STANDARD OF THAI HERBAL MEDICINE No. 3

Standard of Thai Herbal Medicine

Senna alata (L.) Roxb.

Medicinal Plant Research Institute
Department of Medical Sciences
Ministry of Public Health, Thailand
ISBN 974-7549-37-9

Standard of Thai Herbal Medicine

Senna alata (L.) Roxb.

Medicinal Plant Research Institute
Department of Medical Sciences
Ministry of Public Health, Thailand

ISBN 974-7549-37-9

Standard of Thai Herbal Medicine : *Senna alata* (L.) Roxb.

Advisors	Narongsakdi Aungkasuvapala, M.D.	Director-General, Department of Medical Sciences
	Suphan Srithamma, M.D.	Deputy Director-General
	Thaweephol Dechatiwongse Na Ayudhya	Expert on Standardization and Control of Quality Herbal Medicine
	Malee Bunjob	Expert on the Production of Herbal Products
	Somchai Sangkitporn, M.D.	Director, Medicinal Plant Research Institute

Chairperson Pranee Chavalittumrong

Editors Anchalee Chuthaputti
Jaree Bansiddhi
Yenchit Techadamrongsin

Authors Pranee Chavalittumrong
Yenchit Techadamrongsin
Jaree Bansiddhi
Anchalee Chuthaputti
Pranom Dechwisissakul
Suthida Chaiyaraj
Saranya Tharaswaeng
Aimmanas Attawish
Duangpen Pattamadilok
Wiranut Chaibunruang

ISBN 974-7549-37-9
First edition September 2002, 5000 copies
Photographers Jaree Bansiddhi, Wichien Wongsupalak
Printing Design Bansuansil Publishing
Printed by E.T.O. Press, Bangkok

Thai government has always regarded Thai medicinal plants as valuable domestic resource and national heritage. Hence, it is a government policy to utilize Thai medicinal plants for the health of Thai people both in the form of folk remedies in the primary health care and in the form of more developed healthcare products. The value-added healthcare products from medicinal plants, namely health food, herbal drinks, dietary supplements, herbal medicines and cosmetics are not only useful for domestic consumption but also for export to help solve the country's economic problem.

As the Department of Medical Sciences (DMSc), Ministry of Public Health is responsible for consumer protection and research and development in health sciences, one of our responsibilities is to conduct complete-cycled research and development of medicinal plants in order to support the proper and safe use of good quality herbal medicines. An important area of the R&D on medicinal plants of the DMSc is to set up the national "Standard of Thai Herbal Medicine" for the quality control of medicinal plant materials and for the improvement of the quality of the commonly used herbal raw materials and finished products. Furthermore, the standard specifications of medicinal plants are also used to control the quality of herbal products that will be tested for toxicity, therapeutic efficacy and safety in the process of new drug development. The quality control of medicinal plants and herbal medicine not only serves as a means of consumer protection but also helps Thai herbal products and raw materials gaining more acceptances abroad.

Senna alata (L.) Roxb., or "Chum-Het-Thet" in Thai, a medicinal plant found in every region of Thailand, is one of the medicinal plants promoted in the primary health care and selected as one of the herbal medicinal products in the National List of Essential Drugs. The leaf of *S. alata*, when taken as herbal infusion or tea, is useful as a laxative, and when used externally in the form of topical cream, is useful for fungal infection of the skin. Hence, *S. alata* has a great potential for the development into herbal medicines to replace imported modern medicines and for export. The DMSc therefore set up the quality standard of *S. alata* as a means to control and improve the quality of *S. alata* raw material and herbal medicine.

While "Standard of Thai Herbal Medicine : *Senna alata* (L.) Roxb." is the first book in the English version of the Standard of Thai Herbal Medicine book series, the Thai version of this book is the third in the book series published by the DMSc. The first and the second of the book series in Thai are the Standard of Thai Herbal Medicine for *Andrographis paniculata* (Burm.f.) Nees and for *Curcuma longa* L. (turmeric) published in 1999 and 2001, respectively. This book series should give general knowledge and scientific information to the public on how the standard of herbal medicine and the quality control of medicinal plant materials are established. DMSc hopes that "The Standard of Thai Herbal Medicine : *Senna alata* (L.) Roxb." will be useful for the healthcare personnel, medicinal plant growers, people in the herbal medicine business and industry, and the general public alike as a guideline for the development of good quality raw materials and herbal products from *S. alata* in the future.

Narongsakdi Aungkasuvapala, M.D.
Director-General
Department of Medical Sciences
September, 2002

Senna alata (L.) Roxb. is the currently accepted nomenclature of the plant previously named *Casaia alata* L.[1-4], also known as ringworm senna, candle bush or candlestick senna, or in Thai as "Chum-Het-Thet"[2]. *S. alata* has a long history of use as a folk medicine[5-8] and its therapeutic efficacy is well recognized, as a consequence, it is officially accepted in the national pharmacopoeias in many countries[5,6,9]. Even though the indications of *S. alata* leaf may vary in different countries, the two indications mostly acknowledged in the folk medicine are "laxative"[5-9], since it contains the anthraquinone glycosides commonly found in several genera, such as *Senna* and *Cassia*[4,9-12], e.g. *Senna alexandrina* Mill., *Senna tora* (L.) Roxb. and *Cassia fistula* L., and "topical antifungal" for the treatment of dermatophyte infection[5,6]. Both indications of *S. alata* have been scientifically proven *in vitro* and in animal studies. *S. alata* extracts were shown to exhibit laxative activity by stimulating the movement of gastrointestinal tract[7,13] and possess antifungal activity against dermatophytes[14-21]; these two pharmacological activities therefore support its therapeutic claims. However, microbiological studies showed that *S. alata* is either weakly active or inactive against *Candida albicans*, a yeast-like fungus which causes oral thrush[14,16,21-24].

Clinical reports in humans also confirmed the therapeutic efficacy of *S. alata* as a laxative[25] and a topical antifungal[26,27]. Based on the above-mentioned scientific evidence, *S. alata* was therefore selected as one of the herbal medicines in the National List of Essential Drugs of Thailand for the treatment of constipation, ringworm, athlete's foot, and tinea versicolor[28].

In addition, there have been many reports on other pharmacological activities of *S. alata* leaf extracts conducted *in vitro* or in animals, but no clinical studies to support such therapeutic efficacy or to establish appropriate doses for such indications. Hence, the following reported pharmacological activities of *S. alata* have not yet been clinically accepted, e.g. antibacterial[29-31], antiviral[32], anti-inflammatory[33,34], analgesic[35], hypoglycemic[36], and antioxidant activities[37,38].

Therapeutic efficacy of any herbal medicine relies on the quality of the raw materials and the manufacturing process, quality control is therefore essential to ensure the quality of herbal medicine which usually faces the problem of the inconsistency of the active principle content. In order to produce a high quality herbal medicine, one needs to have good basic knowledge in various aspects about that herb, and people with expertise in various fields are involved. Initially, the plant growers need to know the right species, varieties and cultivars of the plant, the cultivation process, the proper period of time to collect the part used of each plant, as well as appropriate post-harvesting process. Then the quality of the raw materials and herbal medicines must be determined at the beginning, during and after the manufacturing process. According to the principles of Thai traditional medicine, the quality of an herbal raw material is decided by its appearance, smell and taste, or other traditional testing procedures. In contrast, the

scientifically based quality control of an herbal raw material is performed by qualitative and quantitative analyses according to the previously stated quality standard of each medicinal plant material.

"The Standard of Thai Herbal Medicine : *Senna alata* (L.) Roxb." is the first English version of the book series published by the Department of Medical Sciences, Ministry of Public Health, Thailand. The content of this book can be divided into three parts. The first part is the "General Information about the Quality Control of Medicinal Plant Materials" aiming to inform those who are not familiar with the concept to better understand what the "Quality Standard of Herbal Medicine" is about and how it is set up. The second part is devoted to the Quality Standard of *Senna alata* Leaf as raw material for the production of laxative. The third part is the Appendix covering the information about the definition and the preparation of some reagents used in the quality control of *S. alata* leaf based on Thai Herbal Pharmacopoeia Volume I.

The medicinal plant growers, healthcare personnel and the manufactures of *S. alata* products can all benefit from this book, which covers cultivation and harvesting techniques, post-harvest handling techniques to prevent the plant material from moisture and bacterial contaminations. For the analytical chemists, this book provides the analytical procedure for the quality control of *S. alata* raw material based on the Standard of Thai Herbal Medicine described in the Thai Herbal Pharmacopoeia. It is hoped that this book will serve as a means to promote and improve the production of good quality herbal medicine from *S. alata* in Thailand and for foreigners who are interested in Thai herbal medicine to better understand about Thai herbs and our herbal products.

GENERAL INFORMATION ABOUT THE QUALITY CONTROL OF MEDICINAL PLANT MATERIALS

The quality control of medicinal plant materials, which are used worldwide as folk medicine or raw materials for the pharmaceutical industry, has always been one of the main concerns of the World Health Organization (WHO). Therefore, WHO organized the meetings of experts from various countries to establish internationally accepted guidelines for assessing the quality of medicinal plants so that they can be used by the regulatory agency in each country to set up the national quality specifications of medicinal plant materials.

Based on the WHO and other related documents[5,9,39-44], this chapter summarizes general information about the quality control of medicinal plant materials that all parties involved in the production of herbal medicine need to understand in order to manufacture good quality, effective and safe products. The topics covered in this chapter are as follows: -

1. General description of the plant

1.1 Local name
1.2 English name
1.3 Scientific name
1.4 Scientific synonym
1.5 Morphological description of the plant
1.6 Geographical distribution and local abundance
1.7 Habitat
1.8 Part used
1.9 Chemical constituents
1.10 Preparation of crude drug
- Cultivation
- Harvesting
- Post-harvest handling
- Packaging and storage

2. Quality specification

2.1 Official definition
2.2 Description of crude drug
2.3 Identification
2.3.1 Pharmacognostic characteristics
- Macroscopical description
- Microscopical description
  - Histological characteristics
  - Description of powdered drug

2.3.2 Chemical characteristics
- Preliminary test
- Confirmatory test

2.4 Foreign matter
2.5 Moisture
2.6 Total ash
2.7 Acid-insoluble ash
2.8 Solvent extractives
2.9 Main/active constituents
2.10 Microbial contamination
2.11 Pesticide residue contamination
2.12 Arsenic and heavy metal contamination
2.13 Radioactive contamination

3. Indication
4. Toxicity
5. Contraindication
6. Warning
7. Preparation used and dose

The significance of the above-mentioned topics to the proper use of medicinal plants is described below.

General Description of the Plant

Names

In order to select and use the right kind of medicinal plant, it is necessary to know the correct scientific name of the plant. Local names of the plants often cause confusion because one plant usually has different local names in different regions of the country or different plants sometimes have the same local names. Hence, if wrong kind of a plant is mistakenly used, not only the required therapeutic efficacy will not be achieved but toxicity may also occur instead. The correct scientific name of a plant not only helps us to use the right kind of plant but is also useful for searching of the scientific information about that plant.

Morphological description of the plant and habitat

The information about morphological description of the plant and its habitat is helpful for saving the time to search and collect the plant, or for the planning of cultivation process to maintain adequate and sustainable supply of the plant.

Part used

To obtain the required therapeutic efficacy of the medicinal plant, one needs to know which part of the plant is used, whether it is the root, the leaf, the flower, the fruit or the seed or others. Different parts of the plants contain different amount of the active constituents. Hence, if wrong part of the plant is used, not only one cannot benefit from the plant but toxicity may occur instead.

Chemical constituents [42]

Several groups of phytochemicals exist in different parts of a plant and in different kinds of plants. For the development of a plant-derived medicine, it is necessary to know which compound is the active chemical constituent of the plant. The groups of chemical constituents commonly found in medicinal plants are as follows.

Alkaloids This group of nitrogen-containing phytochemicals are bitter and alkali in nature and usually possesses pharmacological activities. Some examples of alkaloids are atropine from *Datura metel*, strychnine from *Strychnos nux-vomica* or snake wood, morphine from opium, and quinine from cinchona.

