Standard of Thai Herbal Medicine

Andrographis paniculata (Burm.f.) Wall. ex Nees

Medicinal Plant Research Institute
Department of Medical Sciences
Ministry of Public Health, Thailand
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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 herbal healthcare products, namely health food, herbal drinks, dietary supplements, herbal medicine and cosmetics are not only useful for domestic consumption but also for export to help the country's economy.

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 medi-

cines not only serves as a means of consumer protection but also helps improve the quality of Thai herbal products and raw materials in an attempt to gain more domestic and international acceptances.

Andrographis paniculata (Burm.f.) Wall. ex Nees, or "Fa-thalai-chon" 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. Clinical studies in Thailand show that the aerial parts of *A. paniculata* are useful for the treatment of sorethroat (pharyngotonsillitis) and non-infectious diarrhea; therefore, *A. paniculata* herbal medicine is recommended for such indications. Other research reports indicate that extracts of *A. paniculata* can also reduce symptoms of common cold and show antioxidant activity. Hence, *A. paniculata* 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 *A. paniculata* as a means to control and improve the quality of *A. paniculata* raw material and herbal medicine.

"Standard of Thai Herbal Medicine: *Andrographis paniculata* (Burm.f.) Wall. ex Nees" is the second in the English edition of the Standard of Thai Herbal Medicine book series, while the Thai edition of this book was the first in the book series in Thai that was published by the DMSc in 1999. The first of the book series in English is the "Standard of Thai Herbal Medicine: *Senna alata* (L.) Roxb."

published in 2002. 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 : *Andrographis paniculata* (Burm.f.) Wall. ex Nees**" 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 *A. paniculata* in the future.

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February, 2003

Andrographis paniculata (Burm.f.) Wall. ex Nees, known in English as "creat" and in Thai as "Fa-thalai" or "Fa-thalai-chon", is an annual herb in the Family Acanthaceae grown and cultivated abundantly in India, China, and many parts of tropical Asia including Thailand[1,2]. The dried aerial parts of *A. paniculata*, also known as *Andrographis* herb, have a long history of use as traditional medicine in India and China. Traditionally, this herb has been used in China for influenza with fever, sore throat, aphthous ulcers, cough, colitis, dysentery, and urinary tract infection, carbuncles, sores, and venomous snake bite[3]. In India it has been used as a remedy for snake bites, insect bites, dysentery, stomachache, diabetes, hepatoprotective, fever, influenza, and bronchitis[4]. *A. paniculata* is official in the pharmacopoeias of China and India as well as in Thailand[3-5] and WHO has recently published the monograph of "Herba Andrographidis" in the second volume of WHO Monographs of Selected Medicinal Plants[6].

Extracts of *A. paniculata* and its active constituents, collectively called diterpene lactones, possess various pharmacological activities that appear to support several of its traditional claims. Based on clinical evidence; however, *A. paniculata* is officially accepted in the National List of Essential Drugs of Thailand and recommended for the primary health care for only two indications, namely for the treatment of pharyngotonsillitis and non-infectious diarrhea[7-8].

There have been several clinical trials conducted to prove the efficacy of *Andrographis* herb and its extract to relieve symptoms of cold and upper respiratory tract infection; the results so far strongly support this therapeutic effect[9-16]. Initially, clinical trial conducted in Thailand by Thamlikitkul and colleagues showed that on day 3 of the treatment of adult patients suffering from pharyngotonsillitis with *Andrographis* herb, containing 6% of total lactones calculated as andrographolide, at the dose of 6 g per day, it was as effective as paracetamol in relieving fever and sore throat[9]. The following clinical trials conducted by researchers from Sweden and Chile using standardized extract of *Andrographis* herb containing 4% or 5% andrographolide at the dose of 1020-1200 mg/day[10-12] confirmed therapeutic efficacy of this herb to alleviate various symptoms of common cold and uncomplicated sinusitis. Regarding the preventive effect of *Andrographis* herb against common cold, it was found that the occurrence of common cold was significantly less in the group of schoolchildren taking 200 mg/day of standardized extract of *Andrographis* herb containing 4% andrographolide than in the placebo control group on the third month, but not the first or the second month of the treatment[13]. In addition, recent reports also showed that standardized extract of *Andrographis* herb in fixed combination with the extract of *Eleutherococcus senticosus* could also relieve various symptoms of cold and upper respiratory tract infections[14-17].

As for the mechanism of action, additive immunomodulatory activities of diterpene lactones, e.g. andrographolide and its derivatives, which are the active principles of this herb, may partly be responsible for this therapeutic effect[17,18].

Moreover, well-established anti-inflammatory and anti-pyretic activities of *Andrographis* herb can also account for this efficacy as well[19-23]. The anti-inflammatory effect of *Andrographis* herb and its diterpenoids have been shown to be mediated by various possible mechanisms of action[19,23-27].

Regarding the antidiarrheal activity of *A. paniculata*, several *in vitro* and *in vivo* pharmacological studies provided scientific evidence to support this therapeutic claim[24-28]. It was found that extracts of *Andrographis* herb and diterpene lactones possess spasmolytic activity when tested in isolated tissues of animal intestine and stomach[28-31], and high doses of 85% ethanolic extract of *Andrographis* herb could partially antagonize the diarrheal effect of castor oil and magnesium sulfate in mice[31]. In another study, different strains of *E. coli* were used to produce thermolabile (LT) and/or thermostable (ST) enterotoxins that could induce diarrhea in animal models by stimulating adenylate cyclase or guanylate cyclase, respectively[32]. It was found that alcoholic extract of *A. paniculata* could inhibit *E. coli* enterotoxins-induced intestinal secretory response. The antisecretory effect of andrographolide and neoandrographolide was as effective as 1 mg loperamide against LT and LT/ST enterotoxins, while andrographolide was more effective than loperamide against ST enterotoxin-induced diarrhea[32]. Hence, scientific evidence suggests that the antidiarrheal effect of *Andrographis* herb be mediated by several mechanisms of action.

Clinical trial conducted in Thailand comparing the efficacy of *Andrographis* herb with tetracycline for the treatment of acute diarrhea and bacillary dysentery in 106

patients indicated that *Andrographis* herb was effective for decreasing diarrheal stool and fluid input. The duration of diarrhea appeared to be shorter than the tetracycline control group but the difference was not statistically significant. The dose of 1 g every 12 hours for 2 days was more effective than 500 mg every 6 hours for 3 days. In addition *Andrographis* herb was more effective in the treatment of shigellosis than cholera[33].

Several microbiological studies were conducted to determine if the therapeutic effect of *Andrographis* herb to relieve sore throat and diarrhea was due to antibacterial activity[34-37]. *A. paniculata* extracts[35,36] and several diterpene lactones[34,37] as well as serum[34,35] or urine[34] samples of human subjects taking therapeutic doses of *Andrographis* herb were tested against bacteria that can cause diarrhea or upper respiratory tract infections, e.g. *Salmonella*, *Shigella*, *E. coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Streptococcus*, *and Bacillus subtilis* spores. Taken together, the results showed no or very weak antibacterial activity of those tested substances. Therefore, the therapeutic efficacy of *Andrographis* herb for the relief of the symptoms of cold and upper respiratory tract infections or for the treatment of non-infectious diarrhea is not likely due to the antibacterial activity.

Even though preclinical evidence to support antimicrobial activity of *A. paniculata* was still conflicting, other clinical studies conducted in Thailand indicated that *Andrographis* herb or its extract was as effective as antimicrobial agents for the prevention of urinary tract infection and for the treatment of periodontitis[38-40]. In the former study, *Andrographis* herb was used to prevent urinary tract

infections after extracorporeal shock wave lithotripsy. It was found that after 1 month of treatment, *Andrographis* herb (100 mg three times daily) was as effective as cotrimoxazole (50 mg twice daily) or norfloxacin (200 mg twice daily) to reduce pyuria, hematuria and proteinuria[38].

In the latter study, a group of researchers of Mahidol University, Thailand developed *A. paniculata* gel that was found to exhibit antibacterial activity against *Porphyromonas gingivalis*, a type of periodontitis-causing bacteria. Clinical studies showed that the gel given by subgingival administration was effective as an adjunct in the treatment of adult periodontitis[39,40]. The development of *A. paniculata* gel won a Silver Medal at the Brussels Eureka 2001 50th World Exhibition of Innovation, Research and New Technology, and an Outstanding Invention Award 2002 in the field of Chemical Science and Pharmacy from National Research Council of Thailand.

