ Maxi Thermal Cycler (ESCO). The amplified products were detected by 1.2% agarose gel electrophoresis in TAE buffer and visualized with ethidium bromide staining.

DNA barcode sequencing

The amplified specific fragments of the samples were sequenced and the sequences were tested for showing nucleotide differences among species called sequence alignment using MEGA software version 4 (Tamura et al., 2007). The sequences were submitted to GenBank and the corresponding accession numbers are listed in Table 1.

RESULTS

Species diversity

Senna species distribute throughout Thailand, so the usages have been occurred in all regions of Thailand. They are commonly used as shade plants, ornamentals, food and traditional medicines in both the natural plants and prepared forms. Fourteen *Senna* species were found throughout Thailand: *S. alata* (L.) Roxb., *S. alexandrina* Mill., *S. fruticosa* (Mill.) Irwin and Barneby, *S. garrettiana* (Craib) Irwin and Barneby, *S. hirsuta* (L.) Irwin and Barneby, *S. occidentalis* (L.) Link, *S. pallida* (Vahl) Irwin and Barneby, *S. siamea* (Lam.) Irwin and Barneby, *S. sophera* (L.) Roxb., *S. spectabilis* (DC.) Irwin and Barneby, *S. sulfurea* (DC. ex Colla) Irwin and Barneby, *S. surattensis* (Burm.f.) Irwin and Barneby, *S. timoriensis* (DC.) Irwin and Barneby, and *S. tora* (L.) Roxb. These dried specimens were kept at BK herbarium with collector numbers Chaveerach 687-700.

Usage information

Senna species are commonly used in the natural form for food, shade, ornament or traditional medicine. Certain species, for example *S. alata* and *S. alexandrina* used in both natural and prepared forms as herbal infusions. A study of the ethnobotany of *Senna* was carried out by interview and the ways in which the genus is used to enhance Thai people's lives are shown subsequently.

S. alata (L.) Roxb.

Thai name: *Chumhet thet*

Common names: Candelabra bush, Candle bush, Candle tree, Christmas candle, Emperor's candlesticks, Ringworm bush and Ringworm tree. It is widely used mostly leaves in all regions of Thailand in both the natural and prepared state as an herbal infusion, capsule, powder and cream which are commonly sold in department stores, traditional drugstores and the Chaophraya Aphaiphubet hospital, Prachin Buri province. The four pharmacological activities of this species lead to its use in four common treatments: for constipation (laxative activity), abscesses and wounds, inflammation, and a dermatophyte infection called ringworm (Figure 1A).

Traditional uses: Treating abscesses and wounds: add water to cover 1 handful of twig and leaf pieces, boil until water is reduced to 1/3. Clean abscesses and wounds every morning and evening with the boiled water. Treating constipation: Put 12 to 15 sun or fire dried leaflets in some water and then boil. Drink once before breakfast or before bedtime. Alternatively, eat 2 to 3 soft-boiled inflorescences with chili sauce.

Treating ringworm: Grind fresh leaves to a powder with or without a little water or grind an equivalent amount of leaves, garlic and red lime (calcium carbonate) then smear the mixture on ringworm-affected skin twice a day

until ringworm disappears. It is more effective if one scrapes the skin surface of the affected area before smearing on the powder.

For ornamentation: Used as ornamental planting in office areas, campuses, and parks due to the tree's beautiful shape and yellow inflorescences. Additionally, the leaf and bush shape are considered dignified and attractive.

Caution: Fresh leaves should not be used medicinally, they must be fire or sun dried to eliminate substances that cause side effects of nausea. Manufactured or processed uses: A number of products are manufactured from the dried *S. alata* leaves and pods. It is used in commercially prepared non-prescription drugs where capsules of the cream, powder and infusion of the plant are used. These products are prescribed in Thailand for the treatment of constipation, skin inflammation and ring worm and are often found online as well.

S. alexandrina Mill.

Thai name: *Ma kham khaek*

Common names: Senna, Alexandria senna, Alexandrian senna, Indian senna, Tinnevely senna

It is widely used in all regions of Thailand in both the natural and prepared state as herbal infusions and capsules of the pre-fruiting leaves and young pods which are commonly sold as *S. alata*.

