

Pharmacognostic Specifications of *Acanthopanax trifoliatum* leaves

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ABSTRACT

Introduction: *Acanthopanax trifoliatum* or Phak-pam has been popularly used as both medicinal plant and food plant in Northern of Thailand. **Objective:** The present study aims to investigate the pharmacognostic specifications to authenticate *A. trifoliatum* leaves. **Methods:** Leaves of *A. trifoliatum* from 12 sources throughout Thailand were collected, then morphological and physicochemical determinations following WHO guideline of quality control methods for medicinal plant materials were used to investigate leaves samples. **Results:** Morphological identification revealed remarkable microscopic characters were rosette crystals of calcium oxalate and anisocytic stomata. Physico-chemical parameters including; loss on drying, total ash, total acid insoluble ash, water content, ethanol soluble extractive and water soluble extractive values were found to be 1.08 ± 0.17 , 10.52 ± 1.33 , 1.67 ± 0.30 , 9.87 ± 1.32 , 6.76 ± 4.41 and 6.67 ± 0.57 , respectively. *A. trifoliatum* leaves contained traces of volatile oil. **Conclusion:** This study provided useful information of pharmacognostic specification that could be used for authentication, identification and quality control of *A. trifoliatum* leaves.

Keywords: *Acanthopanax trifoliatum*, Phak-pam, Pharmacognostic specification, Physicochemical specification

Introduction

Acanthopanax trifoliatum, also known as Phak-pam, belongs to the Araliaceae family [1]. Its leaves have been used as both medicinal plant and food plant in local Thai cuisine. As a medicinal plant, decoction of *A. trifoliatum* leaves was used to treat tuberculosis and lung hemorrhage and as a tonic to improve general weakness [2]. *A. trifoliatum* leaves extracts had anti-inflammatory effects in acute and chronic inflammation [3], as well as showed positive results on cognitive and emotional deficits within olfactory bulbectomized mice [4]. Its essential oil from leaves also had antimicrobial effect [5]. However, there was no report for *A. trifoliatum* leaves authentication. In this study aims to investigate the pharmacognostic specifications to authenticate *A. trifoliatum* leaves following the World Health Organization guideline of quality control method [6]; including macro- and microscopic, loss on drying, total ash, acid- insoluble ash, water content, volatile oil content, ethanol and water-soluble extractive values and TLC fingerprinting profiles.

Methods

Plant material

A. trifoliatum leaves samples were collected from 12 different geographical sources in Thailand. The samples were authenticated and compared to herbarium at Queen Sirikit National convention, Chiang Mai, Thailand. Voucher specimens were deposited at Department of Applied Thai Traditional Medicine, School of Integrative Medicine, Mae Fah Luang University, Chiang Rai, Thailand. After removal of any foreign matters, all samples were dried in Solar Green House and grinded by using mechanical grinder into powders.

Pharmacognostic specifications

Morphological identification

Macroscopic identifications including size, shape and other visible characteristics were observed with naked eye or using a magnifying glass. Microscopic identifications were observed both transverse section and ground powders under a microscope to identify tissue and cell structures.

Physio-chemical determination

All pharmacognostic parameters were determined using quality control method for medicinal plant materials following WHO guideline [6]. Weighed 3 g of dried samples in crucibles, then dried at 105 °C until constant weight was obtained to determine loss on drying. After that, 3 g of dried samples was burnt at 500 °C for 6 h, placed crucibles in a desiccator for 30 min, then observed and weighted the carbonless ash to obtain total ash. Added 2 N of HCl, 25 ml into the remaining ash, gently boiled in water bath for 5 min and burnt it at 500° C for 6 h, then filtrated with ash less filter paper (No. 40) and then placed the crucibles in a desiccator for 30 min to obtain acid insoluble ash. Water content was determined using Azeotropic method, weighed 25 g of dried samples, then added 200 ml of water-saturated toluene; after the water completely separated from water-saturated toluene, measured and calculated the water volume. The volatile oil content was measured by using Clevenger distillation method, weighed 50 g of dried samples then added 200 ml of water; after volatile oil completely separated from water, measured and calculated volatile oil volume. Determination of extractive value, weighed 5 g of dried samples, then macerated with 70 ml of solvents (water or 95% ethanol); shaken with orbital shaker for 6 h, left on standing for 18 h then filtrated with filter paper rapidly. Pipetted adjusted volume to 100 ml, then dried it on a hot water bath at 105 °C until the constant weight was appeared. All samples were done in triplicate. The results were reported by grand mean $\pm$ pooled standard deviation. For TLC fingerprinting, the ethanol extract was determined using Silica gel 60 F₂₅₄ plate as stationary phase and a mixture of acetone: hexane (3:7) as mobile phase. It was developed in TLC chamber saturated with a solvent system. After the development of mobile phase, the plate was removed and allowed it to dry, then observed spot on the plate under ultraviolet light (254 and 365 nm) and daylight.

Results

Figure 1, 2, and 3 demonstrated morphological identification of *A. trifoliatius* leaves.