In addition to the above-mentioned clinical studies, there have been many reports on other pharmacological activities of extracts of the aerial parts of *A. paniculata* or its diterpene lactones conducted *in vitro* or in animals. However, there have been no clinical studies to support such therapeutic efficacy or to establish appropriate doses for such indications yet. Hence, the following reported pharmacological activities of *A. paniculata* have not yet been clinically accepted in Thailand, e.g.

* ***Anti-hepatotoxic activity*** In *in vitro* and *in vivo* studies[41-51], aqueous or alcoholic extracts of *A. paniculata* leaf or andrographolide showed hepatoprotective effect against various hepatotoxic agents, i.e. carbontetrachloride[41,43,44,48],

galactosamine[42], paracetamol[42,45,47], ethanol[46], and hexachlorocyclohexane (BHC)[49-51]. Andrographolide was more potent than silymarin, the standard hepatoprotective agent from milk thistle *(Silybum marianum)*, to prevent paracetamol-induced decrease of bile volume and bile contents[47], while andrographolide and neoandrographolide were as effective as silymarin to reduce the formation of the degradation products of lipid peroxidation and the leakage of glutamate pyruvate transaminase and alkaline phosphatase in the serum of carbontetrachloride-treated mice[48].

* ***Antioxidant effect*** It was found that extracts of *A. paniculata* possessed antioxidant activity[52-55] and neoandrographolide could scavenge superoxide free radicals[56].

* ***Antihyperglycemic activity*** Ethanolic extract of *A. paniculata* showed antihyperglycemic activity in streptozotocin diabetic rats[55,57].

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 right 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 analysis according to the previously stated quality standard of each medicinal plant material.

This English edition of "**The Standard of Thai Herbal Medicine : *Andrographis paniculata* (Burm.f.) Wall. ex Nees**" is one of the Standard of Thai Herbal Medicine book series published by the Department of Medical Sciences, Ministry of Public Health, Thailand. The content of this book can be divided into two 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 the aerial parts of *A. paniculata* as raw material for the production of herbal medicine for the relief of fever and sore throat in pharyngotonsillitis and for the treatment of non-infectious diarrhea.

The medicinal plant growers, healthcare personnel and the manufactures of *A. paniculata* 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 contamination. For the analytical chemists, this book provides the analytical procedure for the quality control of *A. paniculata* 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 *A. paniculata* 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 standard specifications of medicinal plant materials. Based on the WHO and other related documents[5,58-64], 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

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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 and precaution**
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, 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 also occur.

Chemical constituents[61]

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 follow:-

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. anthraquinone glycosides from senna leaf, ringworm 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 use 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 have 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 cut on the plant. 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[61-63]

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 plants
- Harvesting of the part used
- Post-harvesting 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 manufacturers, 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**[63]

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 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 as 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[63,65]

The identification of 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 standard 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[58,59,63,66]

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 not more than 2% of foreign matter.

Moisture[58,59,63]

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[58,59,63]

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[58,59,63]

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[59,63]

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 material 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[67]. 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.

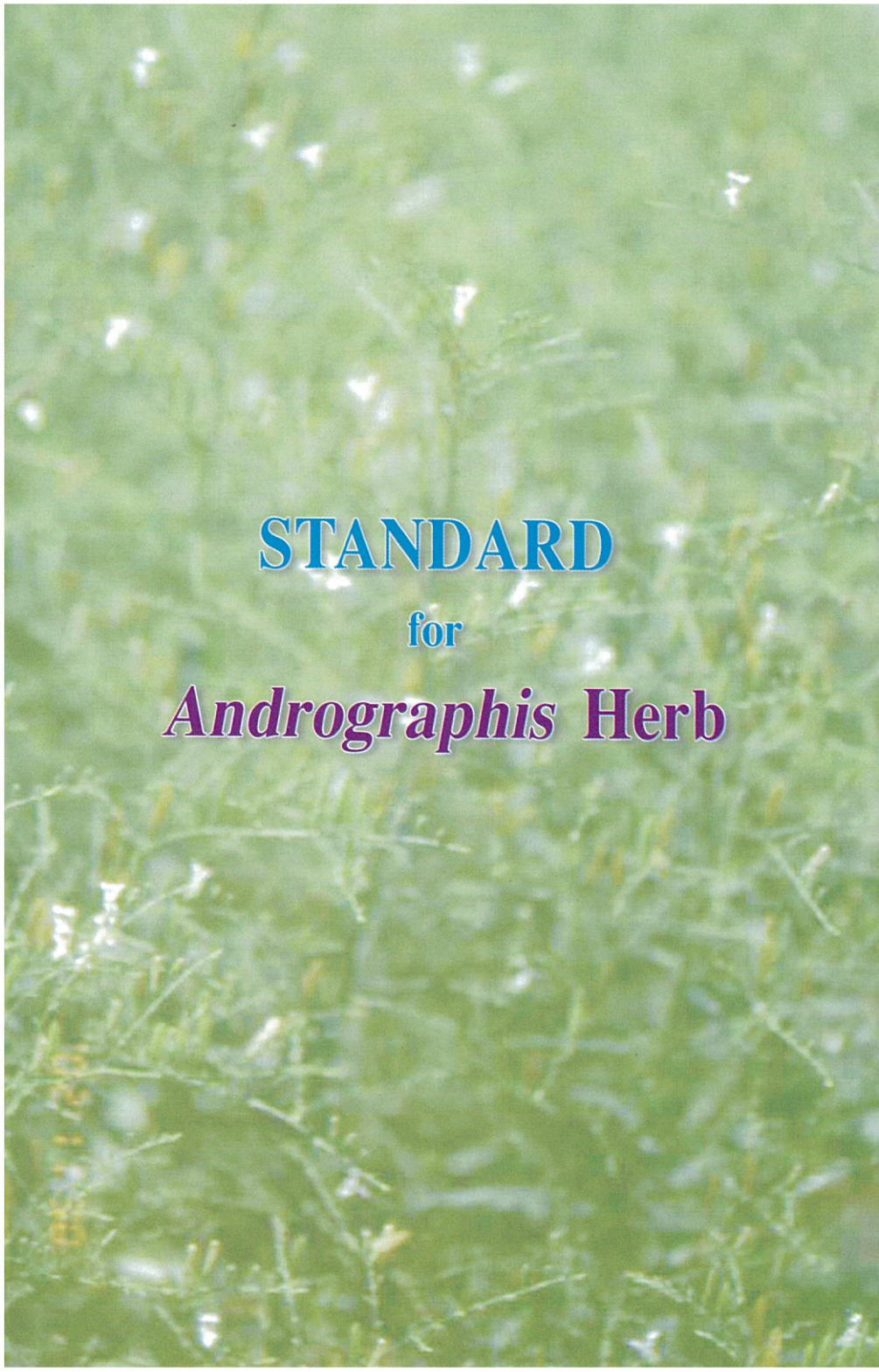
Pesticide residue contamination Nowadays pesticides are widely used in agricultural practice. These pesticides can therefore contaminate in herbal materials and accumulate in the body upon its long-term consumption. Hence, limits of pesticide residues allowed in medicinal plant materials are set for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

