

PHARMACOGNOSTIC STUDY OF *HYDNOCARPUS ANTHELMINTICUS* SEEDS ENDEMIC TO THAILAND

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ABSTRACT: The pharmacognostic specifications of *Hydnocarpus anthelminticus* Pierre ex Laness. (Flacourtiaceae) seeds were performed on 15 samples from 6 different geographical regions throughout Thailand. The evaluations were performed according to the World Health Organization (WHO) guidelines for herbal standardization. Microscopic investigation revealed numbers of aleurone grains, sclereid of seed coat and oil droplets. The mean contents of foreign matter, total ash, acid insoluble ash, moisture, ethanol-soluble extractive, water-soluble extractive and loss on drying were 0.04, 1.61, 0.29, 8.83, 23.93, 3.95 and 7.38 %, respectively. TLC fingerprints were done by using the suitable mobile phase systems which were chloroform : methanol : acetic acid : water (50 : 30 : 8 : 3) or heptane : diethyl ether : acetic acid (70 : 30 : 2). This study is the first reported on pharmacognostic study of *H. anthelminticus* Pierre ex Laness. seeds cultivated in Thailand.

Keywords: Pharmacognostic specification, *Hydnocarpus anthelminticus*

INTRODUCTION

Hydnocarpus anthelminthicus Pierre ex Laness belonged to family Flacourtiaceae is widely cultivated in Southeast Asia, mainly in China, Taiwan, Indonesia, Malaysia, and Thailand [1, 2]. It has been commonly known as Chaulmoogra, Tua-hong-chi (Chinese), or Kra-bao (Thai). Chaulmoogra oil or hydnocarpus oil isolated from seeds has been widely used in Asia such as China, Burma, and India for leprosy treatment [3]. The most active components in this oil were chaulmoogric acid [4] (C₁₈H₃₂O₂) and hydnocarpic acid [5] (C₁₆H₂₈O₂) which have been reported to against *Mycobacterium leprae* [6]. Nowadays, chaulmoogra oil has been added in cosmetics in order to be skin's moisture, treat of acne, and heal the wound. This study aimed to evaluate pharmacognostic specification of *H. anthelminthicus* Pierre ex Laness dried seed in Thailand for standardization of medicinal crude drug which has been used as raw material in various health supplement products.

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MATERIALS

Fifteen samples of *Hydnocarpus anthelminthicus* Pierre ex Laness ripened fruits were collected from 6 regions around Thailand, which were Bangkok, Buriram, Chiang Mai, Chumporn, Lampang, Nakorn Pathom, Nakorn Panom, Nakorn Sawan, Nakhon Sri Thammarat, Nong Khai, Ratchaburi, Rayong, Surat Thani, Ubon Ratchathani, and Uthai Thani provinces. All these samples were further authenticated by comparison with the herbarium specimen (SN 1422 and SN 1105) at the Princess Sirindhorn Plant Herbarium Bangkok, Thailand. Freshen seeds were kept in -20°C prior to use in the experiments. The powdered drug was performed by drying these seeds in an oven (60°C), grinding and then sifting through a 250 micron sieve. All these dried samples were kept in air-tight plastic containers at room temperature (30°C) for further analysis.

METHODS

These seeds from each region were analyzed individually in triplicate. The pharmacognostic evaluation of *H. anthelminthicus* Pierre ex Laness seeds was performed by method described in

Bunrathep S, Jongrungruangchok S, Vipunngun N, Songsak T, Ruangrungrasi N, Palanuvej C. Pharmacognostic study of *Hydnocarpus anthelminthicus* seeds endemic to Thailand. J Health Res. 2014; 28(1): 65-9.

World Health Organization (WHO) guideline standard methods [7, 8].

Macroscopic and microscopic examination

Each fresh fruit was macroscopically examined by visual inspection of physical properties such as texture, size, and color, along with other organoleptic tests. For microscopic examination, the fresh samples and powdered drug were considered for cell and tissue characteristics under microscope equipped with micrometer.

Foreign matter determination

Sample (50 g) will be spread in a thin layer, and the piece of foreign matter will be sorted out by visual inspection. The powdered foreign matter will be sifted through a 250 micron sieve. All portion of foreign matter will be pooled and weighed.