Glycosides The molecules of glycosides are composed of two parts, namely the glycone or the sugar part and the aglycone or the non-sugar part. Several glycosides are used as medicines; e.g. anthraquinnone glycosides from ringworm senna leaf, senna leaf or aloe are useful as laxative.

Volatile oils or essential oils are oily liquids with characteristic odors, which are usually pleasant and volatile at room temperature. Main constituents of volatile oils are terpenoids. Some volatile oils that have medicinal uses are, e.g. clove oil, which is used as a carminative, antiseptic, and local anesthetic to relieve toothache; peppermint oil, which is useful as a carminative to relieve bloating; and eucalyptus oil, which is used as an expectorant and antiseptic.

Tannins are a group of phytochemicals with astringent taste and hence are used as an astringent to treat mild diarrhea. Tannins can be found in *Acacia catechu*, leaf and fruit of guava (*Psidium guajava*) and fruit of *Terminalia*

chebula, etc.

Flavonoids are a group of plant constitutents, which usually has colors, e.g. carthamin with red color from *Carthamus tinctorius* petal, luteolin with yellow color from *Lonicera japonica* flower, chrysin with light yellow from the bark of *Oroxylum indicum*. Some flavonoids can help strengthen the vascular wall, e.g. rutin and quercetin.

Steroids belong to a group of compounds with chemical structures similar to steroid hormones and steroidal anti-inflammatory agents. Hence, some plant steroids, e.g. diosgenin from *Costus speciosus*, are used as the precursors for the semi-synthesis of steroid hormones.

Terpenoids are another group of compounds commonly found in plants. Terpenoids are the main constituents of volatile oils, e.g. limonene and citronellol.

Gums are sticky and gummy substances secreting from the cuts on some plants. Certain gums, e.g. gum acacia, gum tragacanth, are used for the pharmaceutical preparation of suspensions.

Other chemical constituents commonly found in medicinal plants are fat, carbohydrates, proteins, amino acids, enzymes, vitamins, resin, and balsam.

Preparation of crude drug [42-44]

The preparation of crude drug as raw material is a very important step for the production of herbal products. One should keep in mind that no matter how good the analytical procedure is, it cannot guarantee the quality of the crude drug or the finished product if the active constituent is destroyed prior to the analysis.

The preparation of crude drug can be divided into 4 steps, namely: -

- Cultivation of medicinal plant
- Harvesting of the part used
- Post-harvest handling of crude drug
- Packaging and storing of crude drug

Each above-mentioned step is very crucial to the quality of the herbal raw materials and involves people in various fields of expertise, namely agronomists, medicinal plant growers, crude drug buyers and sellers, herbal drug manufactures, health care personnel, and others. Each person in his or her own way can contribute to the quality of herbal raw materials, which eventually will benefit the consumers' health.

- **Cultivation of medicinal plants**

The quality of each crude drug usually depends on a particular group of active constituents and its content, which is affected by the species or variety or cultivar of the medicinal plant selected, environmental conditions, methods of appropriate cultivation, and husbandry techniques.

- **Harvesting of the part used**

It is important to know the part used of each plant that will serve as an herbal raw material for the production of herbal products, the age of the plant, the time of the day to harvest, and appropriate harvesting technique. Generally, the part used should be harvested from the fully mature plants using the flowering period as the indicator of plant maturation.

General guidelines for harvesting of different parts used of medicinal plants

- **Whole plant or aerial part** of annual or biennial herbs should be harvested at the beginning of the flowering period and harvesting should be done in the morning.
- **Root or rhizome** should be harvested at the dormant period or during winter until summer. For each kind of medicinal plant, it is necessary to determine the most appropriate age of the plant for harvesting to achieve the desired content of active constituent.
- **Leaf** Usually the early mature leaves should be harvested before they are fully mature and they should be harvested in the morning.
- **Stem bark** should be harvested during summer or the beginning of the rainy season. The age of each plant appropriate for harvesting should be determined.
- **Wood** should be harvested during the late period of rainy season until winter. The wood of certain medicinal plants can be harvested any time of the year.
- **Flower** should be harvested before blooming or at the beginning of the blooming period. However, there are certain plants that the flowers are harvested at full bloom. The harvesting of the flower is generally done in the morning.
- **Fruit** should be harvested when it is fully mature.
- **Seed** should be harvested when the fruit is fully mature.

- **Post-harvest handling** [44]

This step of raw material preparation is aimed at controlling the quality of each medicinal plant material after the part used is appropriately harvested. Inappropriate handling of harvested plant material may result in the degradation of active constituents and the decrease of raw material quality. Post-harvest handling is composed of two steps, namely:-

☆ *Cleaning of the herbal raw material* After harvesting, any foreign matters or contaminants that are not the part used of the plant should be removed and discarded. The plant material should then be cleaned with clean water, and if required, it should be cut, chopped or sliced into appropriate sizes. Certain herbs may need to be heated, steamed, or boiled at this step.

☆ *Drying* Herbal raw material that has high moisture content is not only susceptible to microbial contamination but its active constituents are also prone to chemical degradation. Hence, herbal raw material has to be dried under appropriate conditions, namely:-

Sun dry or air dry Herbal raw material may be dried under the shade or under direct sunlight depending on the types of the herbs.

Oven dry The oven suitable for drying herbal raw materials should have an exhaust fan. Temperature should be set according to the part used of the plant, e.g. for flower, leaf, whole plant or aerial part, the temperature should be about 35-45°C, while stem bark, wood, root or fruit should be dried at 40-60°C.

- **Packaging and storage**

Appropriate packaging and storage help maintain the quality of herbal raw material and prevent it from moisture or microbial contamination, or infestation by insects. Small amount of herbal raw material should be kept in tightly closed amber glass jar with the name of the herb, the amount (weight), and the date that the raw material is prepared or kept on the label. Large amount of raw material should be divided into several proportions and separately kept in clean and well-closed plastic bags, gunnysacks, or appropriate sized containers instead of storing the whole batch in one large container. The opening and closing of a large container several times will increase the chance of microbial contamination and increase the moisture content of the raw material, which will, in turn, lower its quality. The stored herbal raw material should be kept in a cool dry place with good ventilation and it should be sun dried or oven dried periodically every 2-3 months. In general, herbal raw material should be used within one year, or longer depending on the types of herbs.

Quality Specification of Medicinal Plant Materials/Crude Drug

Official definition

In setting up the quality standard of a medicinal plant material used for herbal medicine preparation, it is necessary to give the official definition of the plant so that the readers know which plant the quality standard is for. The official definition of the plant includes the scientific name, part used, and, if known, the minimum amount of

the active constituent that should be present in the plant material.

Description of crude drug

Sometimes we cannot obtain fresh plant material to prepare our own crude drug and we may have to purchase dried or powdered crude drug from suppliers instead. Certain crude drugs are hard to identify or differentiate if we do not have previous experience with those crude drugs before. It is also possible that we might end up buying wrong kind of crude drug because there are several Thai herbs with the same common names. Furthermore, some dishonest plant growers or suppliers may use other plants to replace the actual one or use as an adulterant; hence, special care must be taken when purchasing medicinal plant materials. Quality standard of a medicinal plant material therefore contains "description of crude drug" to explain how one can identify each plant material using its color, odor, taste and shape.

Identification of crude drug [44,45]

The identification of a plant material or crude drug is based on two main characteristics; namely pharmacognostic characteristics and chemical characteristics.

Pharmacognostic characteristics are the detailed description of specific characteristics of the plant material. Plant materials of which their pharmacognostic characteristics have already been identified would then be kept as reference specimens for the identification of sample plant materials in the future. Pharmacognostic characteristics can be divided into two types.

Macroscopical description is the detailed description of the plant material that can be observed visually or under magnifying lens, i.e. shape, size, surface characteristics, texture, wrinkle and fracture characteristics, and appearance of the cut surface, etc.

Microscopical description is the detailed description of the plant material, as observed under microscope, which can be divided into two characteristics, namely: -

Histological characteristics describe the orientation of cells and their organelles in the tissues of the part used of the plant. Histological examination of plant tissues is performed by cross-sectionally or longitudinally dissecting the part used of the plant into very thin slices and staining with appropriate dyes.

Description of powdered drug as seen under microscope is an important part of the quality standard of a plant material. The findings that cells or organelles of certain plant materials, though their tissues are ground into powder, can retain special microscopic characteristics of the particular plants; make the characteristics of the powdered drug a useful tool for plant identification.

Chemical characteristics Medicinal plants contain varieties of phytochemicals. Knowing the class of the active constituent of a plant and establishing the chemical procedures to identify such compounds is crucial and useful for research and development purpose, consumption purpose, commercial purpose, and sometimes for plant identification purpose. Consequently, chemical characteristics are an indispensable part of the quality standard of medicinal plant materials. Two types of chemical tests are commonly used, namely: -

Preliminary test is the procedure to chemically detect the group of active constituent(s) by color reaction, formation of precipitate, or any other chemical reactions that are useful tools for more sophisticated testing of the chemical component further.

Confirmatory test is the chemical procedures or techniques to determine the composition of the group of active constituents previously identified in the preliminary testing. There are several analytical methods that can be employed but chromatography is most commonly used. Chromatography can be classified into various techniques, e.g. thin-layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), etc. The choice of techniques depends on the group of active constituents to be analyzed. TLC is one of the most commonly used techniques for setting up the quality specification of a medicinal plant material because it is a rapid and economical procedure to identify its chemical components as compared to other procedures.

Foreign matter [39,40,44,46]

Foreign matter means anything else besides the part of the plant that is used for the preparation of herbal medicine.

- *Organic foreign matter*, e.g. other parts that are not the part used from the same plant, parts of other plants, animal parts including fecal matters.
- *Inorganic foreign matter*, e.g. gravel, rock, soil, sand, etc.

Generally, medicinal plant material should contain no more than 2% of foreign matter.

Moisture [39,40,44]

In general, moisture content of plant material should not be higher than 10%, except for certain plants of which their appropriate moisture contents are set at a higher level. There are two methods to determine the moisture content and the most appropriate method for each plant material must be used.

Gravimetric method determines the moisture content by heating the plant material in the oven until completely dry and the "loss on drying" weight of the plant material is determined as the moisture content. This method is simple and suitable for plant materials containing no other volatile substances but water.

Azeotropic distillation method determines moisture content by measuring the water content obtained by distillation. This method is more complicated and costly than the first method. It is suitable for plant materials containing volatile substances other than water, e.g. herbal materials containing volatile oils.

Ash [39,40,44]

The amount of two types of ash obtained after burning the plant material in a muffled furnace must be determined and used as another indicator of plant material quality, namely: -

Total ash which is the sum of the amount of physiological ash derived from plant tissues and non-physiological ash derived from other foreign matters, e.g. rock, soil, sand, etc. Generally, the amount of total ash should be between 1-20%.

Acid-insoluble ash is used to determine the amount of inorganic foreign matters, e.g. gravel, soil, sand, contaminated in the plant material. In general, the amount of acid-insoluble ash should be between 1-10%.

Solvent extractives [39,40,44]

The amount of solvent extractives obtained by extracting a plant material with an appropriate solvent is a means to determine the amount of the active constituent to control the quality of a medicinal plant material when a more specific assay procedure cannot be established.

Main/active constituent

If the active constituent of a plant material is known and the procedure for the quantitative analysis can be established, the amount of the active constituent will serve as a much better indicator of the quality of medicinal plant material than the content of solvent-soluble extractive.

Contamination [40,44]

Medicinal plant materials containing no contaminants or lower amount of contaminants than the specified limits would be safe for long-term consumption. A medicinal plant material may be contaminated with various types of contaminants, which can significantly lower its quality, namely: -

Microbial contamination Care must be taken to prevent medicinal plant materials from microbial contamination or toxin contamination from certain types of fungi, e.g. aflatoxin. Each country including Thailand has set up microbial limits allowed for medicinal plant materials. In

Thailand, microbial limits of herbal medicines are published in the Thai Pharmacopoeia[44]. If gamma irradiation is required to kill microorganisms contaminated in plant materials, it must be performed with great care and under the close supervision of authorized personnel from responsible office.