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
Andrographis Herb

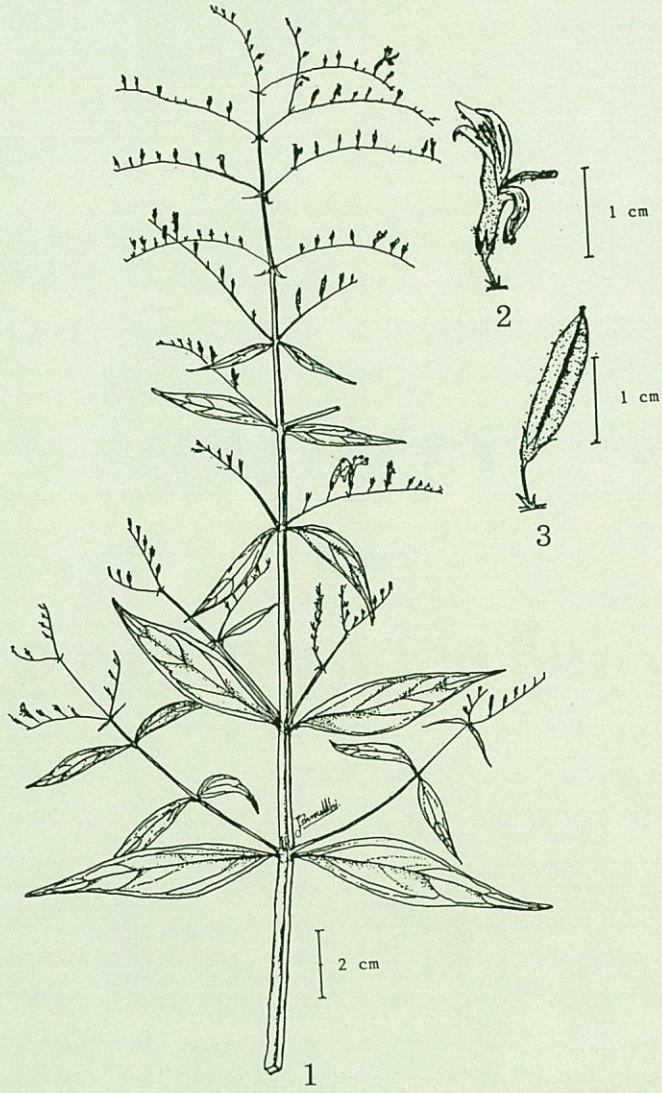


Figure 1 *Andrographis paniculata* (Burm.f.) Wall. ex Nees
1. twig 2. flower 3. fruit

Standard for Andrographis Herb

General Description of the Plant *Andrographis paniculata* (Burm.f.) Wall. ex Nees

Name

Local names : Fa thalai, Fa thalai chon, Namlai pangporn, Sam sip dee, Ya kan ngu [1,5,68]

English names : Creat, Green chireta, Kalmegh, King of bitters[6,69]

Scientific name : *Andrographis paniculata* (Burm.f.) Wall. ex Nees[2,4,6,69-72]

Scientific synonyms : *Justicia paniculata* Burm.f.[6,64,72]
J. latebrosa Russ.[6]
J. stricta Lam. ex Steud.[6]

Family : Acanthaceae

Morphological description[70-72]

A bitter annual herb, 30-100 cm tall; stem acutely quadrangular, manifestly thickened above the nodes, much branched. *Leaves* simple opposite, lanceolate to narrowly ovate, 2-12 cm long and 1-4 cm wide, attenuate at base and acute to acuminate at apex, glabrous, margin entire to slightly undulate; upper leaves often bracteiform; petioles 2-8 mm. *Inflorescences* patent, terminal and axillary in lax panicle, 10-30 cm long; bracts small, lanceolate 2-3 mm long. *Flowers*, whitish, with pedicels 1-7 mm long. *Calyx* 5-partite, segments small linear, 3-4 mm long, glandular-pubescent. *Corolla* tube narrow, almost straight, about 6 mm long, limb not shorter than the tube, conspicuously bilabiate, segments 5; upper lip broadly cuneate, 2 fids oblong, white with a yellowish top, 7-8 mm long; lower lip broadly cuneate, 3 fids, oblong, white with violet markings, about 6 mm long. *Stamens* 2 inserted in the throat and far exserted, filaments narrow with a broadened base, about 6 mm long and anthers basally bearded, deep purple. *Superior ovary* 2-celled, 3-7-ovuled per cell; style far exserted; stigma entire. *Fruit* a loculicidal capsule narrowly oblong, erect, 1-2 cm long and 2-5 mm wide, acute at both ends, compressed, longitudinally furrowed on the broad faces, young slightly thinly glandular-hairy, mature subglabrous. *Seeds* small slightly subquadrate, rugose, yellowish-brown to deep brown. (Figure 1)

Geographical distribution and habitat

A. paniculata is probably indigenous to India and China. It is widely distributed in the tropical and subtropical Asia, southeast Asia[6,64,73], abundantly naturalized and

occasionally cultivated as a medicinal plant. It has been introduced to Central America and the West Indies. This plant can grow from the tropical lowland up to 1,600 m altitude[2], but is more commonly found at lower elevations.

It is often found on the open location, waste places, roadsides, to shady area[2]. It grows well in sunny on a wide range of soils[21,74] which should retain adequately moisture, with moderate fertility.

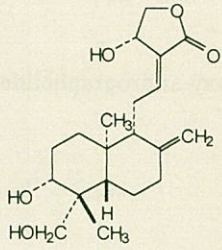
Part used

Aerial parts.

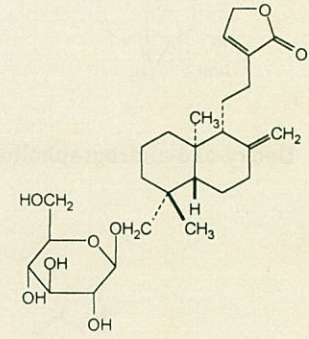
Chemical constituents

The aerial parts contain the major lactone compounds, namely andrographolide, neoandrographolide, deoxyandrographolide and deoxy-didehydroandrographolide. Other minor lactones in the aerial parts are deoxy-oxo-andrographolide, dideoxy-andrographolide, deoxy-methoxy-andrographolide, andrographiside and deoxyandrographiside. Together with lactone compounds, flavone such as oroxylin, wogonin and andrographidine A; steroidal saponins; tannins; phenol and organic acid are found in this plant.

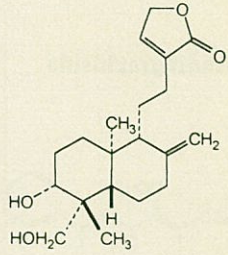
The leaves contain andrographolide, neoandrographolide, deoxyandrographolide, homoandrographolide, andrographan, andrographon and andrographosterin.



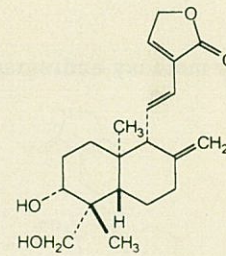
Andrographolide



Neoandrographolide

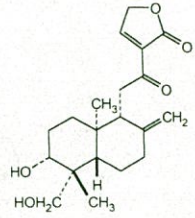


Deoxyandrographolide

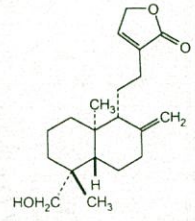


Deoxy-didehydroandrographolide

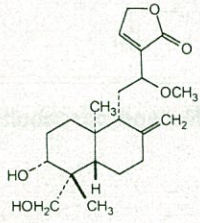
Figure 2 Chemical structures of active constituents in *Andrographis* herb



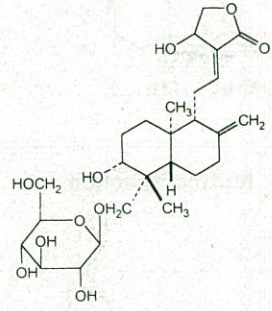
Deoxy-oxo-andrographolide



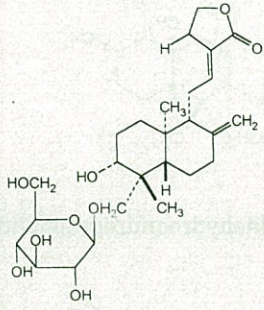
Dideoxy-andrographolide



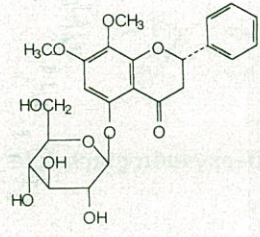
Deoxy-methoxy-andrographolide



Andrographiside



Deoxyandrographiside



Andrographidine A

Figure 3 Chemical structures

Preparation of crude drug

Cultivation

Presently *A. paniculata* is widely cultivated in Thailand, and the research-based knowledge on the cultivation and propagation of this plant is ongoing for the establishment of appropriate national guidelines of the Good Agricultural Practice (GAP) of *A. paniculata.* In the meantime, the following general technical information and some research-based data should be useful for the growers.

Propagation

Method of propagation

- ***Asexual propagation*** : It can be cloned by stem cutting[21,75], and *in vitro* culture technique[76].
- ***Sexual propagation*** : It can be propagated by seeds[21].

Propagated material

The stem cutting materials should consist of 2-3 nodes taken from the one year old plant[2]. It is best to select a cutting material from the upper portion of the plant rather than from the portion near the ground where the material could possibly be contaminated with soil pathogens.

In nature this plant reproduces primarily by seeds. The seedling propagation is an efficient, economical and widely used propagation method for planting *A. paniculata* in Thailand[21]. Nowadays, there are some records[77-81] about the variation of the phenotype, and genotype of this plant, the selection should be directed to the plant material with a higher content of important active constituents.