Total ash and acid-insoluble ash content determination

The ground sample (3 g, accurate weighed) will be placed in a previously ignited and tarred crucible. The sample will be spread in an oven layer and ignited by gradually increasing the heat to 500-600 °C until white ash was obtained. The ash will be then cooled in a desiccator and weighed without delay. The crucible containing total ash will be added 2M (70 g/l) HCl (25 ml). The crucible will be then added with watch-glass, and the mixture will be boiled gently for 5 minutes. The watch-glass will be rinsed with hot water (5 ml), and this liquid will be added into the crucible. The insoluble matter will be collected on ashless filter paper and washed with hot water until the filtrate become neutral. The filter paper containing the insoluble matter will be transferred to the original crucible, dried on hot plate, and ignited to constant weight. The residue will be allowed to cool in a desiccator, and weighed without delay.

Ethanol-soluble extractive determination

The ground sample (5g, accurate weighed) will be macerated with absolute ethanol (100 ml) in a closed conical flask in shaking bath for 6 hours and allowed to stand for 18 hours. The extracted will be filtered rapidly to avoid loss of ethanol. The filtrate (20 ml) will be evaporated to dryness in a tarred small beaker and then dried with heat to constant weight.

Water-soluble extractive determination

The ground sample (5g, accurate weighed) will be macerated with distilled (100 ml) in a closed conical flask in shaking bath for 6 hours and allowed to stand for 18 hours. The extracted will

be filtered rapidly to avoid loss of ethanol. The filtrate (20 ml) will be evaporated to dryness in a tarred small beaker and then dried with heat to constant weight.

Loss on drying determination

The ground sample will be accurately weighed (5 g) in a small beaker and then dried at 105 °C to constant weight.

Moisture content determination

The ground sample (50 g) in water-saturated toluene (200 ml) will be subjected to azeotropic method distillation. As soon as the water will be completely distilled, the inside of the condenser will be rinsed with toluene, and the distillation will be continued for 5 minutes. The heat will be then removed, and the receiving tube will be allowed to cool to room temperature. The water and toluene layers will be allowed to separate, and then the volume of water will be read off.

Volatile oil content determination

Volatile oil distillation will be performed on the sample (100 g) in water (600 ml) using a Clevenger apparatus. The heat will be removed, and the receiving tube will be allowed to cool to room temperature after the complete distillation. The volatile oil and water layers will be allowed to separate, and then the volume of volatile oil will be read off.

Thin-layer chromatographic identification

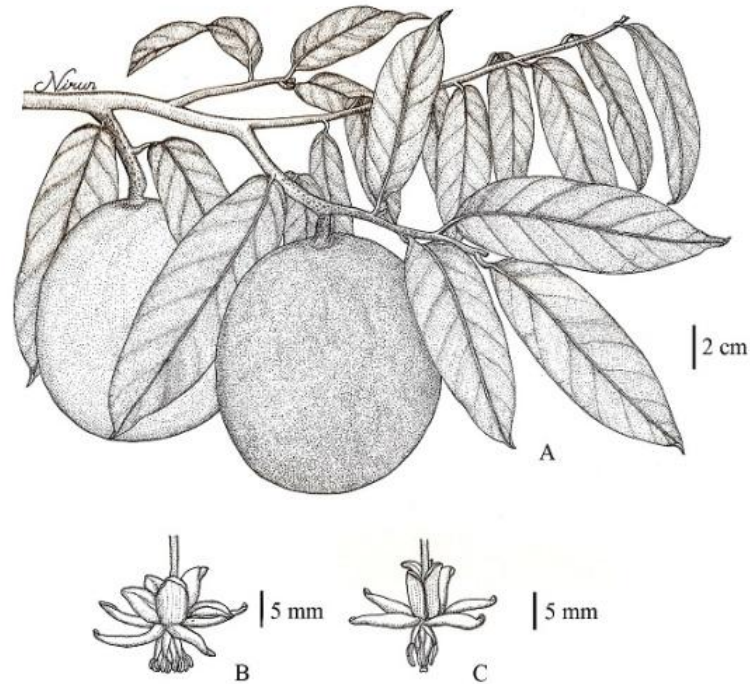
The ground sample (1g) was macerated with methanol or distilled water (20 ml) for 12 hours, then the extract was filtered and evaporated. The residue was dissolved in methanol (0.5 ml) and then applied on TLC plate (Silica gel GF₂₅₄ precoated plate). TLC plate was developed in suitable mobile phase, then allowed to dry in air, and examined under UV₂₅₄ and 365 nm, following by spraying with 10 % sulfuric acid in methanol, and heated in hot-air oven (120°C) for 10-15 minutes.