In Thailand, this species is commercially grown on farms as an annual in amphur Wang-muang, Saraburi province, it is propagated by seeds near the end of the rainy season. Leaves are harvested 2 to 3 times per year starting at 2 to 3 months after sowing and then in 1 month intervals. Pods are harvested last after 4 months. Leaves and pods are sun dried, and then manufactured into different medicinal products (Figure 1B).

Traditional uses: For treating constipation: Put dried 1 to 2 handful of leaves (about 3 to 10 g) in water, boil, and drink the water or grind 1 to 2 handful dried leaves to a powder, add the powder to hot water to make an infusion. Alternatively, to relieve stomach pain, boil 4 to 5 dried pods with water or boil 10 to 15 dried pods with ginger (*Zingiber officinale*) and salt, and drink the water.

Manufactured or processed uses: Capsules and infusions are manufactured from the dried *S. alexandrina* leaves and pods for commercially prepared non-prescription drugs for the treatment of constipation. These products can be found on many Thai websites.

S. fruticosa (Mill.) Irwin and Barneby

Thai name: *Khi lek yawa*

Common name: twin-flowered cassia.

Rarely used in all regions of Thailand as an ornamental in offices and parks due to its beautiful yellow

inflorescences. The plants are seen in Queen Sirikit Botanic Garden, Maerim, Chiang Mai province (Figure 1C).

S. garrettiana (Craib) Irwin and Barneby

Thai names: *Samae san*, *Khi lek san* and *Khi lek kao*

Common name: Manatapat

It is widely used in all regions of Thailand for shade, ornamentation, food, and traditional medicine. The tree has yellow flowers that alternate with glossy light green leaves when young and glossy dark green when mature. The glossy leaf is a character distinguishing this species from *S. siamea*.

Traditional uses: Medicine: The heartwood is boiled with water with the decoction used as drinking water to treat constipation, expectorant and emmenagogue.

Food: Young shoots and leaves are cooked in a dish called *kaeng khi lek* (a kind of curry) just as with *S. siamea*. Shade and ornamentation: It is commonly planted at road side and farms interchangeably with *S. siamea* as an ornamental plant and shade (Figure 1D).

S. hirsuta (L.) Irwin and Barneby

Thai names: *Phong pheng*, *Chang chud* and *Dab phit*

Common names: Woolly Senna, Senna and Woolly
Widely used in all regions of Thailand as a traditional medicine. It spreads in the wild by seeds following the rainy season as weed.

Traditional uses: To treat poisoning: Northern Thai hill tribes usually grate the root into water to be drunk to treat poisoning from food such as toxic mushrooms or vegetable. All plant parts boil with water for women after giving birth to drink treatment all poison by eating, drinking and smelling. The local Thais in Ladkoo village, tambon Bandu, amphur Muang, Loei province uses stem with the bark removed, as they say the leaves and bark have a bad smell. This de-barked stem is soaked in water until the medicinal substances dissolve into the water, and then drunk to treat poisoning and drunkenness similar to the northern Thai and hill tribes (Figure 1E).

S. occidentalis (L.) Link

Thai name: *Khi lek phi* and *Chum het lek*

Common names: Coffee weed and *Coffea senna*

A weed, it is widely distributed in unoccupied regions of Thailand, where it grows in conjunction with *S. sophora*. All plant parts smell bad, so its use is not widespread. In the country side of northeastern Thailand (Khon Kaen, Roi Et and Loei provinces) steamed mature pods are sometime eaten (Figure 1F).

S. pallida (Vahl) Irwin and Barneby

Thai name: *Lueang ostrelia*

Common name: Twin-flowered cassia

It is widely used in all regions of Thailand as an ornamental in offices, campuses, and parks due to its admired inflorescences. It is a small shrub with yellow flower that is commercially propagated from the branches (Figure 1G).

S. siamea (Lam.) Irwin and Barneby

Thai names: *Khi lek*, *Khi lek ban*, *Khi lek kaen*, *Khi lek luang* and *Khi lek yai*.

Common names: Cassod tree, Thai copper pod, Siamese senna, Iron wood, Yellow cassia and Thailand shower.

It is widely used in all regions of Thailand in both natural and processed forms for shade, ornamentation, cooking, and traditional medicine. The yellow-flowered tree is usually planted with other *Senna* species namely *S. garrattiana* and *S. spectabilis*.

Traditional uses: For treating constipation: Boil about 4 to 5 handfuls of fresh or dried leaves with water and drink the water before breakfast.