Seed selection

It requires a good planning in order to grow *A. paniculata* that produces a high yield of aerial parts as the raw material for the production of herbal medicine. Initially, a good quality and a large quantity of seeds must be collected so that enough healthy seedlings can be selected for cultivation.

In collecting seeds of *A. paniculata*, the mature seeds should be collected from unbroken ripening fruits on the standing plants that are vigorous, without any disease or insect infestation. Normally, seeds of this plant achieved physiological maturity about 30 days after anthesis. The fruits 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 fruits should be placed on canvas, tray, or screen and allow to dry in open air for 1-3 weeks[76,82,83] before separating the seeds from the fruits and debris. Only the fully mature healthy seeds with brownish red or dark-brown in color, free from pest or diseases, should be selected[21].

Seed storage

Generally after harvesting, seeds should be stored for the next planting season or longer, and mass propagated in a highly efficient manner. 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.

Normally there are many types of seed storage[76], e.g. open storage without moisture/temperature control, sealed containers, conditioned storage, and moist/cool 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. For optimum storage, well-winnowed and cleaned seeds must be dried to moisture content of less than 10%[76]. 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. A preliminary research was conducted by the Department of Horticulture, Faculty of Agriculture, Kasetsart University in Bangkok to observe the viability of mature *A. paniculata* seeds that were stored for 12 months at ambient temperature had the germinating rate of only 22.75% even though they still retained viability[84].

Seed germination test and seed health test [76,83]

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.

Normally primary dormancy of the seeds of *A. paniculata* often occurs by the internal controls that exist or develop during ripening and for a period of time after harvesting, such as inhibitor and seed covering, that prevent germination. Usually good quality seeds for large-scale cultivation should have a germination percentage of at least 85%[21].

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 test should be performed to determine the presence of pathogens that may impact on seed germination and plant development[76,83].

Seed propagation

Seed propagation involves careful management of germination conditions and propagation facilities. Using good quality seeds is an important factor. The seeds should germinate rapidly and vigorously to withstand any possible adverse conditions in the seedbed. Generally after harvesting, *A. paniculata* seeds are in the dormant state, which may cause unequal germination and a large variation of the age of the plant population in the field under natural condition. Therefore, some forms of pre-treatment must be given to the seeds to overcome such dormancy before propagation[76,83].

Breaking dormancy Generally, the treatments are as follows;

Scarification is any process of breaking, scratching, mechanically altering, or softening the seed covering to make it permeable to water and gases, e.g.

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 (80-100°C) and the seeds are soaked in the gradually cooling water for 12-24 hours.

Stratification is a method of handling dormant seeds by exposing water-absorbed seeds to cool temperature for a certain period of time to allow the embryo to go through "after ripening" process, e.g.

Refrigerated stratification Dry seeds should be fully absorbed with water prior to refrigerated stratification. The usual stratification temperature is 0-10°C. The time required depends on the kind and the lot of the seeds.

Other treatments to overcome dormancy and initiate germination in viability testing, e.g.

Pre-chilling : Place absorbed seeds at 5-10°C for 5-7 days prior to germination.

Pre-drying : Subject dry seeds to 37-40°C for 5-7 days prior to germination.

Potassium nitrate : Place absorbed seeds in germination trays or petri dishes, and moisten the substratum with 0.1 to 0.2% potassium nitrate solution.

Seed soaking : Soak the seeds in water before planting in order to shorten the time of emergence in the seedbed.

A preliminary research was conducted by the Department of Horticulture[84], Faculty of Agriculture,

Kasetsart University in Bangkok to observe seed dormancy and to compare the effect of different treatments on the germination rate of *A. paniculata* seeds stored at the ambient temperature for 2 months and 12 months. Germination and seed vigor were observed during 28-day period after propagation. It was found that the seeds stored for 2 months gave the best germination rate of 82.75% when they were initiated by soaking in water at room temperature for 24 hours, while the germination rate of 80.25% was obtained when they were pre-chilled at 10°C for 1 month. For the seeds stored for 12 months, the best germination rate of 91.75% was obtained when they were initiated by pre-chilling at 10°C for 1 month, then exposed to 45°C for 48 hours. When those seeds were subjected to only pre-chilling or only exposed to 45°C for 48 hours, the germination rate was 88.25 and 71%, respectively. In contrast, when the seeds were not previously pre-chilled but were soaked in room temperature water for 24 hours or incubated at 45°C for 4 hours before propagation, such treatments were unable to break dormancy or induce higher germination rate. However, one report[85] stated that *A. paniculata* seeds had slight dormancy which could be easily removed by treating with 0.5% potassium nitrate solution.

Seed propagation systems

Seed propagation can be carried out by two general systems, namely field seeding in the location where the plant is to remain and propagation in protected condition or seedbed and transplanting to a permanent location.

Preparation of seedbed

Generally the seeds should be cultured in the proper environmental conditions for good uniform germi-

nation to enable the plants to grow at the same rate. The cultivation in a plantation requires 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 seedbed should be at a slightly shady location, and has a loose but fine physical texture that produces close contact between seed and soil so that moisture can be supplied continuously to the seeds. The soil should have suitable organic matters and provides 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. Sanitation during propagation should also be considered[76].

Seedbed for the propagation of *A. paniculata* seeds 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[21].

Preparation of seeding

A. paniculata seeds should be initiated by a proper method as previously mentioned[84] or the seeds should be soaked for 24 hours and dried before being sown, normally germination will start after 1 week[2]. Otherwise, the seeds should be immersed in hot water at 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[21]. It is critical to predetermine the proper rate of seed sowing in order to obtain a desired plant density. It can be estimated by the following formula[76]:

$$A = \frac{B}{(CxDxE)}$$

When

A = weight of the seeds per unit area (rate of sowing)
B = density (number of plants/unit area) desired
C = number of seeds/unit weight (seed count)
D = percent of germination rate expressed as a decimal
E = percent of purity expressed as a decimal

The rate of sowing obtained is a minimum and should be adjusted to account for expected losses in the seedbed, determined by previous experience at that site. However, avoid using too many seeds per seedbed area because the competing seedlings will become spindly, thin, and unhealthy and do not transplant well.

Sowing of seeding material

The newly germinated seeds previously mixed with the sand may be either spread over the surface of the seedbed, or drilled into closely spaced rows. Then use a rake to cover them up to a depth of about 1 cm. Cover the seedbed with suitable mulch[86], e.g. 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. After sowing the

seeds, water the seedbed. Some mulch should be removed after one-month germination to avoid elongation of the seedling.

Seedbed husbandry

Under a warm and humid condition, during propagation procedures of young seedlings, sanitation should be considered to avoid various pathogens, insect pests and weed problem that can sometimes be devastating.

Watering After sowing the seeds, if there is no rain, the seedbed should be irrigated regularly with water fountain in the morning and the late afternoon until seedlings emerge.

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[87-89] should be applied at a minimum effective level in accordance with the recommendation from the manufacturer and the regulatory 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

One to two months old seedlings from which 7-14 leaves have sprouted should be transplanted into the growing plots or in plastic bags and tended until the seedlings are strong enough for transplantation in the 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 seedbed prior using a spade to dig up the seedlings, allowing as much soil to cling to the roots as possible. This will help the seedlings to recover sooner, and will result in a higher rate of survival[21].

Site selection and preparation of growing area

To select the 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 cultivation *A. paniculata* should be a moist and slightly opened, or slightly shaded location, with abundant organic matters and loose soil, as well as adequate and constant soil moisture. However, it has recently been reported that leaves exposed to bright sunlight had up to 35% lower chlorophyll and 30% higher oxalic acid concentration[90]. Normally, a pH range of 5.5 to 7 is suitable for the growth and development of the plant.

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 weeds, 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 plots: For large growing

area, the raised plots should be considered if planting is done during the rainy season, or on a low land or the flood-plain with bad drainage. The plots should be prepared by digging the soil with a hoe and raise the height of a plot to about 15-20 cm and the width of about 1-2 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, they should be separated by walkway 30-50 cm wide to provide easy access. It is advisable to irrigate plots one day before planting to avoid any risk of exosmosis of the seedlings. Plots are to be irrigated immediately after planting.