Statistical analysis

The data are presented as group mean \pm SD. The calculations were performed using Microsoft Excel data sheet.

RESULTS AND DISCUSSION

The macroscopic characteristic of *Hydnocarpus anthelminticus* Pierre ex Laness was shown in Figure 1. It is a dioecious, small to medium-sized tree, 10-20 (-30) m tall, outer bark lenticellate, grayish-black, inner bark brown; leaves ovate-lanceolate to ovate-oblong, 10-33 cm x 3-7 cm,



A. Fruiting branch of *Hydnocarpus anthelminthicus* Pierre ex Laness.
 B. Male flower C. Female flower

Figure 1 The macroscopic characteristic of *H. anthelminthicus* Pierre ex Laness

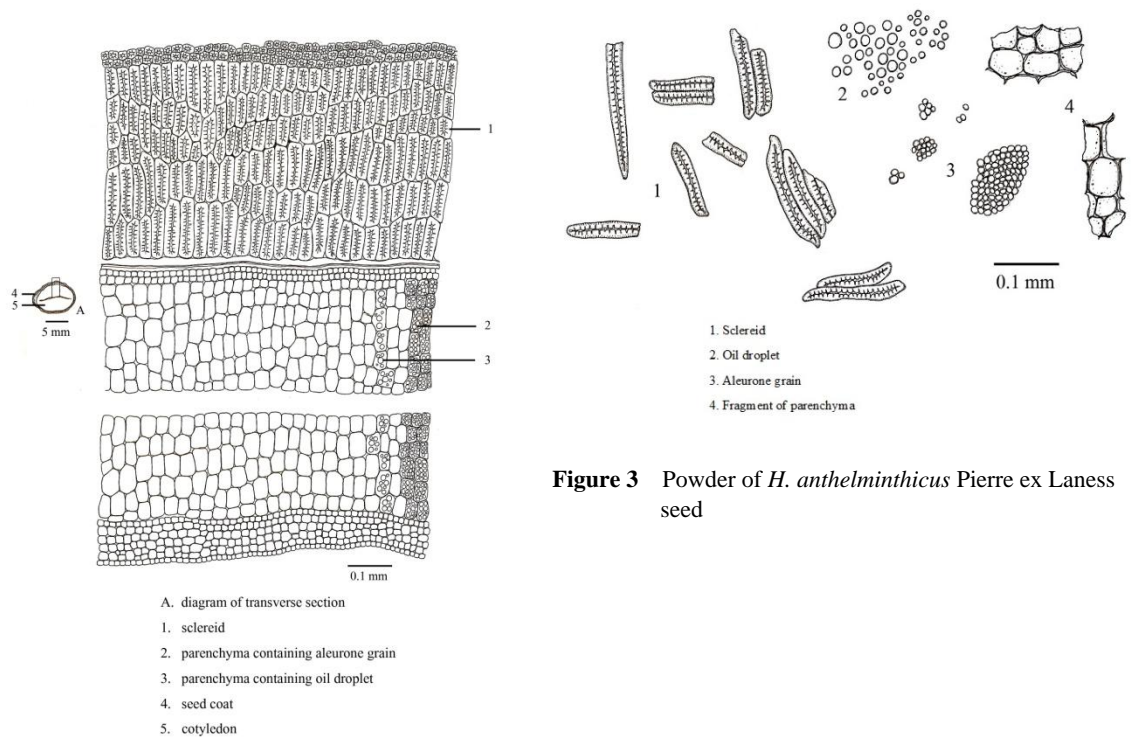


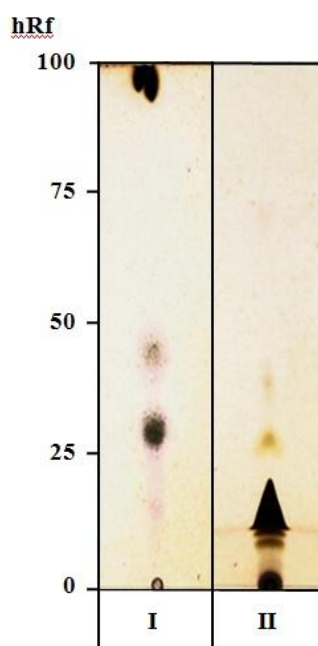
Figure 3 Powder of *H. anthelminthicus* Pierre ex Laness seed