For treating insomnia: Boil 30 g dried leaves or 50 g fresh leaves and drink the water before bedtime.

For food: Young shoots and leaves are used for cooking a dish called *kaeng khi lek* which is found in two forms – with and without coconut milk – that is very popular eating throughout Thailand.

Manufacturing and processed uses: Capsules and tablets of *S. siamea* are popular alternative medicine choice in Thailand for their laxative, relaxing, and sleep-promoting properties (Figure 1H).

S. sophora (L.) Roxb.

Thai names: *Phak wan ban*, *Phak khet* and *Phak khlet*.

Common names: Senna and African senna.

Widely distributed in unoccupied regions of Thailand as a weed. It always grows in conjunction with *S. occidentalis*. The young shoots and fruits eaten as a soft boiled vegetable with chili sauce are very popular among rural Thai in northeastern and northern Thailand. Additionally, it is used as an ornamental since it is an attractive bush with beautiful yellow flowers (Figure 1I).

S. spectabilis (DC.) Irwin and Barneby

Thai names: *Khi lek American* and *Khi lek wan*

Common name: Spectacular cassia

As it is considered less bitter than *S. siamea* (*khi lek*), *S. spectabilis* is called *khi lek wan* (sweet khi lek) in the rural areas of Northeastern Thailand such as Nanglek village, amphur Kosumpisai, Mahasarakham province where it replaces the *S. siamea* in the cooked dish *kaeng khi lek*. In addition, it is a popular road side ornamental tree with a long flowering period with beautiful yellow inflorescences, usually planted interchangeably

with *S. siamea* and sometimes *S. garrettiana* (Figure 1J)

S. sulfurea (DC. ex Colla) Irwin and Barneby

Thai names: *Trueng badan* and *Song badan*

Common name: Smooth senna

It is a widely used shrub in all regions of Thailand solely as an ornamental in offices, around a house, and parks due to its beautiful year-round inflorescences, full of yellow flowers. It is commonly planted on road sides and traffic island interchangeably with *S. surattensis* which are often mistaken for *S. sulfurea*. It is commercially propagated both vegetative from branches via air layering and seed germination (Figure 1K).

S. surattensis (Burm.f.) Irwin and Barneby (Figure 1L)

Thai name: *Song badan*

Common names: Scrambled egg tree, Scrambled egg tree bush and Scrambled egg tree plant.

It is widely used in all regions of Thailand similarly to *S. sulfurea* as an ornamental in offices, home regions, and parks due to its beautiful year-round inflorescences, full of yellow flowers. It is commonly planted on road sides interchangeably with *S. sulfurea*. It is commercially propagated both vegetative from branches via air layering and seed germination.

S. timoriensis (DC.) Irwin and Barneby

Thai names: *Khi lek lueat*, *Khi lek pa* and *Khi lek daeng*

Common name: -

It is widely distributed in all forest regions of Thailand. Young leaves and flowers can be used as vegetable by soft boiling and eating with chili sauce. The heartwood is used as a traditional medicine by women to stimulate menstrual blood flow (Figure 1M).

Traditional uses

To stimulate blood flow (emmenagogue): Dip 5 to 6, 2.5 to 4.0 × 12.5 cm slices of heart wood in a pot of boiling water, and drink the water as an emmenagogue and diuretic for women.

S. tora (L.) Roxb.

Thai names: *Chumhet thai*, *Chumhet na* and *Chumhet lek*

Common names: Sickle senna and Foetid cassia

Widely used in all regions of Thailand in the natural state as a tea. It is a weedy small shrub in wild areas.

Traditional uses: Boil 10 to 15 g fire dried seeds with water, then drink the water before bedtime or after breakfast to treat constipation. Boil 15-30 g dried leaves with water and then drink as another constipation treatment. It can also be used to help relax and promote restful sleep by grinding 10 to 15 g fire dried seeds to a powder, adding it in hot water and drink. Five to



Figure 1. Plant specimens: *Senna alata* (A), *S. alexandrina* (B), *S. fruticosa* (C), *S. garrettiana* (D), *S. hirsuta* (E), *S. occidentalis* (F), *S. pallida* (G), *S. siamea* (H), *S. sophera* (I), *S. spectabilis* (J), *S. sulfurea* (K), *S. surattensis* (L), *S. timoriensis* (M) and *S. tora* (N).

fifteen gramme fire dried seeds boiled with water and drinking act as a diuretic (Figure 1N).