Planting

The climatic and environmental condition of the tropical region of Thailand is suitable for growing plants at any time of the year. *A. paniculata* is very adaptable, it can grow luxuriantly and starts flowering in the open area during the early rainy season[91,92]. Moreover, the rain will also help saving the cost of watering the plants.

Methods of Planting

Similar to the methods of seed propagation, two general planting methods can be used to cultivate this plant, the details are described as follows:-

1. Direct field seeding This method can be used for hobbyists or home planting. It is also suitable for planting along with other main farm crops, which can provide shade for newly germinated and young seedlings, or for planting in the moist area with no weed problem. Field seeding may be broadcast (i.e. spaced randomly over the entire area) or drilled at given spaces.

Broadcast sowing The prepared growing areas or plots should be evenly sown by the square meter with the newly prepared germinated seeds. If after 1 month, seedlings grow at a high density, competing seedlings should be removed. However, manual weed control cannot be easily performed.

Growing in row The rows prepared for row sowing should be about 30 cm apart. The advantages of row planting are reduced damping-off, better aeration, easier transplanting and weeding.

Growing in row at given spaces The prepared growing plots should be marked by placing stick marks leaving 15-20 cm spacing between the holes and 30-40 cm between rows. Dig the holes 2-5 cm in size, then drop 5-10 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. This method uses the least number of seeds[21,91].

Cost and labor requirements for direct field seeding is lower than those for 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 to permanent location : Transplant seedlings or remove plastic bags from the seedlings and place them in the prepared growing plots previously marked by placing stick marks leaving 15-20 cm spacing between plants and 30-40 cm between rows. Dig the holes 10-15 cm in size, then line the bottom of the holes with manure at the rate 100-150 g per hole, and well corpulate with the soil[21]. It is advisable to water plots

one day prior to planting to avoid any risk of exosmosis of the seedlings. Then put one seedling/hole and cover up the hole with pulverized soil, tamp it down tightly against the seedlings. Mulch around the seedlings to maintain soil moisture and to prevent weeds from growing. Plots are to be irrigated immediately after planting.

Husbandry

Watering

Initially if it is sunny and there is no rain, water the plants once or twice every day until they are 1-2 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. A lacking of water over a period of several days will cause the plants to wither and will stunt their growth, prompting them to flower rather sooner than normal. Furthermore, the plants will not be able to absorb certain nutritional elements, resulting in a violet leaf symptom.

Fertilizers

Fertilizers are generally uneconomical for the soil containing sufficient organic matters. Although the experiments showed that macro-nutrient application, e.g. nitrogen (N) or phosphorus (P), influences yield predominantly through its influence on biomass production[78,93,94] all fertilizing agents should be applied sparingly and in accordance with the needs of the plants and supporting capacity of the soil. Fertilizer should be applied in such a manner as to minimize leaching.

To improve soil fertility and structure, general garden fertilizer may be applied to the planting bed before

planting, and water immediately and thoroughly. Fertilizer may be applied at different intervals as follows[21]:

- Before transplanting or sowing, line the bottom of each hole with 100-150 g/hole or 0.5-1 kg/m^2 of manure, and mix it well with the soil.

- When the plants are 2 months old, apply 150 kg/per Rai* of chemical fertilizer formula 15-15-15, 16-20-0 or 30-20-10.

- From 3 months onwards, apply 150 g/m^2 or 30 g/plant of chemical fertilizer formula 15-15-15 or 30-20-10; or 150 g of manure/plant

To apply fertilizer, sow it around the hole 10 cm from the plant, or sow it in parallel 10 cm from the row, 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 plant or row 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.

Weed control

To achieve economic yields, competing weeds around the plants 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 insect control

The plant is almost free from any diseases and

* Rai is a Thai unit of area amounts to 1,600 m^2, 6.25 Rai = 1 ha, or 2.5 Rai = 1 acre

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

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 diseases and insects known to attack the plant[21,95], but are of minor concern are as follows :

Stem and root rot This disease is caused by the fungi *Fusarium* sp. The disease can be mechanically controlled by cutting off and immediately destroyed the affected parts. For more serious disease, the recommended chemical pecticides[87] can be used to control the fungi. When using these chemicals, strictly follow the recommended instructions on the label.

Ants Ants often eat young seedlings, they should be mechanically controlled by destroying both the ants and the egg mass. If serious outbreak occurs, spray with the recommended insecticides[88,89]. When using these chemicals, strictly follow the recommended instructions on the label.

Harvesting

The aerial parts that will be used as the raw material for the preparation of herbal medicine are generally harvested only when the plants have achieved early maturity,

or the plants are beginning to blossom[21]. Normally the plants at flowering stage are 90-120 days up to 140 days old after sowering[92,96], depending on the environment.

Preliminary researches carried out by several institutes showed that the aerial parts should be harvested from the beginning of the flowering period[96] until prior to 50% of blooming[21]. However, it was found that the contents of four diterpenes (percent w/w) in the leaves of cultivated plants collected monthly were varied through out the year[97].

Normally, the aerial parts of *A. paniculata* that will be used as raw material for the preparation of herbal medicine are harvested at the required amount when needed. The active constituents are high prior to flowering; hence, harvesting should not be done in late full bloom[21,91]. In Thailand, the plant grows well and produces the highest yield during the rainy season through to the early cool season. The harvesting can be done three to four times a year if sown in early rainy season. The subsequent harvest can begin again about 60-100 days after the previous harvest[85,97].

The aerial parts should be harvested using pruning shears or a sickle to cut off at the base leaving 10-15 cm stem for plant regeneration, and the collected plants should be put in proper container. This method is quick, does not damage the raw material and suitable for transfer[21]. It was reported that a well maintained crop grown during rainy season could yield about 2,000 to 3,000 kg of fresh herb per Rai[21].

It was found that many samples of *A. paniculata* collected from different growing areas and during different collection periods contained higher than 6% total lactones,

the minimal content stated in the quality specification[98-100]. 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[100,101].

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 good quality, but also safe raw material. Cutting devices, harvesters or other machines must be cleaned 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 harvested material, rinse the uncut aerial parts with clean water, then cut into segments and leave on a clean surface and allow the water to drain. Spread the raw material evenly on a clean utensil such as a winnowing basket or tray[21]. Damaged raw material must be discarded during post-harvest inspection.

Drying process

The raw material should 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[21]. Otherwise, cover the raw material with a clean white cloth to protect from dust and wind, then dry in the sun until they are completely dried.

Packaging and storage

Keep the dried raw material in clean well-closed containers, e.g. tightly tied and appropriately labeled plastic bags. If only a small amount of the raw material 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 raw material should not be kept longer than one year.

Nowadays, organic food and organic natural products are well received by consumers worldwide. Therefore, the growers and producers of *A. paniculata* should take this opportunity to produce high value, 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 production has already been established a few years ago[102].

Quality Specification of *Andrographis* Herb

Definition

Andrographis Herb consists of the dried aerial parts of *Andrographis paniculata* (Burm.f.) Wall. ex Nees (Family Acanthaceae) containing not less than 6.0% by weight of total lactones, calculated as andrographolide[5,103].

Description of crude drug

Color, dark green; odour, slight and specific; taste, extremely bitter.

Identification

Pharmacognostic characteristics

Macroscopical description *Andrographis* herb occurs as a mixture of dried, broken, crisp, mainly dark green lanceolate leaves and quadrangularly stem; capsule fruits and small flowers occasionally found.

Microscopical description

- ***Histologic characteristics***

  Leaves Surface and transverse sections of the leaf through lamina and midrib region show the following characters (Figure 4):-

  1. **Upper epidermis,** a layer of cells, slightly wavy-walled; lithocyst, large cell containing cystolith; glandular trichomes present; unicellular and multicellular trichomes rarely seen; stoma absent.