Pesticide residue contamination Nowadays pesticides are widely used in agricultural practice. These pesticides can therefore contaminate in herbal materials and accumulate in the body upon long-term consumption. Hence, limits of pesticide residues allowed in medicinal plant materials are set for most commonly used pesticides.

Arsenic and heavy metal contamination Plant materials may be contaminated with arsenic and heavy metals from environmental pollution. For the safe use of plant materials, WHO therefore recommends that the amount of arsenic and heavy metals, e.g. cadmium and lead, be determined as a part of the quality control process of plant material.

Radioactive contamination Nowadays radioactive substances have been used for various purposes and nuclear accidents sometimes occur. The spreading of radioactive substances into the global ecological system may result in radioactive contamination to medicinal plant materials in certain areas of the world. WHO in collaboration with other concerned agencies have therefore made recommendation on this subject.

Other information

Other pieces of information that will be useful for the safe and effective utilization of herbal medicine and should be give to the consumers are **indication, toxicity, contraindication, warning, precaution, dosage form and strength, and dosage.**

STANDARD

for

Senna alata Leaf

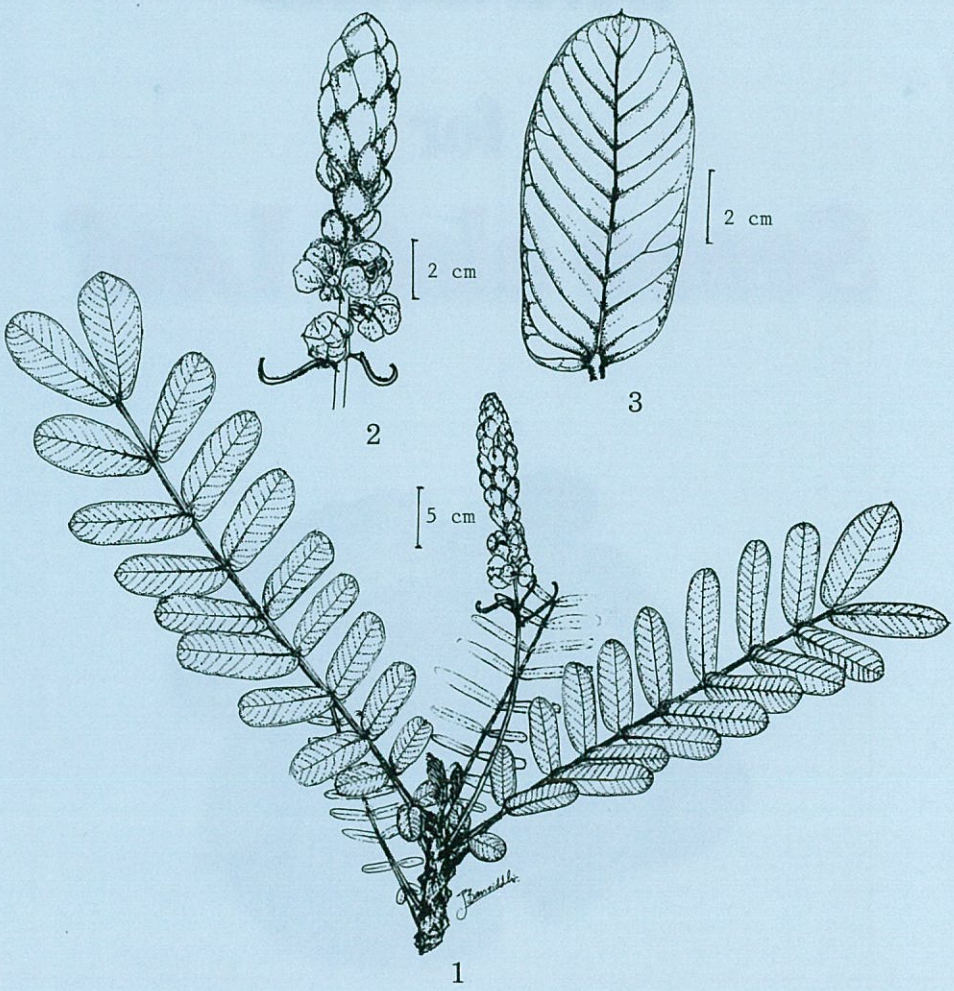


Figure 1 *Senna alata* (L.) Roxb.

1. twig 2. inflorescence 3. leaflet

Standard for *Senna alata* Leaf

General Description of the Plant *Senna alata* (L.) Roxb.

Names

Local names : Khi khak, Chum het thet, Chum het yai, Lap muen luang, Mak kaling thet[2]

English names : Acapulco, Akapulko[48], Candelabra bush[2], Candle bush[1,48], Candlestick senna[1], Christmas candle[49], Empress candle plant[49], Impetigo bush[1], Ringworm bush[2,50], Ringworm senna[50], Ringworm shrub[49], Seven golden candlesticks[4,49,50]

Scientific name : *Senna alata* (L.) Roxb.[51,52]

Scientific synonyms : *Cassia alata* L.[50-52]
Cassia bracteata L.f.[51]
Herpetica alata (L.) Raf.[52]

Family : Leguminosae

Morphological description [51-53]

A shrub 1-2 m tall, sometimes up to 5 m; horizontally spread branches. Stipules auriculate-deltoid, 6-15 mm, persistent. *Leaves* spirally or alternate arranged; paripinnate with 8-20 pairs of leaflets; petiole robust, 2-3 cm; rachis 30-60 cm. *Leaflets* oblong-obovate or oblong-elliptic, 5-15 cm long and 3-7 cm wide; base rounded or cordate; apex rounded or almost notched, with a short sharp point; margin entire; petiolules robust, 2-3 mm. *Inflorescence* racemose, axillary and terminal, dense, robust, many-flowered, 20-50 cm long and 3-4 cm wide; bracts strobilate, at first enveloping the flowers, yellow, broadly ovate, caducous, 2-3 cm long and 1-2 cm wide; flower bright yellow; pedicels 5-10 mm. *Sepals* 5, orange-yellow, oblong, subequal, 1-2 cm long and 6-7 mm wide. *Petals* 5, subequal, bright yellow, ovate-orbicular, 1.6-2.4 cm long and 1-1.5 cm wide, short-clawed. *Stamens* 9-10; 2 large with stout filaments 4 mm long and anthers 12-13 mm opening by apical pores; 4 with filaments 2 mm long and anthers 4-5 mm opening with apical pores; reduced stamens 3-4. *Superior ovary* puberulous, ovules many; style filiform; stigma small. *Pod* dehiscent, sharply tetragonal, blackish, winged, 10-15 cm long and 1.5-2 cm wide, wings 4-8 mm wide. *Seeds* many up to 50, shining, flattened, slightly quadrangular, 7-8 mm long and 5-8 mm wide. (Figure 1)

Geographical distribution and habitat

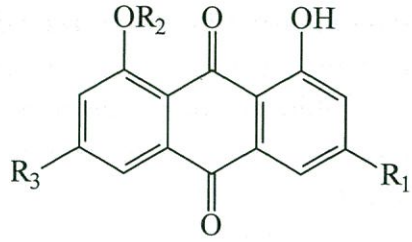
Senna alata is native to tropical America such as South and Central America[4,48], now pantropical, abundantly naturalized and occasionally planted[4,5,52]. It occurs from the tropical lowland up to 2,100 m altitude[4,51,53], but is more common at lower elevations. It is reported to tolerate an annual rainfall from 600 mm to 4,300 mm and average yearly temperatures of 15-30°C[4]. It has a wide ecological amplitude, along river blanks, margins of ponds, ditches and wet areas[4,52,54], including the water logged areas[7]. It grows well in full sun on a wide range of soils, which should retain moisture adequately, particularly aggressive in areas where there is a high water table, prefers rather open, not too dry habitats[4].

Part used

Leaf

Chemical constituents

Senna alata leaf contains free aglycones of hydroxyanthracene derivatives, e.g. rhein, emodin, aloe-emodin, chrysophanol, and isochrysophanol, and glycosides of rhein, aloe-emodin and physcione. It also contains kaempferol, β-sitosterol and sennosides A, B, C, and D[9]. (Figure 2)



Rhein : R_1 = COOH, R_2 = R_3 = H
Rhein-8-glucoside : R_1 = COOH, R_2 = $C_6H_{11}O_5$, R_3 = H
Emodin : R_1 = CH_3, R_2 = H, R_3 =OH
Aloe-emodin : R_1 = CH_2OH, R_2 = R_3 = H
Chrysophanol : R_1 = CH_3, R_2 = R_3 = H
Physcione : R_1 = CH_3, R_2 = H, R_3 = OCH_3

Figure 2 Chemical structures of active constituents in *Senna alata* leaf

Preparation of crude drug

Cultivation

Presently *S. alata* is not yet grown in industrial agriculture in Thailand, and research-based knowledge on the cultivation and propagation of this plant is still insufficient for the establishment of appropriate national guidelines of the Good Agricultural Practice (GAP) of *S. alata.* However, the following general technical information and research-based data should be useful for the growers.

Propagation

Method of propagation[48, 55,56]

- ***Asexual reproduction:*** propagation by stem cutting
- ***Sexual reproduction:*** propagation by seeds

Propagation material by sexual reproduction

Propagation by seeds is the means by which the plants reproduce in nature and one of the most efficient and widely used propagation methods for planting *S. alata* in Thailand[55]. Research on the improvement of *S. alata* cultivar is however still lacking.

Seed selection[7,57,58]

The fully mature seeds should be collected from unbroken ripening pods on the standing plants, before the pods dehisce, shatter, or drop to the ground. Usually, the pods should be collected when the humidity is low, and avoids harvesting during or after the rain because it will increase the susceptibility of the seeds to diseases and lower the seed vigor. The collected pods should be placed on canvas, tray, or screen and allow to dry in open air for 1-3 weeks[57,58] before separating the seeds from the pods and debris. Only the fully mature healthy seeds with shiny dark-brown or dark grayish-brown color, free from pest or diseases, should be selected[7]. For optimum storage, well-winnowed and cleaned seeds must be dried to moisture content of less than 10%[57]. It was reported that unripe green, but fully-grown seeds did not germinate [59].

It requires a good planning in order to grow *S. alata* that produces a high yield of leaves as the raw material for the production of herbal medicine. Initially, a very large quantity of seeds must be prepared so that enough healthy seedlings can be selected for cultivation. The seeds should be cultured in the proper environmental conditions for good uniform germination to enable the plants to grow at the

same rate. The selected seedlings must be erect, strong, healthy, and free from pathogens or insects[7,57].

Seed storage

Seeds are usually stored from harvest until the next planting season; hence, attempt should be made to control every condition that helps retain seed viability, e.g. the rate of physiological change of the seeds, and the environmental conditions of storage, primarily temperature and humidity. The control of moisture content of the seed is probably the most important factor in seed longevity and storage. Rapid drying can cause shrinkage and cracking, some of these injuries are internal and not noticeable, but causing low viability after storage.

Seeds can be stored in bulk, but more often are stored in smaller lots in bags, sealed cans, or moisture-tight packets, which maintain the seeds at low moisture content. It was reported that if the seeds contain more than 10-20% moisture, heat would be generated during storage in closed containers leading to the impairment of seed viability or loss of germinating ability. The storage conditions that maintain seed viability are low moisture content of the seed, low storage temperature, and modification of the storage atmosphere. For example, the most effective storage is to dry seeds to 3-8% moisture, place in sealed containers, and store at temperatures of 1-5°C with 50% relative humidity[57,58].

In Thailand *S. alata* seeds only need to be stored from harvest until the next planting season. Generally after harvesting, seeds of legumes have a dormant period of 1-

6 months, depending on temperature and storage condition. According to the preliminary research by the Division of Medicinal Plant Research and Development, Department of Medical Sciences, Thailand, it was found that fully mature *S. alata* seeds that were stored for 12 months at ambient temperature had the germination rate of only 34.25%[60].

Seeds germinating test[57,58]

Seed germination test, which is a method of judging seed viability, is essential for successful seed propagation. Viability is expressed by the germination percentage, which indicates by the number of normal seedlings produced by a given number of seeds within a specified period of time. The test can also tell the percentages of abnormal seedlings or ungerminated seeds. The seeds that fail to germinate might be because they are dormant, dead, or moldy, etc. Usually good quality seeds for large-scale cultivation should have a germination percentage of at least 85%[7].