Barcoding and molecular markers

DNA extraction and DNA barcoding were successfully performed in all studied samples with the *trnH-psbA*

spacer regions. The sizes of bands are different at about 400 to 600 bp as shown in Figure 2. The different banding size of each species is called a species specific marker sequence. The alignments were tested for showing nucleotide differences among *Senna* species and shown by genetic distance. These tag sequences have been submitted to the GenBank database with the accession numbers listed in Table 1.

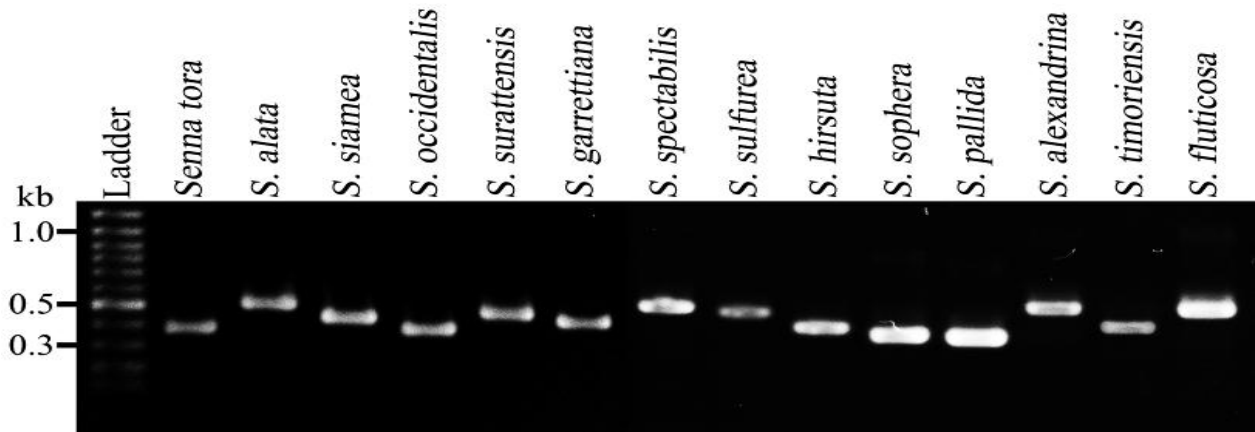


Figure 2. DNA barcode fragments as a species-specific marker from *trnH-psbA* spacer region of the fourteen *Senna* species.

DISCUSSION

Senna in Thailand was studied by Larsen and Larsen (1984) who state that there are three native Thai species including *S. siamea*, *S. timoriensis* and *S. garrettiana* and fifteen exotic species namely *S. alata*, *S. alexandrina*, *S. bicapsularis*, *S. fruticosa*, *S. garrettiana*, *S. hirsuta*, *S. occidentalis*, *S. pallida*, *S. septemtrionalis*, *S. singueana*, *S. sophera*, *S. spectabilis*, *S. sulfurea*, *S. surattensis*, and *S. tora*. In our investigation, fourteen species were found, all except *S. bicapsularis*, *S. septemtrionalis* and *S. singueana*. This may be because they are not culturally popular, rarely seen, or they have disappeared from Thailand. The two native Thai species, *S. siamea*, and *S. garrettiana* especially the first species are noted for being used to cook the dish *kaeng khi lek* for many generations.

All fourteen species found are currently popular with Thais for ornamentation, cooking and traditional medicine in both natural and processed states. *S. pallida*, *S. sulfurea* and *S. surattensis* are used only as ornamentals. They are generally found planted on roadsides, traffic islands, and home and office gardens. Despite being used only ornamentally, they likely contain important medicinal compounds similar to the anthraquinone and glycosides of the other *Senna* species that could be used to improve human health and should be studied further. The assumption will be that *Senna* species may contain identity medicinal substances, with the amount depending on growing conditions and habitat.