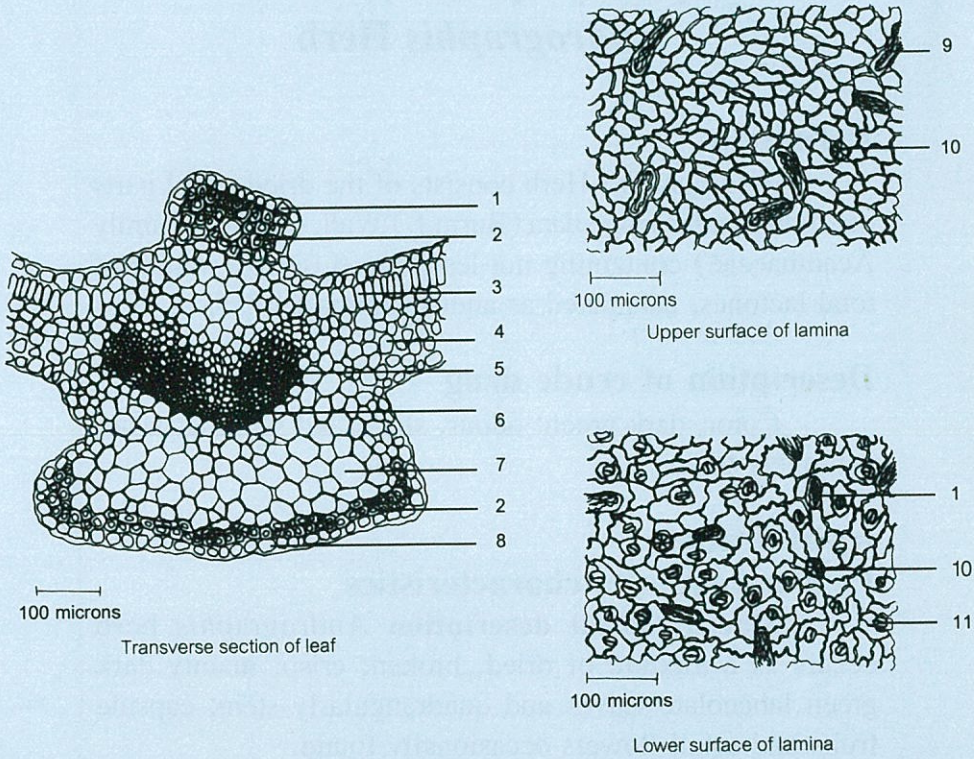


Figure 4 Surface view and transverse section of *A. paniculata* leaf

1. upper epidermis
2. collenchyma
3. palisade cell
4. spongy cell
5. xylem
6. phloem
7. parenchyma
8. lower epidermis
9. lithocyst
10. glandular trichome
11. stoma

2. **Collenchyma,** occurs in the midrib, beneath upper and lower epidermis.
3. **Palisade,** a layer of columnar cells, containing chloroplast.
4. **Spongy,** large thin-walled and slightly rounded parenchymatous cells, containing chloroplast.
5. **Vascular bundle,** composed of lignified xylem in the upper part and phloem in the lower part; vessel, spiral, scalariform, reticulated, pitted.
6. **Lower epidermis,** a layer of wavy-walled cells; stomata diacytic; lithocysts and glandular trichomes present; multicellular trichomes rarely seen.

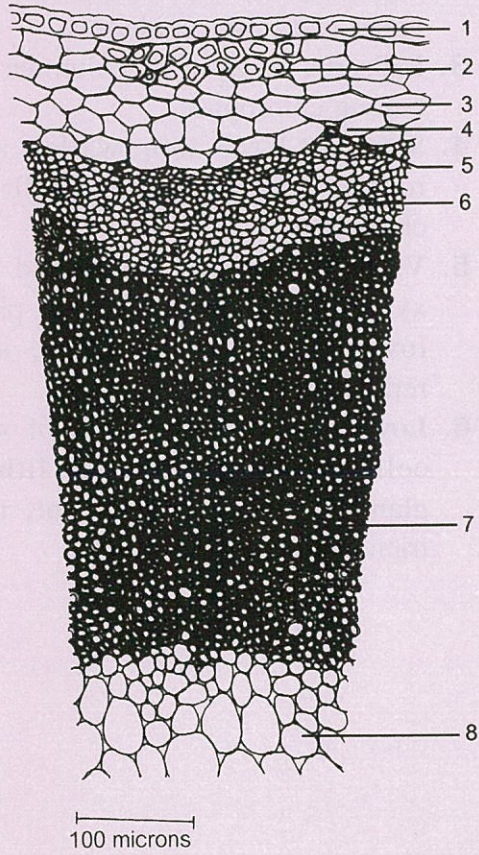


Figure 5 Transverse section of *A. paniculata* stem

1. epidermis
2. collenchyma
3. parenchyma
4. endodermis
5. fiber
6. phloem
7. xylem
8. pith

Stem Transverse section of the stem shows the following characters (Figure 5): -

1. **Epidermis,** a layer of cells, covered with cutin; stomata diacytic; lithocysts and glandular trichomes present; multicellular trichomes rarely seen.
2. **Collenchyma,** densely found at the corner of stem.
3. **Parenchyma,** 2-6 layers of cells, containing chloroplast, slightly elongated on outermost.
4. **Endodermis,** a layer of thick-walled cells; fiber, one cell or two in a group, embeded in this layer.
5. **Phloem,** thin-walled cells.
6. **Xylem,** thick-walled cells, lignified; vessel spiral, scalariform, reticulated, pitted; fibers.
7. **Pith,** large thin-walled cells.

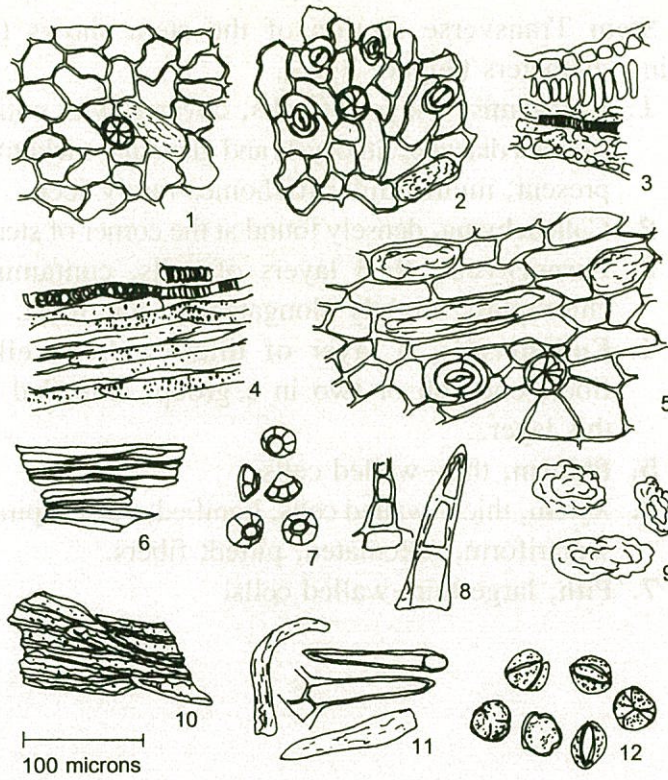


Figure 6 Powdered drug *of A. paniculata* aerial parts

1. fragment of upper epidermis in surface view with lithocyst and glandular trichome
2. fragment of lower epidermis in surface view with stomata, lithocyst and glandular trichome
3. fragment of lamina in sectional view
4. vessels spiral, scalariform and reticulate
5. epidermis of stem in surface view
6. fibers
7. glandular trichomes
8. multicellular trichomes
9. cystolithes
10. fibro-sclereid of pericarp
11. unicellular trichomes
12. pollen grains

- ***Description of powdered drug***

The characteristics of the powdered drug are dark green in color, slight and specific odor, and extremely bitter taste. The following microscopic characters are more to less respectively found (Figure 6).

1. Fragments of epidermis in surface view, both or either upper epidermis or lower epidermis showing lithocysts, glandular trichomes, stomata, but absent stoma in upper epidermis.
2. Fragments of lamina in sectional view in some parts or entire showing upper epidermis, palisade, spongy, vascular bundle, lower epidermis.
3. Fragments of vessels, spiral, scalariform, reticulated, pitted.
4. Fragments of stem in surface view showing stomata, lithocysts, glandular trichomes.
5. Fragments of fiber.
6. Glandular trichomes.
7. Multicellular trichomes of stem.
8. Cystolithes
9. Fibro-sclereid of pericarp
10. Unicellular trichomes of petal.
11. Pollen grains

Chemical identification

Preliminary test

To about 1 g of the sample, in powder, add 20 ml of ethanol, boil in a water-bath and filter. To the filtrate, add 300 mg of decolorizing charcoal, stir and filter. Test the sample solution using the following reagents: -

1. Kedde reagent which is a 1:1 mixture of Kedde A reagent (2 per cent w/v solution of 3,5-dinitrobenzoic acid in methanol) and Kedde B reagent (5.7 per cent w/v solution of potassium hydroxide in methanol). To 1 ml of sample solution, add 2-3 drops of Kedde reagent, a purplish red color develops.