The seeds that will be used as the starting material for organic cultivation should be of best quality and as free as possible from contamination and diseases in order to promote healthy plant growth. The seed health testing[57,58] should be performed to determine the presence of pathogens that may impact on seed germination and plant development.

Seed propagation

The seed coat of *S. alata* is thick and hard, consequently the seeds are impervious to water, gas permeability, or leaching of a germination inhibitor, causing

the delay of seed germination or 'dormancy'. Some forms of pre-treatment must therefore be given to the seeds to overcome such dormancy before propagation [57,58].

Breaking dormancy[7,59,61] Generally, the treatments are as follows: -

1. ***Acid scarification*** Dry seeds are immersed in concentrated sulfuric acid (H_2SO_4) at a volume ratio of about one part seed to two parts acid. Treatment time may vary with different kinds of seeds or seed lots.
2. ***Hot water scarification*** Drop the seeds into four to five times their volume of hot water (80 to 100°C) and the seeds are soaked in the gradually cooling water for 12 to 24 hours.
3. ***Mechanical scarification*** Chip hard seed coats by rubbing with sandpaper, cutting with a file, or cracking with a hammer, etc.

A preliminary research was conducted by the Department of Horticulture, Kasetsart University in Bangkok to compare the effect of different methods of breaking dormancy on the germination percentage of mature *S. alata* seeds 3 days after propagation. It was found that the seeds scarified by soaking in 80°C water for 10 minutes had germinating rate of 93%, while those soaked in concentrated H_2SO_4 for 20 minutes had germinating rate of 91%. By trimming off the seed coat at the opposite side of microphyle, a lower germinating rate of 85% was observed. In addition, research also showed that soaking the seeds in water for 24 or 48 hours was unable to break dormancy or induce germination[61].

Preparation of seedbed

Generally the cultivation of plants in a plantation require the production of a large number of homogeneous healthy seedlings that are free from diseases or pests; hence, seedbeds must be prepared for seed propagation. A good seedbed should have a loose but fine physical texture that produces close contact between seed and soil so that moisture can be supplied continuously to the seed. Such a soil should have suitable organic matters and provide adequate aeration. The surface soil should be free form clods and be of a texture that will not form a crust. The subsoil should be permeable to air and water with good drainage and aeration. Adequate soil moisture should be available to carry the seeds through the germination and early seedling growth stages, but the soil should not be waterlogged or anaerobic. Sanitation during propagation should also be considered[57].

Seedbed for the propagation of *S. alata* seedlings should be prepared by digging the soil with a hoe and raise the height of a plot to about 10-15 cm and the width of about 1 m to ensure good drainage. The length of the plot will depend on the number of seedlings to be transplanted. If there are several plots, leave a space about 50 cm wide between each plot to provide easy access. The seedbed soil should be broken into small clods and evenly spread to make a smooth surface[7].

Preparation of seeding

S. alata seeds should be immersed in hot water 80-100°C for about 10 minutes to accelerate the germination rate, then mixed with the sand at a ratio of 1:1-2, wrapped

in thin cloth, watered, and kept under the shade. The seeds will swell up and germinate in about 1-2 days[7]. It is critical to predetermine the proper rate of seed sowing in order to obtain a desired plant density[57]. The number of seeds for propagation per area of seedbed should be over-estimated, i.e. for the seeds with a germination rate of 85% or above, about 100-200 seeds per square meter should be used. However, avoid using too many seeds per seedbed area because the competing seedlings will become spindly, thin, and unhealthy and do not transplant well[7]. It is desirable to obtain a high percentage of healthy seedlings for the required size for field planting[57].

Sowing seeding material

Sow the newly germinated seeds previously mixed with the sand over the seedbed and use a rake to cover them up to a depth of about 1 cm. Cover the seedbed with straw or cogon leaves to provide shade, maintain soil moisture, and lessen the impact of heavy rainfall or water, which often causes the seeds to float resulting in weak seedlings[7].

Seedbed husbandry

Watering After sowing the seeds, water the seedbed. If there is no rain, keep daily watering the seedbed, in the morning and the late afternoon.

Diseases and pest control Sometimes insects and stem rot can damage the seedlings. However, the use of pesticides and highly toxic chemicals should be avoided if possible. When necessary, recommended products should be applied at a minimum effective level in accordance with the recommendation from the manufacturer and the regula-

tory agencies of both the grower and the end-user, or pesticides from natural source should be used instead.

Weeding Manual weeding is preferred to prevent damage to the seedlings or disturbance of their growth.

Transplanting of seedling

After 30 days the seedlings will sprout 5-7 leaves. Transplant them into the growing plot or in plastic bags and tend until seedlings are strong enough for transplantation in growing area. Great care must be taken when transplanting the seedlings not to damage them or break off their roots. It is best to water the plot first and then use a spade to dig up the seedlings, allowing as much soil to cling to the roots as possible. This will help them to recover sooner, and will result in a higher rate of survival[7].

Site selection and preparation of growing area

To select cultivation site, one should consider the risk of soil, air, and water contamination with toxic substances, i.e. heavy metal residues, hazardous or toxic chemicals, and select the sites free from the problem of pests or weeds.

The selected site for *S. alata* cultivation should be in the opened and sunny rather than shaded location, with abundant organic matters and loose soil, as well as adequate and constant soil moisture.

Land preparation: Practically, the plant growing area should be ploughed during late summer until early rainy season to control the weeds and to prepare the soil for cultivation in the rainy season. In the area where there is a

low density of weed, only one plough should be sufficient, while the area with high weed density or hard soil surface should be ploughed twice. The first plough is to get rid of the weeds and expose the soil to the sun for about 1-2 weeks to eliminate pests and plant pathogens. The second plough is to further loosen the soil and ease the removal of wood debris or weeds.

Preparation of growing holes: For large growing area, the growing holes should be marked by placing stick marks leaving about 3 meters spacing between seedlings and about 4 meters between rows. Dig the holes 40 x 40 x 40 cm in size, then line the bottom of the holes with manure 0.5 kg per hole, and well corpulate with the soil[7].

Planting

The climatic and environmental condition of the tropical region of Thailand is suitable for growing plants at any time of the year. However, the plants usually thrive best in the open when planted during the early rainy season. Moreover, the rain will also help saving the cost of watering the plants.

S. alata is very adaptable, it can grow at a fast rate achieving full size within one year[48]. Two general planting methods that can be used to cultivate this plant are described as follows: -

Methods of cultivation

1. *Field seeding :* This method can be used for planting *S. alata* along with other main farm crops, which can provide shade for newly germinated and young *S. alata*

seedlings, or for planting in the marshy land with no weed problem. Dig 30 x 30 x 30 cm holes, 3-4 m apart, and carefully pulverize the soil if compacted. Plant 5 to 6 previously scarified seeds in each hole, and cover with pulverized soil to 1 cm height. Mulch over the seed site to prevent weed competition and to maintain soil moisture. Cost and labor requirement for field direct seeding is lower than that of transplanting seedlings. However, the major problem of field seeding is the losses of seeds and young plants that result from predation by insect pest, diseases and from drying, hot weather, and weeds.

2. *Transplanting seedlings :* Transplant seedlings or remove plastic bags from the seedlings and put them in the holes at the rate of 1 seedling/hole. Cover up the hole with pulverized soil, tamp it down tightly against the seedlings, and water the seedlings soon afterwards. Tie the seedlings to sticks to support them from falling down. Mulch around the seedlings to maintain soil moisture and to prevent weeds from growing.

Husbandry

Watering

Initially if it is sunny and there is no rain, water the plants once or twice every day until they are 2-3 months old. Then keep watering every 2-3 days and later every 5-6 days. Watering frequency will depend mainly on the season and the level of soil moisture.

Fertilizers

Fertilizers are generally uneconomical for the sufficient organic-matter soil. All fertilizing agents should be applied sparingly and in accordance with the needs of the plants and supporting capacity of soil. Fertilizer should be applied in such a manner as to minimize leaching.

In case to improve soil fertility and structure, apply general garden fertilizer to the planting bed at 3-month intervals, and water immediately and thoroughly. Fertilize heavily after annual pruning. Fertilizer may be applied at different intervals[7] as follows: -

- When transplant seedlings, line the bottom of each hole with 0.5 kg of manure, and mix it well with the soil.

- When the plant are 1-2 months old, apply 1 tablespoonful (about 30 g) per plant of chemical fertilizer formula 16-20-0 or 30-20-10, once a month.

- From 3 months onwards, apply 200-300 g of chemical fertilizer formula 15-15-15 or 30-20-10, mixed with 1-2 kg of manure/plant every 3 months. Otherwise, general garden fertilizer (10-30-10) could be applied instead[48].

It is not necessary to apply fertilizer or manure during winter (December - February) because the pods and branches of *S. alata* stop growing by then. Apply fertilizer again after pruning the plants at the beginning of the rainy season (around May).

To apply fertilizer, sow it around the hole as far as the bush's perimeter, plough the soil to cover it, and water

immediately and thoroughly.

Ploughing

When the plant is at least 3 months old, plough around and towards the bole using a hoe and make a furrow around it about as far as the perimeter marked by the branches of the plant in order to retain water and to facilitate the application of fertilizer.

Stem supporting

Stem supporting should be provided when the plants are at least 3 months old and during rainy season to prevent them from falling down by heavy rains and strong wind by tieing them tightly to supporters. It is necessary to retie them once in a while to prevent the rope or cord from cutting into the stems and possibly damage them.

Pruning

In Thailand, *S. alata* grows rapidly in the rainy season with full sun on a wide range of soil. It flowers from late rainy season to cool season or during October to February[54]. Start pruning when the old branches begin to wither and become dry in the dry season starting from January or when the plants are infested with disease in order to prevent the disease from spreading. Using pruning shears or a knife cut these branches back to about 30 cm from the ground[48]. With proper irrigation and the start of the new rainy season, this pruning procedure assures optimum increase of new branching and blooming during the following season. Plants will become rangy if not drastically pruned.

Weed control

To achieve economic yields, competing weeds around the bush must be eliminated. Hand weeding is often attempted when the plants are young. For older plants, a hoe or a spade is preferred for weed control and ploughing at the same time. Herbicide application should be avoided.

Diseases and pest control [7,48,62-64]

So far there has been no report of the diseases or pests that affect long-term health or cause serious damage to *S. alata*.

Pesticide and chemical plant protection products should be avoided as much as possible. When necessary, approved plant protection products should be applied at the minimum effective level in accordance with the recommendation from the manufacturer and the regulatory agencies of both the grower and the end-user.

A few pests and diseases known to attack the plant, but are of minor concern are as follows: -

Diseases

- Leaf spot This disease is caused by the fungi *Pseudocercospora cassiigen*. It destroys the leaves by causing brownish-black spot on them. This fungus is spread by wind and water. The disease can be mechanically controlled by cutting off the affected parts and immediately destroyed. If the disease is serious, chemicals such as mancozeb or carbendazim can be used to control the fungi.

When using these chemicals, be sure to follow the instructions on the label.

- Powdery mildew This disease is caused by the fungi *Oidium* sp. It destroys the leaves, leaving white powdery spots on the surface. This fungus is spread by wind and water. The disease can be mechanically controlled by cutting off the affected parts and immediately destroyed. For more serious disease, chemicals such as dinocap, sulphur or benomyl can be used to control the fungi. When using these chemicals, be sure to follow the instructions on the label.

- Sooty mold This disease is caused by black mold (fungi) which circulate in the air and live on sweet mist that comes from excreta of aphids, appearing as black glaucous ashes on leaves. They are widespread during the cool season. The disease can be mechanically controlled by cutting off the affected parts and immediately destroyed. If the disease is serious, chemical such as carbaryl may be used. When using these chemicals, be sure to follow the instructions on the label.