Senna species used as traditional medicines commonly contain anthraquinones and glycosides. The medicinal properties of *S. alata* derive from the anthraquinones and glycosides it contains including rhein, aloe emodin, and physcione; the free aglycones such as emodin, aloneemodin, chrysophanol and isochrysophanol; plus kaempferol, sitosterol and sennosides (Campbell and Cooper, 1955; Rao et al., 1975; Harrison and Garro, 1977; Rai, 1978; Smith and Sadaquat, 1979). The laxative activity of *S. alexandrina* derives from its

sennoside, aloe emodin, and dianthrone glycoside contents which are anthraquinone derivatives and glycosides (Okada, 1940; Erspamer and Paolini, 1946; Joy et al., 1998). The pharmacological activity of *S. garrettiana* derives from the many anthraquinone derivatives it contains such as chrysophanol and cassialoin. Additionally, it contains many other medicinal substances including aloe emodin, aloin, betulic acid, cassialoin, cassigarol (A-G), chrysophanic acid, chrysophanol dianthrone, piceatannol, piceatanol, protococatechuic aldehyde, quercetin, rhamnetin, rhamnocitrin (Mahidol University 1992). The many anthraquinones derivatives of the young shoots and leaves of *S. siamea* produce this species' pharmacological activities that lead to it being used to treat constipation.

Young and mature leaves contain anhydrobarakol which relieves anxiety, promote restful sleep, and provides calming and relaxing effect (Mahidol University, 1992; Thongsaard et al., 1996; Pooviboonsuk et al., 2000; Padumanonda and Gritsanapan, 2006; Sakulpanich and Gritsanapan, 2009). The pharmacological activities of the leaves and seeds of *S. tora* are due to its many anthraquinones and glycosides such as aloe-emodin, 1,8-dihydroxy-3 (hydroxymethyl)-anthraquinone, leading to its use for constipation. Additionally, it can be used to help with relaxation and promoting restful sleep (Raghunathan et al., 1974; Koshioka et al., 1978; Mukerji, 1985). Additionally, some *Senna* are used to treat ring worm, abscess and wound treatment. *S. siamea* and *S. tora* contain anhydrobarakol which relieves anxiety, promotes restful sleep, and relaxes (Thongsaard et al., 2001; Maity and Dinda, 2003; Padumanonda and Gritsanapan, 2006). The compounds of the other nine species found in Thailand have not been identified. *S. hirsuta* has a unique effect for *Senna* in that it is used to treat poisoning caused by food, water, alcohol and inhalation. As a precaution, no *Senna* species should be taken by women who are pregnant, breast-feeding or in

their menstrual period. The locals said the people have exhausted supply of water due to purgative properties of the plants. Processed and natural forms of *Senna* are sold as medicines on many Thai websites using Thai name keywords.

The Thai governmental organizations of the Institute of Thai Traditional Medicine, Ministry of Public Health; Office of The Primary Health Care Committee, and The Government Pharmaceutical Organization and others oversee the production of traditional and manufactured medicine. Additionally, some hospitals produce, sell, and guide people in the use of Thai traditional products made from *Senna* species, such as Chaophraya Aphaiphubet hospital, Prachin Buri province and other provincial hospitals.

As *Senna* is often used in highly processed forms in Thailand, barcode markers are a necessary and effective method of determining the species of unidentified medicinal powders, stem cuttings, and dried or ground leaves. Of the standard region studied, *trnH-psbA* is the most effective at distinguishing *Senna* species by different band sizes without having to perform sequencing and alignment for fixing nucleotide differences among the species. It can be called a species specific DNA marker. Additionally, called specific marker, the authors are still calling them as barcodes because of using *trnH-psbA* barcode primers and sequencing. The different DNA fragments are sequenced and compared showing nucleotide differences among the species following the basis of barcoding. The region is quite useful aid in determining the right medicine for the right illness. The tag sequences that we performed and stored in the NCBI database are distinct species specific markers and therefore greatly improve further rapid, accurate and automatable species identification of processed *Senna* parts that have no visible morphological features.

The efficiency of the region is agreed to Hollingsworth et al. (2009) who proposed that the *trnH-psbA* region is a strong candidate for plant barcoding aside from core barcodes such as *rbcL* and *matK*. It is possible to use small sample sizes in molecular studies as quoted by Hillis (1987). Molecular study are usually much smaller than in morphological studies (often as small as a single individual) because of analyses of large sample sizes are often limited by availability of specimens and /or expense of analysis. However, the studied samples were randomly collected leading to have realistic results. DNA barcode which is the specific marker for identifying plant using the standard sequences usually use only one individual sequence for a species used at the family, genus and species level identification.

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