2. Ethanolic potassium hydroxide TS (or 6.5% potassium hydroxide in ethanol) To 1 ml of sample solution, add several drops of ethanolic potassium hydroxide TS until it shows a red color. Set aside for 10 to 15 minutes: the color is changed to yellow.

Confirmatory test

Solution A

Boil 1 g of the sample, in powder, with 20 ml of ethanol on a water-bath for 5 minutes. Add 300 mg of decolorizing charcoal, stir and filter. Evaporate the filtrate under reduced pressure until dryness, and dissolve the residue in 1 ml of warm ethanol (80 per cent).

Standard solutions

Separately dissolve standard substance in 1 ml of ethanol using 2 mg of andrographolide, 2 mg of neoandrographolide, and 4 mg of dehydroandrographolide.

TLC apparatus and solvent

1. Adsorbent-coated glass plate

Use 5 x 20 cm glass plate coated with silica gel GF_{254}, 0.25 mm thick. Dry the plate in the oven at 105 °C for about 1 hr.

2. Developing solvent

Mix chloroform with absolute ethanol at the ratio of 17:3

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 and standard

solutions, 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 15 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 254 nm.

2. Spray the plate with Kedde A reagent and then spray with an excess of Kedde B reagent until the spots can be clearly observed.

Result

The chromatogram obtained from the second detection method is shown in Figure 7. The location, as indicated by hR_f value, and the color of the spots of each type of the lactones in the sample are the same as those of the standard solutions (Figure 7).

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.

hR_f values and the results of the identification of different lactones by both detection methods are summarized[5] in Table 1.

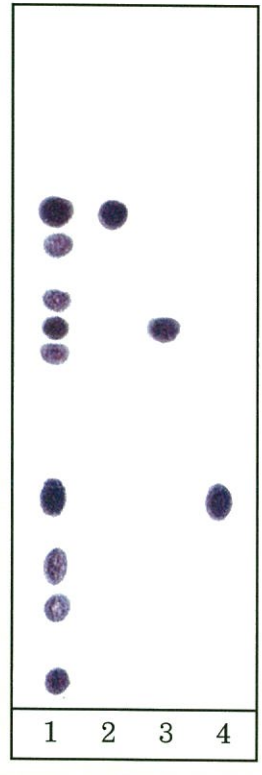


Figure 7 Thin-layer chromatogram of the ethanolic extract of the aerial part of *A. paniculata* as detected by Kedde A and Kedde B reagents.

1 = Sample solution (Solution A)
2 = Standard dehydroandrographolide solution (average $hR_f = 70$)
3 = Standard andrographolide solution (average $hR_f = 54$)
4 = Standard neoandrographolide solution (average $hR_f = 30$)

Table 1 hR_f values and the results of the detection of different lactones in ethanolic extract of the aerial part of *A. paniculata*

Lactones	hR_f value	Detection with	
		UV_{254}	Kedde A/Kedde B
Lactone #1	1-5	-	dark violet
Lactone #2	11-15	quenching	violet
Lactone #3	18-22	quenching	violet
Neoandrographolide	28-32	-	dark violet
Lactone #5	49-51	-	violet
Andrographolide	52-56	quenching	dark violet
Lactone #7	57-59	quenching	violet
Lactone #8	66-68	-	violet
Dehydroandrographolide	69-71	quenching	dark violet

Foreign matter[58,59,66]

Not more than 2.0 per cent w/w

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 to constant weight** [5]

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 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[99]

Not specified

When several parts of a medicinal plant are used, total ashes in different samples usually significantly differ. Hence, only the percentage of acid-insoluble ash will be specified. Since the part used of *Andrographis* herb is the aerial parts consisting of different ratios of the stems, leaves, flowers and fruits in different samples, analytical result showed a significant difference of total ashes between samples of plant materials.

Acid-insoluble ash

Not more than 2.0 per cent w/w[5]

Place 2-4 g of the ground plant material, accurately weighed, in a suitable tared crucible (usually of platinum or silica), previously ignited, cooled and weighed. Incinerate the sample by gradually increasing the temperature,

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

not exceeding 450°C, until free from carbon; cool and weigh. 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 (85 per cent)-soluble extractive

Not less than 13.0 per cent w/w[5]

Macerate 5 g of the air-dried crude drug, coarsely powdered and accurately weighed, with 100.0 ml of 85% 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 85% ethanol-soluble extractive with reference to the air-dried substance.

Water-soluble extractive

Not less than 18.0 per cent w/w[5]

Proceed as directed in 85% ethanol-soluble extractive but using chloroform water as the solvent in place of ethanol.

Total lactones content

Not less than 6.0 per cent w/w of total lactones,

calculated as andrographolide (5,99)

Place about 1 g of *Andrographis* herb in No. 180 powder, accurately weighed, in a 100-ml round-bottomed flask, add 50 ml of ethanol (85 per cent), reflux in a water-bath for 2 hours, and filter. Wash the marc with sufficient amount of ethanol (85 per cent) until the last washing is almost colorless. Combine the washings and the filtrate and allow to cool. Add 1 ml of *Basic lead acetate solution**, set aside for 15 minutes, filter, and wash the precipitate with ethanol until the last washing is no longer green. Combine the washings and the filtrate, add dropwise with swirling 1 ml of a 25 per cent w/v solution of *sodium sulfate* and mix well. Set aside for 1 hour, add 500 mg of decolorizing charcoal, and reflux in a water-bath for 10 minutes. Filter through the Buchner funnel containing 500 mg of decolorizing charcoal and wash with three 2-ml portions of hot ethanol. Combine the washings and the filtrate, add 20 ml of distilled water, allow to cool, and neutralize with 0.1 M sodium hydroxide, using *phenolphthalein TS*** as indicator. Add 5.0 ml of 0.1 M sodium hydroxide VS, reflux in a water-bath for 30 minutes, allow to cool, and titrate with 0.05 M hydrochloric acid VS. Perform a blank determination (Residual Titrations) under the same condition. Each ml of 0.1 M sodium hydroxide VS is equivalent to 35.04 mg of andrographolide, $C_{20}H_{30}O_5$

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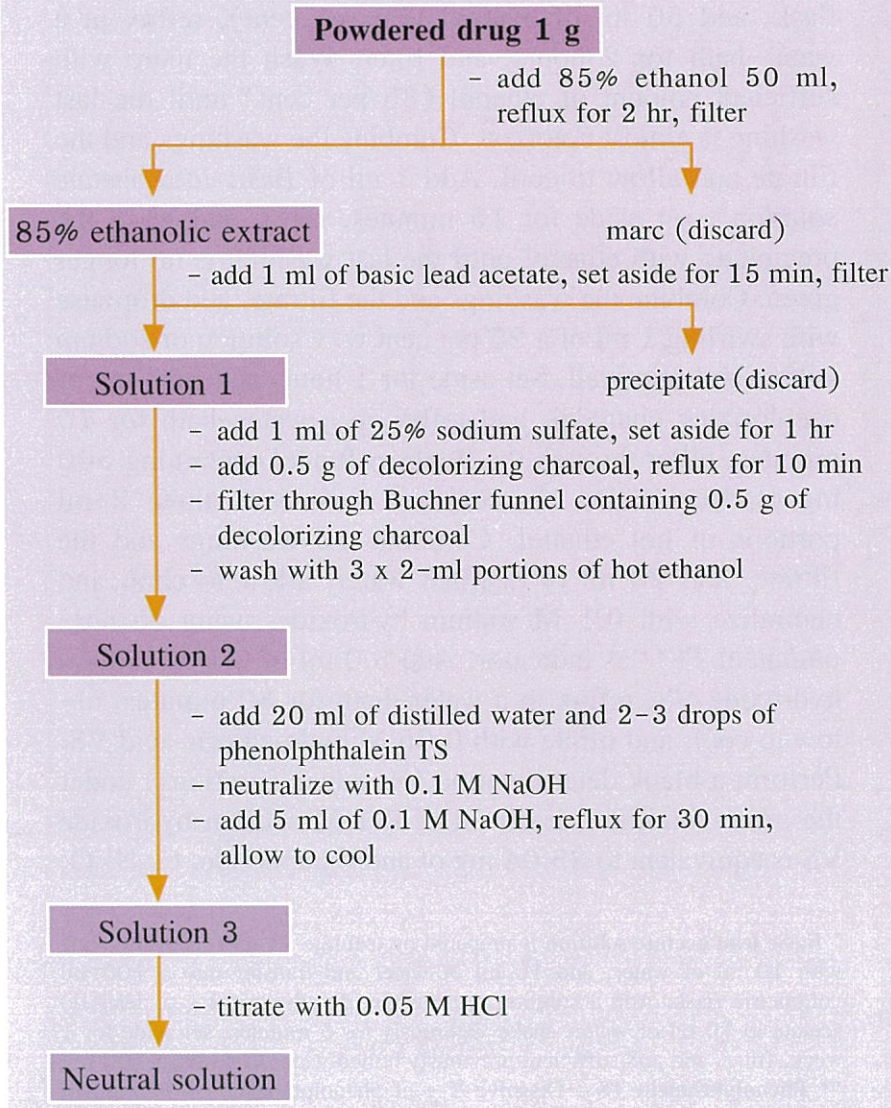
* **Basic lead acetate solution** is prepared by triturate 14 g of lead(II) oxide with 10 ml of water, add 10 ml of water and transfer into a 100-ml volumetric flask. Add a solution prepared by dissolving 22 g of lead(II) acetate in 70 ml of water, shake vigorously for 5 minutes, set aside for 1 week, filter, and add sufficient previously boiled water to produce 100 ml.
** **Phenolphthalein TS** : Dissolve 1 g of phenolphthalein in 100 ml of ethanol (50 per cent).