The major insect pests

- Lepidopterous pest *Hedylepta bacalis* should be mechanically controlled by collecting egg mass and larvae off and immediately destroyed. If serious outbreak occurs, spray the recommended insecticides, e.g. microbial, pyrethroid, triazophos and IGR (Insect Growth Regulator). When using these chemicals, be sure to follow the instructions on the label.

- Aphids The sucking insect normally causes

damage by sucking and feeding at pods or leaves. In addition, the excremental solution from aphids also causes the outbreaks of black mold (fungi) on the leaves. The pest can be mechanically controlled by cutting the plant parts off and immediately destroyed. Avoid spraying with the synthetic pesticide, but use the biopesticides, such as neem solution, instead. In case of heavy infestation, the following effective insecticides, e.g. malathion or chlorpyrifos might be used. Strictly follow the recommended instructions on the label.

- Red mites They are red minute insects but could be observed by nake eyes. The red mites always suck and feed underneath the leaf and are also a virus carrier. In case of infestation, the surface of the leaves becomes coarse. In an advanced state of deterioration, the leaves become smaller, narrower and begin to droop. For disease control, the acaricides such as sulpher and recommended biopesticides should be applied.

Harvesting

S. alata leaves that will be used as raw material for the preparation of herbal medicine are generally harvested by picking only mature leaves that are neither too young nor too old, i.e. those which are 2-3 months old. The leaves should be harvested using pruning shears to cut off the whole leaves and put them in proper utensil. This method is quick, does not damage the leaves and suitable for transfer.

Normally, the required amount of the leaves of

S. alata is harvested when needed. The active constituents are high prior to flowering; hence, harvest should not be done in late full bloom[4,7].

In Thailand, *S. alata* grows well and produces the highest yield during the rainy season through to the early cool season. The plants will wither and produce no leaves during dry season when it is the best time for pruning and watering. Pinching new growth increases branching, creating a fuller canopy, which produces more leaves. Harvest can begin again 2-3 months after the plants have been pruned, when new branches are putting forth fresh leaves. Collect only the lower leaves first and allow younger and upper leaves for next harvest, which should take place 1-2 months later. However, preliminary research carried out by the Department of Medical Sciences shows that the most suitable leaves for drug preparation are those from at least 6-7 month-old plants of which the active constituents reach the desired standard level[7]. However, one should also keep in mind that the content of active principles of raw material derived from different habitats or cultivated at different sites may be considerably varied[65-67].

During harvesting, care should be taken to ensure that no foreign objects, weeds or toxic plants are contaminated in the harvested raw materials, so as to produce not only quality, but also safe raw material. Cutting devices, harvesters or other machines must be clean and adjusted such that contamination from soil particles and microbes are reduced to a minimum during use.

Post-harvest handling

The preparation of raw materials

After removing foreign objects from the leaves, rinse uncut leaves with clean water, then leave on a clean surface and allow the water to drain. Spread the leaves evenly on a clean utensil such as a winnowing basket or tray[7].

Damaged raw materials must be discarded during post-harvest inspection.

Drying process

Cover the leaves with a clean white cloth to protect from dust and wind and dry the leaves in the sun until they are completely dried. The leaves may be dried in a hot-air oven at 50°C for the first eight hours, and then at 40-45°C until they are completely dried[7].

Packaging and storage

Keep the dried leaves in clean well-closed containers, e.g. tightly tied and appropriately labeled plastic bags. If only a small amount of the leaves is prepared, stored in a tightly closed bottle. Put the name of the plant, part used, weight, collection date and the name of the person in charge of raw material preparation on the label. Keep the raw material in a clean, cool and dry place free from pests and inaccessible to rodents, birds, livestock and domestic animals. The leaves should not be kept longer than one year. According to the preliminary research by the Division of Medicinal Plant Research and Development, Department of Medical Sciences, it was found that the amount of the active substances of *S. alata* is reduced by about 20% after one-year storage[65].

Nowadays, organic food and organic natural products are well received by consumers worldwide. Therefore, the growers and producers of *Senna alata* should take this opportunity to produce high priced, more profitable organic raw material or products by following GAP guideline and international organic quality assurance production system.

In developed countries, which are the largest market of organic products, international organic production standard has long been established and followed. In Thailand the national standard of organic plant production has already been established a few years ago [68].

Quality Specification of *Senna alata* Leaf

Definition

Senna alata leaf consists of the dried leaves from the plant *Senna alata* (L.) Roxb. Family Leguminosae containing not less than 1.0% by weight of hydroxyanthracene derivatives, calculated as rhein-8-glucoside[9].

Description of crude drug

Color, greenish brown to brown; odour, slightly aromatic; taste, slightly bitter.

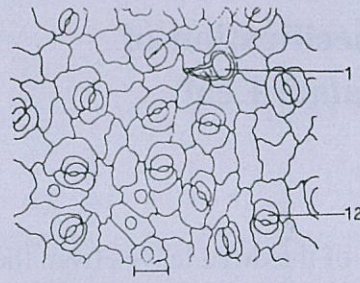
Identification

Pharmacognostic charactreistics

Macroscopical description *Senna alata* leaf occurs as a mixture of entire and broken, greenish brown to brown leaflets and frequently rachillae of the compound leaves. Leaflet oblong to obovate, minutely mucronate at the apex, base oblique, margin entire, pinnate reticulate venation, rigidly subcoriaceous, obscurely downy beneath.

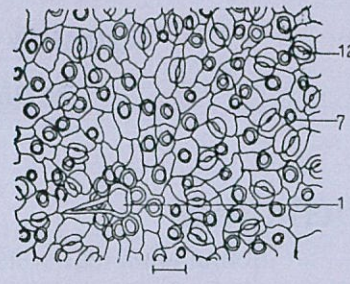
Microscopical description

- ***Histologic characteristics*** Surface view and transverse section of the leaflet under the microscope shows the following characters (Figure 3).



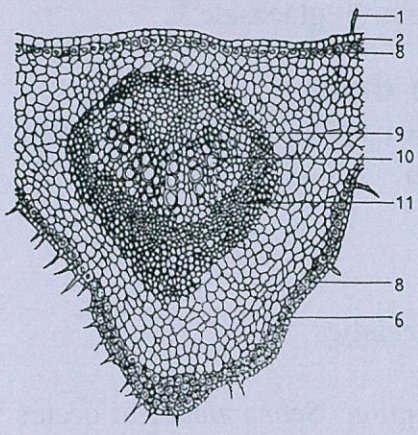
25 microns

Upper surface view of lamina



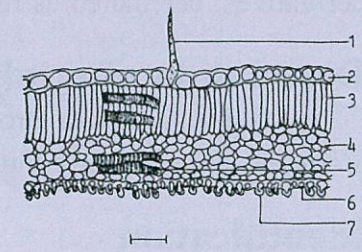
25 microns

Lower surface view of lamina



100 microns

Transverse section of midrib



50 microns

Transverse section of lamina

Figure 3 Surface view and transverse section of *Senna alata* leaf

1. unicellular trichome
2. upper epidermis
3. palisade cell
4. spongy cell
5. vascular bundle
6. lower epidermis
7. papilla
8. collenchyma
9. crystal sheath fibre
10. xylem
11. phloem
12. stoma

Lamina

1. **Upper epidermis** composed of a layer of cells, about 17 microns thick, slightly wavy-walled cells; stoma paracytic, about 17 x 23 microns in size, stomatal index 12.4; covering trichome unicellular, 15-17 microns long; cicatrix slightly rounded, surrounded by cells in radially arrangement; papilla slightly rounded in surface view, rarely seen.
2. **Palisade** composed of a layer columnar cells, about 10 x 57 microns in size, palisade ratio 6.65.
3. **Spongy** thin-walled parenchymatous cells.
4. **Vascular bundle** composed of phloem and xylem surrounded by crystal-fiber; vessel spiral or scalariform.
5. **Lower epidermis** composed of a layer of thick-walled cells, about 10 microns thick, wavy-walled cells; stoma paracytic, about 15 x 19 microns in size; stomatal index 17.8, unicellular trichomes 22-127 microns long, cicatrix and papillae, clearly and densely seen.

Midrib

1. **Upper epidermis** composed of a layer of cells; trichome unicellular, rarely seen.
2. **Collenchyma,** occurs beneath upper and lower epidermis.
3. **Parenchyma** thin-walled slightly rounded cells.
4. **Vascular bundle** composed of phloem on the upper part and xylem on the lower part, surrounded by crystal-fiber; vessel spiral, pitted or reticulate, densely seen.
5. **Lower epidermis** composed of a layer of cells; trichome unicellular, densely seen.

- ***Description of powdered drug***

The characteristics of the powdered drug are greenish brown, slightly odour and slightly bitter taste (Figure 4).

The following characters are more to less respectively found.

1. Fragments of crystal-fiber associated with xylem.
2. Fragments of upper epidermis in surface view showing stomata, cicatrix and palisade.
3. Fragments of lower epidermis in surface view showing stomata, cicatrix and papillae.
4. Fragments of lamina in sectional view.
5. Vessels spiral, pitted and reticulate.
6. Unicellular trichomes 30-56 microns long.
7. Papillae 12-14 microns high.
8. Prismatic crystals of calcium oxalate about 7 microns in size.
9. Epidermis and collenchyma in sectional view.
10. Epidermis and unicellular trichomes of petiole, rarely seen.
11. Parenchyma of petiole containing calcium oxalate crystals.
12. Cluster crystals of calcium oxalate of petiole.

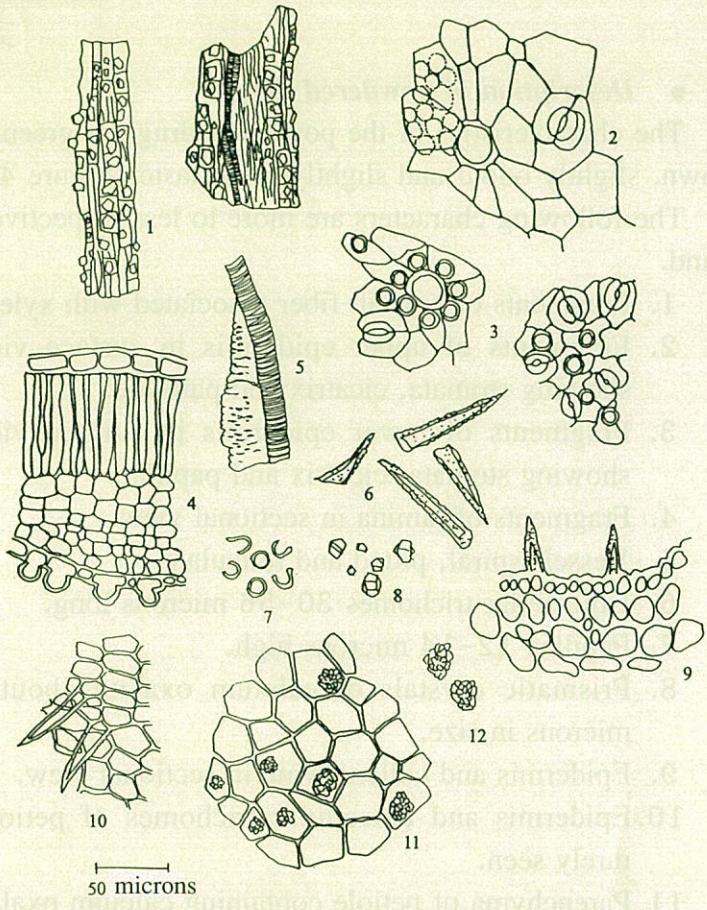


Figure 4 Powdered drug of *Senna alata* leaf

1. crystal fibers
2. surface view of upper epidermis with stoma, cicatrix, and underlying palisade cells
3. surface view of lower epidermis with stoma, cicatrix and papillae
4. transverse section of lamina
5. spiral and pitted vessels
6. unicellular trichomes
7. fragments of papillae
8. prismatic crystals
9. epidermis with unicellular trichomes
10. collenchyma and epidermis with unicellular trichomes in transverse section
11. parenchyma with cluster crystals
12. cluster crystals of calcium oxalate

Chemical identification

Preliminary test[9]

To 100 mg of the sample, in powder, add 25 ml of 2 M hydrochloric acid, heat on a water-bath for 15 minutes, and immediately filter through a plug of cotton wool. Allow the filtrate to cool and shake with 20 ml of ether. Separate the ether layer and shake with 10 ml of *ammonia TS* (see Appendix). The aqueous layer becomes red. (Figure 5)

Figure 5 The result of the preliminary test for anthraquinones

1. Extract of anthraquinones from *S. alata* leaf is yellow in color.
2. After reaction with ammonia, the aqueous layer becomes red.