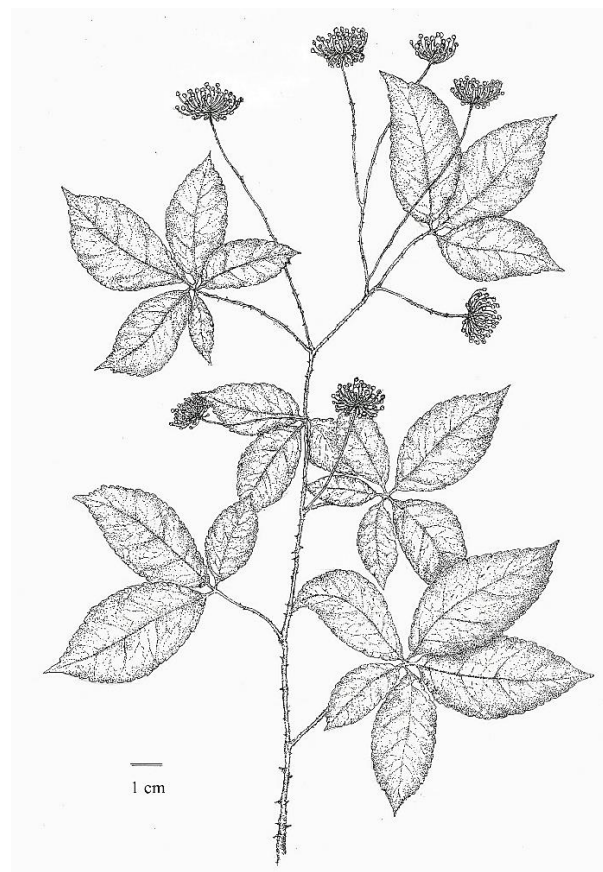


Figure 1 Macroscopic characteristic; whole plant of *A. trifoliatius*

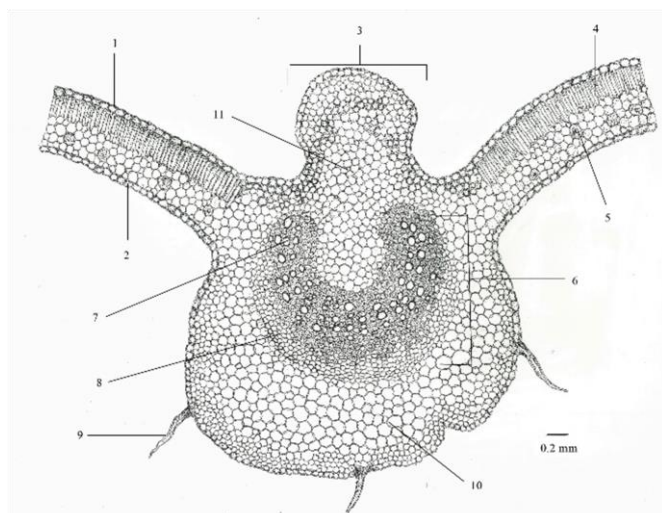


Figure 2 Microscopic identifications; anatomical characteristics (transverse section) of *A. trifoliatius* leaves;

1. upper epidermis;
2. lower epidermis;
3. midrib;
4. palisade parenchyma;
5. spongy parenchyma;
6. vascular bundle;
7. xylem vessels;
8. phloem;
9. covering trichome;
10. parenchymatous cells;
11. collenchyma