Diagram showing the procedure for the determination of the content of total lactones in the aerial parts of *Andrographis paniculata*



Summary of the quality specification of *Andrographis* herb

Items analyzed	Not More Than (% w/w)	Not Less Than (% w/w)
Foreign matter	2.0	
Loss on drying	11.0	
Acid-insoluble ash	2.0	
85% Ethanol-soluble extractive		13.0
Water-soluble extractive		18.0
Content of total lactones, calculated as andrographolide		6.0

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[67] set up the limits for microbial contamination allowed in different types of herbal prepa-

rations. For preparations of crude drugs and mixtures of crude drugs for internal use, the total aerobic microbial count should not exceed 5 x 10^5 cfu (colony forming unit) per g or ml, amongst which the yeasts and moulds count should not exceed 5 x 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 x 10^3 cfu per g or ml. A 10-g or 10-ml sample must be free from *Clostridium* spp. and *Salmonella* spp.; and a 1-g sample must be free from *Staphylococcus aureus*.

Microbial limit tests[60,66,105] 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 *Andrographis* herb[106]. 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. Transfer 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 determined 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[59,107] 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[63,108].

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[5,59,63,108], respectively.

Methods of analysis[5,59] 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[5,63].

Indication

For the treatment of pharyngotonsillitis and non-infectious diarrhea[7].

Toxicity

Acute toxicity study of 50% ethanolic extract of *A. paniculata* in mice showed no sign of toxicity or death when the animals were given the highest dose of the extract orally at 15 g per kg of body weight (g/kg BW)[109].

Six-month toxicity study of powdered *Andrographis* herb was performed in 4 groups of 24 Wistar rats (12 males and 12 females)[109]. The three treatment groups were given the herb suspended in 1% tragacanth suspension at the doses of 0.12, 1.2, and 2.4 g/kg BW/day, which were equivalent to 1, 10 and 20 folds of therapeutic dose (6 g/day), respectively, while the control group received 1% tragacanth suspension. The results showed no significant changes of growth rate, food consumption, clinical signs, hematological, or biochemical parameters in the treatment groups as compared to the control group. In addition, upon gross observation, no differences of the weights or physical appearance of the internal organs were observed. Even though some histopathological changes were observed in certain organs in certain groups of animals, those findings were not dose-related and therefore were not likely to be due to the toxic effect of *Andrographis* herb.

Regarding mutagenicity of *A. paniculata*, *in vitro* studies show that *Andrographis* herb is not mutagenic[6];

instead, it possesses antimutagenic activity[6].

It was reported that *Andrographis* herb showed abortifacient activity in mice and rabbits[6]. A study conducted in China showed that 50% decoction of *Andrographis* herb given intraperitoneally in mice at the dose of 8.3 g/kg BW at different stages of pregnancy could terminate pregnancy[110]. This abortifacient effect of *Andrographis* herb at early pregnancy could be prevented by concomitantly given injection of LH-RH or progesterone injection. Hence, it was postulated that *Andrographis* herb might induce abortion in mice by antagonizing with endogenous progesterone. In contrast, when rats were given *Andrographis* herb extract orally at the dose of 2 g/kg BW during the first 9 days of pregnancy, no interruption of pregnancy, fetal resorption, or decrease of the number of live offspring occurred[6]. In a following study, pregnant rats were given 70% ethanolic extract of *A. paniculata* leaves, containing total andrographolide 6.9%, orally at the doses of 200, 600, and 2000 mg/kg BW, which was equivalent to 30, 90, and 300 folds of therapeutic dose, during the first 19 days of pregnancy. It was found that the extract did not affect the elevated level of plasma progesterone in the pregnant rats[110].

Taken together, it is recommended that *Andrographis* herb should not be used during pregnancy due to the potential antagonizing effect between *Andrographis* herb and progesterone observed in some animal species[6,20,111].

As for male reproductive toxicity studies, scientific evidence obtained so far was conflicting. A report from India showed that dried leaf powder of *A. paniculata* given orally to rats at the dose of 20 mg/day for 60 days caused

cessation of spermatogenesis, regressive and/or degenerative changes of seminiferous tubules, epididymis, seminal vesicle, ventral prostate and coagulating gland, and regression of Leydig cells[112]. Further study revealed that andrographolide at the doses of 25 and 50 mg/kg BW given orally to 3-month-old male rats for 48 days caused impairment of fertility by affecting both spermatogenesis and cauda epididymal spermatozoa[113]. These findings were in contrast with the above-mentioned six-month toxicity study of *Andrographis* herb in rats in which much higher doses of the herb and much longer duration of treatment were used; but no histopathological change of the testis was detected[109]. In addition, standardized ethanolic extract of *A. paniculata*, containing 5.6% of andrographolide, given orally to rats at the doses of 20, 200, and 1000 mg/kg BW for 60 days did not cause any alteration of the weight or histopathological changes of testicles, epididymis, seminal vesicle or prostate[114]. The reason for this discrepancy is still unclear.

Contraindication

1. It is contraindicated in individuals who are allergic or hypersensitive to *Andrographis* herb[6].

2. Due to potential anaphylactic reactions, extracts of *A. paniculata* or should not be given by injection[6,115].

3. *Andrographis* herb should not be used during pregnancy or lactation since the herb showed abortifacient effect in some animal species[6,20,111].

4. Therapeutic efficacy of *Andrographis* herb to

relieve fever and sore throat is likely to be due to its antipyretic and anti-inflammatory activities rather than antibacterial activity since scientific evidence to support the latter activity is still inconclusive. Therefore, to prevent patients who are possibly infected with *Streptococcus* group A from serious complications, such as rheumatic fever, rheumatic heart disease and glomerulonephritis, ***Andrographis* herb should NOT be used to treat sore throat in the following cases**[103]: -

- Patients suffering from *Streptococcus* group A sore throat,
- Patients with known history of glomerulonephritis from group A Streptococcal infection,
- Patients with a history of rheumatic heart disease,
- Patients having sore throat due to bacterial infection with symptoms suggestive of possible group A Streptococcal infection, e.g. enlarged and reddened tonsil covered with yellow, gray or white exudate, high fever, and chill.

Warning

1. It may cause allergic reactions ranging from minor skin rashes, urticaria to anaphylaxis[6,115]

2. *Andrographis* herb may cause stomachache, gastrointestinal discomfort, loose stool or diarrhea, loss of appetite, dizziness, vertigo, or palpitations of the heart in some individuals[6,20,111].

3. Prolonged use may cause numbness or weakness

of the extremities[116].

4. After taking *Andrographis* herb for three days, if the symptom is not better or becomes worse, discontinue use and seek medical attention[103].

Precaution

Extracts of *A. paniculata* may synergize with isoniazid[6].

Preparations used and dose

Dosage forms

Andrographis Herb is available in the forms of capsules, tablets, or pills containing 250 or 500 mg of powdered herb.

Dosage

Non-infectious diarrhea : 0.5-2 g four times daily after meals and at bedtime.

Pharyngotonsillitis : 3-6 g per day in 4 divided doses after meals and at bedtime.

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(Burm.f.) Wall. ex Nees