Confirmatory test [9]

Solution A

Reflux 100 mg of the sample, in powder, with two 50-ml portions of chloroform on a water-bath for 15 minutes. Combine the chloroform extracts and evaporate to dryness. Dissolve the residue in 0.5 ml of chloroform.

Solution B

Add 25 ml of 2 M hydrochloric acid to the marc obtained after the reflux from the preparation of solution A and reflux on the water-bath for 15 minutes, and immediately filter through a plug of cotton wool. Allow the filtrate to cool and shake with 20 ml of ether. Separate the ether layer and evaporate to dryness. Dissolve the residue in 0.5 ml of ether.

Standard solution

Dissolve 1 mg of *rhein* (see Appendix) in 1 ml of methanol.

TLC apparatus and solvent

1. Adsorbent-coated glass plate

Use 5 x 20 cm glass plate coated with silica gel G, 0.25 mm thick. Dry the plate in the oven at 105°C for about 1 hour.

2. Developing solvent

Mix petroleum ether (boiling range 40-60°C) with ethyl acetate and anhydrous formic acid at the ratio of 75:25:1.

3. Chromatographic tank

Add the developing solvent sufficient to have a depth of about 1 cm at the bottom of the tank. Cover the tank and allow the system to equilibrate for about an hour (to let the tank atmosphere become saturated with

developing solvent).

Method

Use capillary tubes to deliver solution A, solution B and standard solution, 5 μl each, and apply onto the TLC plate at points about 2 cm from the lower edge of the plate and at least 1 cm apart, and allow to dry. Place the plate in the solvent-saturated chromatographic tank. Allow the solvent in the chamber to reach the lower edge of the adsorbent, but do not allow the spot points to be immersed. Put the cover in place, maintain the system at room temperature, allow the solvent front to ascend 10 cm above the line of sample application. Remove and air-dry the plate, then examine and locate the spots on the plate using the following methods.

1. Observe under ultraviolet light at the wavelength of 366 nm.
2. Expose the plate to ammonia vapour and observe in visible light.

Result

The chromatogram obtained from the two detection methods is shown in Figure 6. The location, as indicated by hR_f value, and the color of the spots of rhein of the sample are the same as those of the standard rhein solution (Figure 6).

The locations of the color spots on the TLC plate are determined by hR_f (100 R_f) values. R_f (retardation factor or relative front) is the ratio of the distance that each chemical constituent moves over the distance that the developing solvent moves.

The results of the identification of rhein by both detection methods are summarized in Table 1.

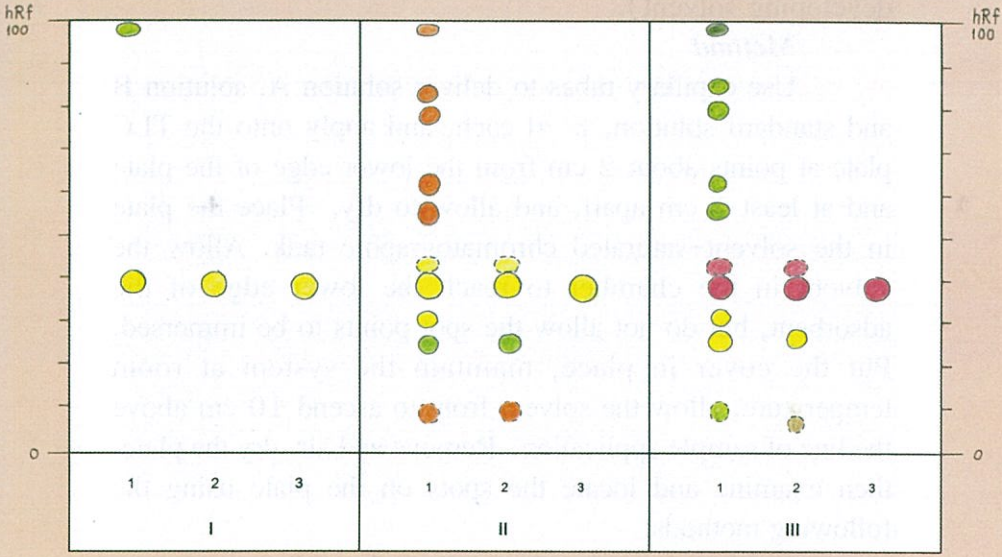


Figure 6 Thin-layer chromatogram of the extracts of the leaf of *Senna alata* (L.) Roxb.

I = Detected under daylight
II = Fluorescence under UV light (366 nm)
III = Visible with ammonia vapour exposure
1 = Solution A
2 = Solution B
3 = Standard rhein solution (average hR_f = 34-38)
○ = Spots observed in all samples of *S. alata* leaf
◌ = Spots observed in some samples of *S. alata* leaf

Table 1 hR_f values of components in the extract of the leaf of *Senna alata* (L.) Roxb.

Solution	Spot	Detection with		
		hR_f value	UV366nm (colour)	Ammonia Vapour (colour)
A	1	4-8	-	light green
	2	25-28	-	yellow
	3	29-32	-	yellow
	4*	34-38	yellow fluorescence	red
	5	53-58	-	light green
	6	60-64	-	light green
	7	74-77	-	light green
	8	78-80	-	light green
B	1	2-4	-	light brown
	2	25-28	-	yellow
	3*	34-38	yellow fluorescence	red

* represents the spot of rhein

Foreign matter

Not more than 2.0 per cent w/w of stems and other foreign matters.

Randomly sampling 100 g of the plant material and spread over a flat container. Separate foreign matter by inspecting with the unaided eye or with the use of 6x lens. Weigh and calculate the percentage of foreign matter present.

Loss on drying

Not more than 11.0 per cent w/w after drying at 105°C for 5 hours.

Accurately weigh 5 g (4 decimal places) of previously mixed crude drugs. Put in an accurately weighed weighing bottle. By gentle, sidewise shaking distribute the test specimen as evenly as practicable. Place the loaded bottle in the drying chamber set at 105°C for 5 hours until the constant weight* is obtained. Calculate the percentage of the weight loss on drying (weight loss is the moisture content of the plant material).

Total ash

Not more than 9.0 per cent w/w

Place 2-4 g of the ground plant material, accurately weighed (4 decimal places), in a suitable tared crucible (usually of platinum or silica), previously ignited, cooled and weighed. Incinerate the sample by gradually increasing the temperature, not exceeding 450°C, until free from carbon; cool and weigh. If a carbon-free ash cannot be obtained this way, cool the crucible and moisten the residue with about 2 ml of water. Dry on a water-bath and then on a hot plate and incinerate to constant weight*. Calculate the percentage of total ash with reference to the air-dried substance.

---

* Constant weight : The weights obtained by two consecutive weighings are not more than 0.5 mg diferrence. The second weighing to determine weight difference is performed after drying or heating the specimen one hour further.

Acid-insoluble ash

Not more than 1.0 per cent w/w

Add 25 ml of 2 M hydrochloric acid in the crucible containing total ash, cover with watch glass and boil for 5 minutes. Collect the insoluble matter on an ashless filter paper, wash with hot water until the filtrate is neutral. Put the filter paper with insoluble matter in the same crucible and ignite at about 500°C until constant weight* is obtained. Calculate the percentage of acid-insoluble ash with reference to the air-dried substance.

Ethanol (50 per cent)-soluble extractive

Not less than 21.0 per cent w/w

Macerate 5 g of the air-dried crude drug, coarsely powdered and accurately weighed (4 decimal places), with 100.0 ml of 50% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and then allowing to stand for 18 hours. Filter rapidly, taking precautions against loss of ethanol, evaporate 20.0 ml of the filtrate to dryness in a tared, flat-bottomed, shallow dish and dry on a water-bath at 105°C to constant weight*. Calculate the percentage of ethanol-soluble extractive with reference to the air-dried substance.

Water-soluble extractive

Not less than 18.0 per cent w/w

Proceed as directed in 50% ethanol-soluble extractive but using chloroform water as the solvent in place of ethanol.

Hydroxyanthracene derivatives content

Not less than 1.0 per cent w/w of hydroxyanthracene derivatives, calculated as rhein-8-glucoside on the dried basis.

Accurately weigh about 150 mg (4 decimal places) of *Senna alata* leaf, in No. 150 powder, and place in a 100 ml round-bottomed flask. Add 30.0 ml of water, mix, weigh, and place in a water-bath. Heat under a reflux condenser for 15 minutes. Allow to cool, weigh and adjust to the original weight with water. Centrifuge and transfer 20.0 ml of the supernatant liquid to a 150-ml separator. Add 0.1 ml of 2 M hydrochloric acid and shake with three 15-ml portions of chloroform. Allow to separate and discard the chloroform layer. Add 100 mg of sodium hydrogencarbonate and shake for 3 minutes. Centrifuge and transfer 10.0 ml of the supernatant liquid to a 100-ml round-bottomed flask with a ground glass neck. Add 20 ml of a 10.5 per cent w/v solution of iron(III) chloride and mix. Heat for 20 minutes under a reflux condenser in a water-bath with water level above that of the liquid in the flask, add 1 ml of hydrochloric acid and heat for a further 20 minutes with frequent shaking, to dissolve the precipitate. Cool, transfer the mixture to a separator and shake with three 25-ml portions of ether previously used to rinse the flask. Combine the ether layers and wash with two 15-ml portions of water. Transfer the ether layers to a 100-ml volumetric flask and dilute with ether to volume. Evaporate 25.0 ml carefully to dryness at low temperature and dissolve the residue in 10.0 ml of a 0.5 per cent w/v

solution of magnesium acetate in methanol. Measure the absorbance of this solution at maximum at about 515 nm, using the magnesium acetate solution as the blank. Calculate the percentage content of rhein-8-glucoside from the expression:

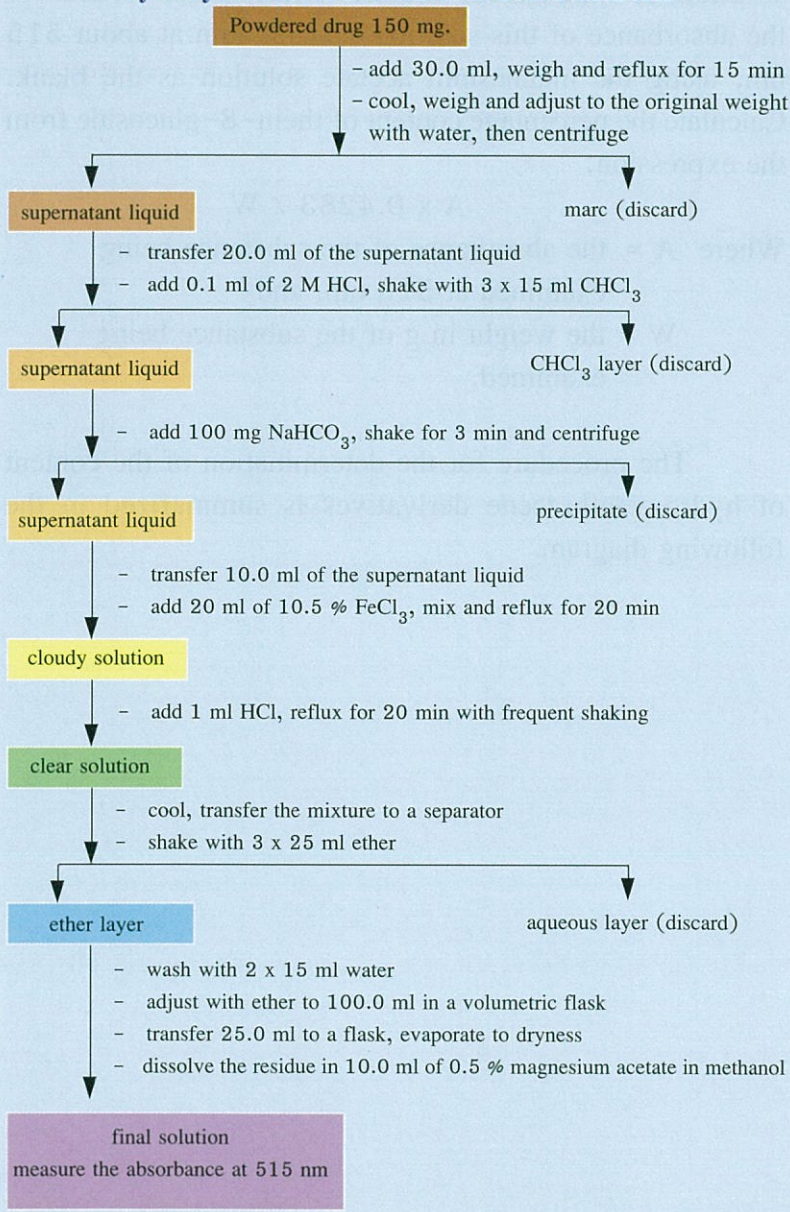
$$A \times 0.4283 / W$$

Where A = the absorbance of the substance being examined at 515 nm, and

W = the weight in g of the substance being examined.