Figure 2 Transverse section of *H. anthelminthicus* Pierre ex Laness seed

Table 1 The constant numbers due to the quality of *H. anthelminticus* Pierre ex Laness seeds

Specification	Mean \pm SD*	Range (Mean \pm 3SD)*
Foreign matter	0.04 \pm 0.04	0 - 0.16
Acid-insoluble ash	0.29 \pm 0.03	0.21 - 0.36
Total ash	1.61 \pm 0.06	1.43 - 1.79
Ethanol-soluble extractive	24.09 \pm 0.60	22.2 - 25.7
Water-soluble extractive	3.95 \pm 0.30	3.07 - 4.82
Loss on drying	7.38 \pm 0.17	6.88 - 7.88
Volatile oil content	0	0
Water content	8.83 \pm 0.22	8.16 - 9.49

* % by weight

**Figure 4** TLC fingerprints of *H. anthelminticus* Pierre ex Laness seed ethanolic extract (I = chloroform : methanol : acetic acid : water (50 : 30 : 8 : 3), II = heptane : diethyl ether : acetic acid (70 : 30 : 2))

coriaceous, glabrous, base cuneate to rounded-obtuse, usually asymmetrical, apex gradually long attenuate; inflorescence a 2-3-branched cyme, few-flowered; flowers 5-merous, white or greenish, scented, female flowers with 5 fusiform staminodes; fruit globose, 8-12 cm in diameter, brownish tomentulose, exocarp 0.2 mm thick, mesocarp yellowish, very hard; seeds 30-50 (-100) per fruit, ovoid, compressed, 1.5-1.8 cm x 1-1.5 cm. By pharmacognostic studies, its seed contains characteristic parenchyma containing aleurone grain and oil droplet, sclereids, and seed coat (Figure 2-3).

The constant numbers due to the quality of *H. anthelminticus* Pierre ex Laness seeds were exhibited in Table 1. The contents of foreign matter, acid-insoluble ash, total ash, loss on drying, and water should be not more than 0.04, 0.29, 1.61,

7.38, and 8.83 %, respectively, whereas the content of ethanol and water extractive should be not less than 24.09 and 3.95 % respectively, meanwhile volatile oil content could not be detected. All these constant number have been reported for the first time in this research.

TLC fingerprints of *H. anthelminticus* Pierre ex Laness seed extracted had been done by using 2 mobile phase systems, which were chloroform : methanol : acetic acid : water (50 : 30 : 8 : 3), and heptane : diethyl ether : acetic acid (70 : 30 : 2) (Figure 4)

CONCLUSION

Chaulmoogra oil or hydnocarpus oil obtained from seeds of *Hydnocarpus anthelminticus* Pierre ex Laness. which belonged to family Flacourtiaceae, had been widely used for leprosy

treatment. This study aimed to perform the pharmacognostic specification of its seeds according to the World Health Organization (WHO) guidelines for herbal standardization. Each fruit of *H. anthelminticus* Pierre ex Laness. composed of 30-50 (up to 100) seeds (1.5-1.8 cm X 1-1.5 cm). The microscopic characteristic of *H. anthelminticus* Pierre ex Laness. seed exhibited number of areurone grains, sclereids of seed coat, and oil droplets. The average constants due to the physical properties of *H. anthelminticus* Pierre ex Laness. which were foreign matter, total ash, acid-insoluble ash, moisture, ethanol extractive, water extractive, and loss on drying were 0.04 ± 0.04 , 1.61 ± 0.06 , 0.29 ± 0.03 , 8.83 ± 0.22 , 23.93 ± 0.06 , 3.95 ± 0.29 and 7.38 ± 0.17 , respectively, whereas volatile oil content could not be detected. TLC fingerprints also reported by using chloroform : methanol : acetic acid : water (50 : 30 : 8 : 3) or heptane : diethyl ether : acetic acid (70 : 30 : 2) as the mobile phase. This is the first reported on phamacognostic study of *H. anthelminticus* Pierre ex Laness. seeds which further applied for the herbal standardization.

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