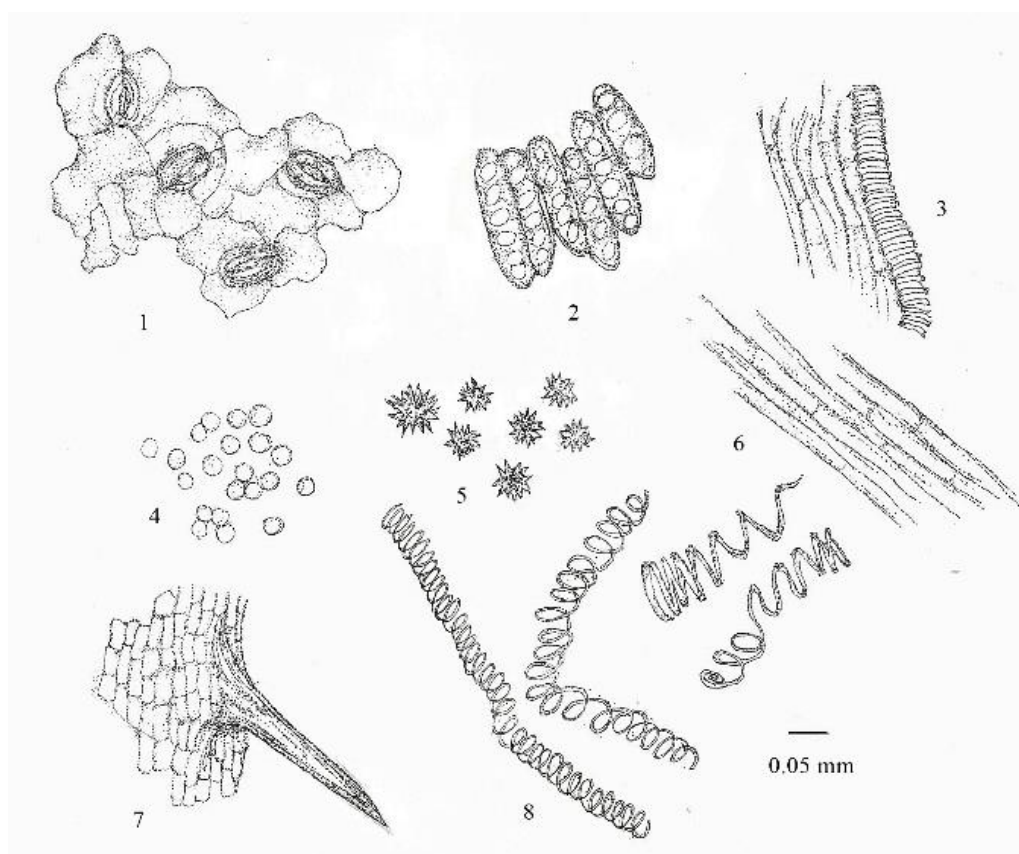


Figure 3 Microscopic identifications; histological characteristics of *A. trifoliatum* leaves powders;

1. anisocytic stomata; 2. fragment of xylem vessels; 3. part of fiber with reticulated vessel; 4. chlorophyll cells; 5. crystals of calcium oxalate (rosettes); 6. fragment of fiber; 7. covering trichome; and 8. spiral vessels

Discussion

Macroscopic and microscopic examinations are the first step to identify and purify the plant materials [6]. The macro- and microscopic examinations of *A. trifoliatum* were shown in figure 1-3. Leaves are green color. Leaf shape is palmately compound with 3-5 leaflets, 2-5 cm wide and 4-10 cm long, papery surfaces with slightly setose, base cuneate, margin serrulate, apex acute or acuminate. Anatomical characteristics of *A. trifoliatum* leaf were shown in figure 2. Specific histological characteristics were rosette shaped of calcium oxalate crystals and anisocytic stomata as shown in figure 3. They could be used as an instrument for the identification in crude drugs. Rosette crystals of calcium oxalate located in the lower epidermis region. Anisocytic stomata located in the leaf surface, usually bounded by three or four subsidiary cells, one of which was noticeably smaller than the others. [7,8].

Pharmacognostic parameters of *A. trifoliatum* leaves were shown in figure 4 and table 1. The water in plant materials can increase microbial growth. The azeotropic method for water content gives a direct quantity of the water present in plant materials. Loss on drying determines both amount of water and volatile [6].

Water content *A. trifoliatum* leaves should not more than 11.42% (w/w). Loss on drying should not more than 1.28 % (w/w). *A. trifoliatum* leaves contained traces of volatile oils. Ash content investigates reveals the degree of foreign inorganic matter for controlling quality of crude drug; the total ash includes amount of both physiological ash, and non-physiological ash (e.g. sand and soil) stick to plant surfaces. Acid-insoluble ash includes amount of silica present [6]. Total ash of *A. trifoliatum* leaves should not more than 13.43% (w/w); acid insoluble ash should not more than 2.36% (w/w). Extractive values revealed both quality and purity of crude drugs [9]. Ethanol soluble extractive of *A. trifoliatum* leaves should not less than 2.01% (w/w); water soluble extractive should not less than 5.69 % (w/w). Thin layer chromatography fingerprinting was presented in figure 4. The R_f value was showed on chromatogram. It could be used as marker characteristics fingerprint profiles of *A. trifoliatum* leaves.

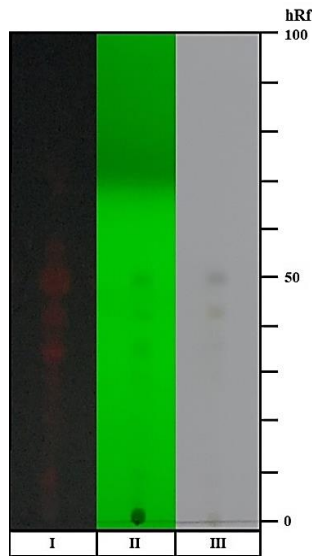


Figure 4 Pharmacognostic specifications *A. trifoliatum* leaves. I, detection under UV light 365 nm; II, detection under UV light 254 nm; and III, detection under daylight

Table 1 Quality parameters

Quality parameters	Grand mean ± pooled SD*	Min-Max*
Loss on drying	1.08 ± 0.17	0.79 - 1.28
Total ash	10.52 ± 1.33	8.57 - 13.43
Acid insoluble ash	1.67 ± 0.30	1.28 - 2.36
Water content	9.87 ± 1.32	7.70 - 11.42
Ethanol soluble extractive	6.76 ± 4.41	2.01 - 15.72
Water soluble extractive	6.67 ± 0.57	5.69 - 7.61
Volatile oil	Trace	Trace

*All samples were done in triplicate

Conclusion

The pharmacognostic specification of *A. trifoliatum* leaves in Thailand was established. This study provides useful information not only for authentication but also quality control and identification both its leaves and specimens.

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