The procedure for the determination of the content of hydroxyanthracene derivatives is summarized in the following diagram.

Diagram showing the procedure for the determination of the content of hydroxyanthracene derivatives in *Senna alata* leaf



Summary of the quality specification of *Senna alata* leaf

Items analyzed	Not More Than (% w/w)	Not Less Than (% w/w)
Foreign matter	2.0	
Loss on drying	11.0	
Total ash	9.0	
Acid-insoluble ash	1.0	
Water-soluble extractive		18.0
50% Ethanol-soluble extractive		21.0
Content of hydroxyanthracene derivatives, calculated as rhein-8-glucoside		1.0

Contamination of medicinal plant materials

The manufacturers of herbal medicines should be aware of the problem of contamination of medicinal plant materials and assays should be performed to determine whether the contaminants exceed the limits in order to control the quality of raw materials and finished products. However, since the procedures to quantitatively analyze various kinds of contamination are very technically specific and beyond the scope of this book, only the principles of the assays will be covered.

Microbial contamination

Thai Pharmacopoeia[47] set up the limits for microbial contamination allowed in different types of herbal preparations. For preparations of crude drugs and mixtures of crude drugs for internal use, which will undergo a process for reduction of count before use (e.g. by pouring over with boiling water); and externally used preparations containing whole or ground crude drugs, the total aerobic microbial count should not exceed 5×10^5 cfu (colony forming unit) per g, amongst which the yeasts and moulds count should not exceed 5×10^3 cfu. The count of *Escherichia coli* should not exceed 50 cfu per g and the count of other Enterobacteria should not exceed 5×10^3 cfu per g. A 10-g sample must be free from *Clostridium* spp. and *Salmonella* spp.; and a 1-g sample must be free from *Staphylococcus aureus*.

Microbial limit tests[41,46,71], can be divided into 3 methods namely: -

- Plate method
- Multiple-tube method
- Membrane-filtration method

Plate method is easy, convenient and accurate. It is suitable for the assay of pharmaceutical preparations as well as ground crude drugs and can be applied for the assay of powdered leaf of *Senna alata*[72]. Therefore, only the plate method will be described in this book.

1. Thoroughly mix 10 g of sample with phosphate buffer at the ratio of 1:10.

2. Serially dilute the above sample solution further with phosphate buffer to obtain the dilutions of 1:100 and 1:1,000 or lower, if necessary.
3. Tranfer 15-20 ml of previously melted tryptic soy agar culture medium that was allowed to cool down to 45-50°C into each petri dish.
4. Pipette 1 ml of each dilution of the sample and transfer into each of two petri dishes. Rotate or tilt the petri dishes to mix the sample with the media and allow the content to solidify at room temperature. Incubate the plates at 30-35°C for 24-48 hours. If the microbial growth is not detected, further incubate until a total of 5-day incubation period is reached.
5. Examine the plates using colony counter, count the number of colonies in the petri dishes with appropriate dilution of sample. Express the average colonies of the two plates in terms of total viable aerobic microbial count per g of sample.
6. Isolate different types of colonies to identify microbial organisms using specific methods in order to determine the type of microbes and whether the counts exceed the microbial limits.

Pesticide contamination

There are four major types of pesticides that should be determine whether they are contaminated in the crude drug, namely: -

1. Organochlorines, e.g. chlordane, DDT, dieldrin, heptachlor

2. Organophosphates, e.g. parathion, malathion, dimethoate

3. Carbamates, e.g. carbaril, methomil

4. Pyrethroids, e.g. cypermethrin, permethrin

Methods of analysis [35,68] basically the assay is performed as follows: -

1. Extract the crude drug sample with appropriate solvent, e.g. acetone, acetonitrile, methanol, etc.
2. Purify the extract by passing through the column of florisil or alumina or charcoal-celite.
3. Determine the type or the amount of pesticides by gas chromatography or high performance liquid chromatography.

If the assay showed that crude drug is contaminated with pesticide residues, WHO guideline on the safety issue of using pesticide-contaminated medicinal plant materials should be followed[44,74].

Arsenic and heavy metal contamination

There is a guideline on arsenic and heavy metal contamination. It is recommended that in 1 kg of herbal products, the amount of contaminated arsenic, cadmium and lead should not exceed 4, 0.3 and 10 mg[9,40,44,74], respectively.

Methods of analysis[9,40] Basically the assay is performed as follows: -

1. Sample preparation. Digest the sample in nitric acid

2. Determine the amount of arsenic and heavy metals by atomic absorption spectrophotometry

Radioactive contamination

The determination of radioactive contamination must be performed by specialized agencies and under the guidelines of the International Atomic Energy Agency (IAEA). It is possible that medicinal plant materials from certain sources may be contaminated with radioactives. For safety reason, WHO therefore recommended that suspected crude drug samples should then be tested for radioactive contamination before use[9,44].

Indication [28]

As a laxative for the relief of constipation.

Toxicity [74,75]

Acute toxicity study of 50% ethanolic extract of *Senna alata* leaf in mice showed that the extract given orally at the dose 15 g/kg BW did not produce toxicity in mice. The doses of the extract that cause death in 50% of the animals or LD_{50} were more than 15 g/kg BW when given orally or subcutaneously, or equal to 8.03 g/kg BW when given intraperitoneally[75].

Chronic toxicity of powdered leaf suspended in 0.5% tragacanth was conducted in rats. The animals were

divided into 4 groups; the control group received 0.5% tragacanth suspension. The three treatment groups received suspension of powdered leaf at the doses of 0.03, 0.15 and 0.75 g/kg/day for 6 months. It was found that the growth rates of all groups of animals were normal and hematological or bichemical examinations showed no evidence of abnormalities or changes that would indicate pathological changes of internal organs. Gross and histopathological examinations of the internal organs and organ weights did not show dose-related abnormalities that could be due to the effect of the powdered leaf of *S. alata*[75].

Contraindication[10-12,28,76]

Senna alata leaf is a stimulant laxative that can relieve constipation by stimulating the contraction of the smooth muscle of the lower bowel. Hence, it is contraindicated in intestinal obstruction or stenosis, acute intestinal inflammation, e.g. Crohn's disease, ulcerative colitis, appendicitis, abdomial pain of unknown origin.

It is contraindicated in children younger than 12 years of age.

Laxative products are contraindicated when abdominal pain, nausea, or vomiting is present.

Warning[9-12,28,76]

1. It may cause nausea, intestinal cramps, uterine contractions, or diarrhea. If these side effects occur, a dosage reduction is required[20,71].

2. Long-term use should be avoided since it may cause dependency, resulting in a decrease of the ability to perform normal bowel functions without the aid of laxatives. Stimulant laxative should not be taken longer than 2 weeks without medical advice.

3. Prolonged and excessive use should be avoided since it may result in excessive loss of fluids and electrolytes, particularly potassium, leading to pathological alterations of the colon, nephritis, renal malfunction, or heart palpitations, which may be potentiated by simultaneous use of cardiac glycosides, thiazide diuretics, and corticosteroids. In addition, chronic use may also cause albuminuria or hematuria.

4. The use of *Senna alata* as a laxative should be avoided in pregnant women or nursing mothers.

Preparations used and dose

Dosage forms of available laxative products of *Senna alata* leaf are as follows: -

1. Dried leaf powder packed in paper teabag - 3 g/bag
   Dose: 1-2 bags of powdered leaf infused in 120 ml of boiling water/bag for 10 minutes, take the infusion once daily at bedtime[25,28].
2. Capsules of powdered leaf - 300-500 mg/capsule
   Dose: 8-13 capsules once daily at bedtime.
   (Dosage of powdered leaf capsules depends on the amount per capsule and the susceptibility of each individual to hydroxyanthracene derivatives.)

Ammonia TS It contains 9.5 to 10.5 per cent w/w of NH_3. Prepare by diluting 400 ml of strong ammonia solution with water to make 1000 ml.

Store in well-closed containers, at a temperature not exceeding 20°C.

Rhein $C_{15}H_8O_6$ = 284.22

DESCRIPTION Yellow needles.

SOLUBILITY Practically insoluble in water; soluble in alkalis and in pyridine; slightly soluble in benzene, in chloroform, in ethanol, in ether, and in petroleum ether.

MELTING RANGE 321°C to 322°C, decomposed at 330°C.

1. McGuffin M, Kartesz JT, Leung AY and Tucker AO. 2000. Herbs of Commerce. 2nd ed. The American Herbal Products Association, USA. p. 134.
2. Phuphathanaphong L, Chayamarit K and Boonthavikoon T (eds). 2001. Thai Plant Names (Tem Smitinand Revised Edition 2001) The Forest Herbarium, Royal Forest Department. Prachachon Ltd., Bangkok. p. 478.
3. Irwin HS and Barneby RC. 1982. The American Cassiinae, A Synoptical Revision of Leguminosae Tribe Cassieae Subtribe Cassiinae in the New World. *Memoirs of the New York Botanical Garden* 35(1) : 1-77.
4. de Padua LS, Bunyapraphatsara N and Lemmens RHMJ. (eds.) 1999. Plant Resources of South-East Asia No.12, Medicinal and Poisonous Plant 1. Prosea Foundation, Indonesia. p. 181, 442-446.
5. Standard of ASEAN Herbal Medicine. 1993. Vol. 1. Aksara Buana Printing, Jakarta. p. 116-128, 459-551.
6. Duong NV. 1993. Medicinal Plants of Vietnam, Cambodia and Laos. Makong Printing. p. 92-93.
7. Division of Medicinal Plant Research and Development.1990. Handbook of Medicinal Plants for Primary Health Care. 3rd ed. Department of Medical Sciences. Text and Journal Corporation, Bangkok. p. 13-35, 42, 46.
8. Perry LM and Metzger J. 1980. Medicinal Plants of East and Southeast Asia. The MIT Press, USA. p. 209-210.

9. Thai Herbal Pharmacopoeia. 1995. Vol. 1. Chumhetthet. Prachachon Co., Ltd., Bangkok. p. 17-23.
10. Blumenthal M, Busse WR, Goldberg A, et al. 1998. The Complete German Commis-sion E Monographs. American Botanical Council, USA. p. 204.
11. Blumenthal M, Goldberg A, Brinckmann J, et al. 2000. Herbal Medicine - Expanded Commission E Monographs. 1st ed. American Botanical Council, USA. p. 341-349.
12. WHO Monographs on Selected Medicinal Plants. 1999. Vol. 1. World Health Organization, Geneva. p. 241-249.
13. Elujoba AA, Ajulo OO and Iweibo GO. 1989. Chemical and biological analyses of Nigeria *Cassia* species for laxative activity. *J Pharm Biomed Anal* 7(12) : 1453-1457.
14. Fuzellier MC, Mortier F and Lectard P.1982.Antifungal activity of *Cassia alata* L. *Ann Pharm Fr* 40(4) : 357-363.
15. Palanichamy S and Nagarajan S. 1990. Antifungal activity of *Cassia alata* leaf extract. *J Ethnopharmacol* 29 : 337-340.
16. Tridech M and Archavakom S. 1994. The study of antifun-gal activity of *Cassia alata* leaf extracts. Special project for the Bachelor of Science Degree in Pharmacy. Faculty of Pharmacy, Mahidol University. 27 pages.
17. Ibrahim D and Osman H. 1995. Antimicrobial activity of *Cassia alata* from Malaysia. *J Ethnopharmacol* 45 : 151-156.
18. Boon-long J and Preugsapan K. 2000. Preliminary testing of the effect of Thai medicinal plant extracts against dermatophytes. Eleventh Annual Conference of the Department of Medical Sciences, Bangkok. p. 29.
19. Boon-long J, Kun-Anake A and Bansiddhi J. 2001. Preliminary testing of extracts from Thai medicinal plants on dermatophytes. *Medical Time* 2(31) : 27-29.
20. Kupitayanant P, Grisanapan W and Wuthiudomlert M. 2001. Antifungal activity of *Cassia alata* Linn. leaf extract. *Thai J Pharm Sci* 25 (Suppl) : 33.

21. Villasenor IM, Canlas AP, Pascua MP, et al. 2002. Bioactivity studies on *Cassia alata* Linn. leaf extracts. *Phytother Res* 16 Suppl 1 : S93-96.
22. Crockett CO, Guede-Guina F, Pugh D, et al. 1992. *Cassia alata* and the preclinical search for therapeutic agents for the treatment of opportunistic infections in AIDS patients. *Cell Mol Biol* 38(7) : 799-802.
23. Srisatjaluk R, Prachayabreud W, Triratana T, et al. 1996. Effects of *Alpinia galanga, Cassia alata*, and *Rhinacanthus nasutus* extracts on *Candida albicans. Mahidol Dent J* 16(2) : 67-74.
24. Vaijayanthimala J, Anandi C, Udhaya V, et al. 2000. Anticandidal activity of certain south Indian medicinal plants. *Phytother Res* 14 : 207-209.
25. Thamlikitkul V, Bunyapraphatsara N, Dechatiwongse T, et al. 1990. Randomized controlled trial of *Cassia alata* Linn. for constipation. *J Med Assoc Thai* 73(4) : 217-222.
26. Imviddhaya S, Nilwises N and Dispraso P. 1987. The use of *Cassia alata, Rhinacanthus nasutus*, and *Brucia javanica* for the treatment of ringworm and tinea versicolor. Final Research Report on Medicinal Plants in "The Medicinal Plants and Primary Health Care Project" under the Support of the UNICEF (1984-1985). The Office of the Primary Health Care. The War Veterans Organization Printing, Bangkok. p. 78-80.
27. Damodaran S. and Venkataraman S. 1994. A study on the therapeutic efficacy of *Cassia alata* Linn. leaf extract against *Pityriasis versicolor. J Ethnopharmacol* 42 : 19-23.
28. National Drug Committee. 2000. National List of Essential Drugs A.D. 1999 (List of Herbal Medicine Products). Agricultural Cooperative of Thailand Press Ltd., Bangkok. p. 24-28.
29. Benjamin TV and Lamikanra A. 1982. Investigation of *Cassia alata*, a plant used in Nigeria in the treatment of skin

diseases. *J Crude Drug Res* 19(2/3) : 93-96.

30. Choudhury HM, Islam SN, Begum K, et al. 1988. Antibacterial activities of the leaves and stem bark of *Cassia alata*. *Bangladesh J Bot* 17(2) : 135-140.
31. Khan MR, Kihara M and Omoloso AD. 2001. Antimicrobial activity of *Cassia alata*. *Fitotherapia* 72 : 561-564.
32. Kantasuk C. 1992. Preliminary test for anti-herpes simplex virus activity of extracts from some Thai medicinal plants. Thesis for the Master of Science Degree in Pharmacy. Department of Microbiology, Chulalongkorn University, Bangkok. 173 pages.
33. Palanichamy S and Nagarajan S. 1990. Anti-inflammatory activity of *Cassia alata* leaf extract and kaempferol 3-O-sophoroside. *Fitotherapia* 61(1) : 44-47.
34. Abatan MO. 1990. A note on the anti-inflammatory action of plants of some *Cassia* species. *Fitotherapia* 61(4) : 336-338.
35. Palanichamy S and Nagarajan S. 1990. Analgesic activity of *Cassia alata* leaf extract and kaempferol 3-O-sophoroside. *J Ethnopharmacol* 29 : 73-78.
36. Palanichamy S, Nagarajan S and Devasagayam M. 1988. Effect of *Cassia alata* leaf extract on hyperglycemic rats. *J Ethnopharmacol* 22 : 81-90.
37. Panichayupakaranan P and Keawsuphan S. 2000. Antioxidants from *Cassia alata* L. leaves. Seminar on the ways and means of medicinal plant development in Thailand. September 13-14, 2000. The National Research Council of Thailand. Bangkok. p. 282-288.
38. Wirachwong P, Burananon V and Kraisintu K. 2000. Antioxidant capacity of Thai medicinal plant extracts. *Thai J Pharm Sci* 24 (Suppl) : 79.
39. Lou ZC. 1980. General Control Methods for Vegetable Drugs. WHO/Pharm/80.502. World Health Organization,

Geneva.

40. Quality Control Methods of Medicinal Plant Materials. 1992. WHO/Pharm/92.559/rev.1 World Health Organization, Geneva.
41. Thai Pharmacopoeia. 1987. Vol. 1. Pt. 1. The War Veterans Organization Press. Bangkok. p. 438, 476-488.
42. Dechatiwongse Na Ayudhya T. 1993. Quality Control of Medicinal Plants. In: Workshop on the Quality Control of Medicinal Plants. Department of Medical Sciences, Nonthaburi. p. 45-53.
43. Techadamrongsin Y, Dechatiwongse Na Ayudhya T and Punyarajun S. 1999. Harvesting, Post-harvesting Handling and Storaging of Crude Drug. Department of Medical Sciences, Nonthaburi. Thailand.
44. Quality Control Methods for Medicinal Plant Materials. 1998. World Health Organization, Geneva. p. 47-63.
45. Jirachariyakul W. (ed.) 2000. Natural Medicine and Products. Text and Journal Corporation Co., Ltd., Bangkok. p. 1-72.
46. British Pharmacopoeia. 1998. Vol. 2.2. The Stationery Office of the British Pharmacopoeia Commission, London. p. A181, A243-252.
47. Thai Pharmacopoeia. 1997. Vol. 2. Pt. 1. P.A. Living Co., Ltd., Bangkok. p. 2064-2065.
48. Clay H and Hubbard JC.1977. The Hawaii Garden, Tropical Shrubs. The University Press of Hawaii, Honolulu. p. 38.
49. Griffiths M. 1994. Index of Garden Plants. The Royal Horticultural Society. Timber Press, Oregon. p. 1074.
50. Quattrocchi U. 2000. CRC World Dictionary of Plant Names. Vol.4. CRS Press, Washington D.C. p. 2459.
51. Larsen K, Larsen SS and Vidal JE. 1984. Leguminosae-Caesalpinoidae. *Flora of Thailand* 4(1) : 108-110.
52. Hou D, Larsen K and Larsen SS. 1996. Caesalpiniaceae.

Flora Malesiana 12(2) : 673-676.

53. Backer CA and Bakhuizen van den Brick RC. 1963. Caesalpiniaceae. *Flora of Java* 1 : 540.
54. Lekhakhun K, Chantaraprasong C, Chaianan C, et al. 1993. Ornamental Plants, for the Honour of Her Majesty the Queen of Thailand. National Identity Promotion Office. Dhansutha Press, Bangkok. p. 166.
55. Pecharaply D, Bansiddhi J, Chansuwanit N, et al. 1998. Folklore Medicinal Plants. (combined edition) Medicinal Plant Research Institute. 5th ed., Rung Ruang Sarn Press Co., Ltd., Nonthaburi. p. 51.
56. Phomtong W. 2002. Plant Propagation. Matichon Press, Bangkok. p. 9-25.
57. Hartmann HT, Kester DE and Davies FT. 1990. Plant Propagation. 5th ed. Prentice-Hall International Inc., London. p. 77-188.
58. Watanaphuti N. 1999. Plants Propagation. O S Printing House, Bangkok. p. 92-162.
59. Wasicky Ri and Wasicky Ro. 1965. Experimental Induction of Embryo Dormancy. *Beitr Biochem Physiol Naturstoffe Festschr* p. 531-539.
60. Department of Medical Sciences. 1992. Annual Report of the Department of Medical Sciences 1992. Arun Printing, Nonthaburi. p. 44.
61. Srisawangwong P, Sukaprakharn S and Paisuksantiwattana Y. 1992. Breaking dormancy of 'Chum het thet' seeds. In : The Project Report of Agricultural Research on Medicinal Plants. Kasetsart University, Bangkok. p. 145-154.
62. Division of Plant Pathology, Department of Agriculture. 1992. Handbook of Plant Pathology Control by Chemical Agents. 2nd ed. Agricultural Cooperative of Thailand Press, Bangkok. p. 6-153.

63. Keinmesuk P, Bansiddhi K, Kitbumrung N, et al. 1999. Insect Pests of Vegetables. Division of Entomology and Zoology. Department of Agriculture. 1st ed. Agricultural Cooperative of Thailand Press, Bangkok. p. 25-63.
64. Division of Entomology and Zoology, Department of Agriculture. 2000. Recommendation on the control of insects and pests 2000. Agricultural Cooperative of Thailand Press, Bangkok. p. 38-86.
65. Dechatiwongse T and Chavalittumrong P. 1988. Chemical Analysis of *Cassia alata* L. *Thai J Pharm Sci* 13(3) : 309-316.
66. Techadamrongsin Y, Boonruad T, Jirawattanapong W, et al. 2001. The Project on the Quality Control of Medicinal Plant Raw Materials and Natural Products. Department of Medical Sciences. Nonthaburi. p. 59-76.
67. Department of Medical Sciences. 2002. Thai Herbs Quality Award and Annual Conference 2002. Department of Medical Sciences. Nonthaburi. p. 4-11.
68. Department of Agriculture, Ministry of Agriculture and Cooperatives. 2001. Declaration of Department of Agriculture, Title : Production Standard for Organic Plants of Thailand. Government Gazette, General Issue. Vol.118 part 36 NG. April 18, 2001. p. 4-10.
69. Dechwisissakul P. 1989. The Pharmacognostical Studies on the Leaves of Chumhetthet (*Cassia alata* Linn.). *Bull Dept Med Sci* 31(1) : 39-46
70. Silapa-archa W and Werawattanametin K. 1977. No.4 *Cassia alata* Linn. From: Thai Crude Drugs; Their Preparations and Specifications. Edited by the Committee on Crude Drugs of Thai Ancient Medicine, Faculty of Pharmacy, Mahidol University. *Mahidol Univ J Pharm Sci* 4(1) : 1-7.
71. The United States Pharmacopoeia. Rev. 23. The National Formulary. 18th ed. 1995. The United States Pharmacopoeial Convention Inc., Rockville, MD. p. 1478-1483.

72. Kanawut W. 1993. The Quality Control of Traditional Medicine. In: Workshop on the Quality Control of Medicinal Plants. Department of Medical Sciences, Nonthaburi. p. 143-155.
73. Thoophom G, Sungvaranond B, Atisook K, et al. 1993. Pesticide and PCB residues in foods, Year 1988-1990. *Bull Dept Med Sci* 35(1) : 1-12.
74. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. WHO/ EDM / TRM / 2000. 1. World Health Organization, Geneva. p. 3-31.
75. Sittisomwong N, Chivapat S, Wangmad A, et al. 1991. Toxicity of *Cassia alata*. *Bull Dept Med Sci* 33(4) : 145-154.
76. McGuffin M, Hobbs C, Upton R, et al. (eds.) 1997. American Herbal Products Association's Botanical Safety Handbook. CRC Press, Boca Raton. p. 177-179.

Standard of Thai Herbal Medicine

Senna alata (L.) Roxb.