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Nutrient and Mineral Content of Six Different Samples of *Hydnocarpus anthelminthicus* Cultivated in Thailand

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Abstract

Hydnocarpus anthelminthicus Pierre ex Laness, a medicinal plant in Flacourtiaceae (Salicaceae) family, is used traditionally as ingredient in Thai herbal medicine for treatment of leprosy and other types of dermatitis and tuberculosis. The objective of this study was to determine the proximate composition and mineral constituents in *H. anthelminthicus* pulps from 6 different agro-climatic regions distributed in Thailand. These pulps were found to contain 1.64-7.24 % of protein; 17.96-26.15 % of fat; 7.99-12.75 % of fiber; 16.90-32.91 % of moisture. The potassium, calcium, iron, sodium, magnesium, and chromium contents of *H. anthelminthicus* pulp in100 g of dry weight were found in the range of 423.95-721.67, 34.72-122.47, 0.91-5.23, 0.82-3.30, 47.79-86.58, and 0.19-1.67 mg, respectively. The contents of water-soluble vitamins: vitamin B1, vitamin B2 and vitamin B12 in *H. anthelminthicus* pulp were 0, 0.123-7.491 and 0-0.275 µg/g of dry weight, respectively. For fat-soluble vitamins: the contents of vitamin A, vitamin D and vitamin E in *H. anthelminthicus* pulp were 0-0.029, 1.049-3.580 and 14.617-58.334 µg/g of dry weight, respectively. The results of present analysis revealed that *H. anthelminthicus* pulps indigenous to different agro-climatic regions of Thailand contained an appreciable amount of nutrients and might be used as a good supplement for some nutrients such as minerals.

Keyword: *Hydnocarpus anthelminthicus,* Krabao, Chaulmoogra, Proximate analysis, Mineral analysis, Vitamin analysis

INTRODUCTION

Hydnocarpus (Flacourtiaceae), a genus of trees, is widely cultivated in Southeast Asia, mainly in China, Taiwan, Indonesia, Malaysia, and Thailand[1,2]. In some parts of the world *H. anthelminthicus* is referred to as the "Chaulmoogra" or "Tua-hong-chi" (Chinese) whereas in Thailand it is known as "Krabao"[3,4]. *H. anthelminthicus* is a deciduous evergreen tree, which grows up to 50 feet or more height. The seeds of this tree yield fatty oils, generally known as the chaulmoogra oil. *H. anthelminthicus* seeds constitute the major fatty acids are hydnocarpic, chaulmoogric and gorlic acids which have parasiticidal anti-leprosy and other types of dermatitis, and anti-tuberculosis[5,6]. The fruits are globose and contains 15-20 seeds. The fresh pericarp is orange-brown[7]. Although the pulps have been used as food for human and animals, there are no published reports for nutrition value of *H. anthelminthicus* pulps which cultivated in Thailand. Thus, the purpose of this study was to assess nutrition values such as protein, fat, water-soluble vitamin,

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fat-soluble vitamin, fiber, potassium, calcium, iron, sodium, magnesium, and chromium contents of *H. anthelminthicus* pulps which planted in Thailand.

MATERIALS AND METHODS

Hydnocarpus anthelminthicus pulps were collected in August – October 2010 from 6 provinces in Thailand, which were Khon kaen, Chanthaburi, Rayong, Mahasarakham, Phranakhonsiayutthaya, and Bangkok. All these samples were further authenticated by comparison with the herbarium specimens (SN 1422 and SN 1105) at the Princess Sirindhorn Plant Herbarium Bangkok, Thailand. The pulps were weighed, freeze-dried, weighed, grounded and kept in air-tight plastic containers at room temperature (30°C) for further analysis. The pulps from each region were assayed and analyzed individually in triplicate. Proximate analysis procedure including the percentage of moisture content, crude protein, crude fat, ash content and crude fiber in the sample (Table 1) were determined by the Association of Official Analytical Chemists methods (AOAC)[8]. Likewise, potassium, calcium, iron, sodium, magnesium, and chromium were determined by the use of atomic absorption spectrophotometer (AAS), Varian SpectrAA 220 (Table 2).

Proximate analysis:

Moisture content: An aluminium dish was placed in a drying oven at 105°C for 2 h and allowed to cool in a desiccator. The aluminium dish was weighed and about 2 g of the powder sample was accurately weighed and placed in the aluminium dish. The sample was dried in a drying oven (Memmert 600, Germany) at 105°C for 3 h. After that it was allowed to cool in a desiccator and then weighed to determine the percentage of dry weight and moisture content.

Ash: A crucible was heated at 150°C for 2 h in a muffle oven and allowed to cool in a desiccator. The crucible was weighed and about 2 g of the powder sample was accurately weighed and placed in the crucible. The crucible with sample was incinerated at low flame and then burnt at 550°C in a muffle oven (Furnace Nabertherm, Germany) for 8 h. After that it was allowed to cool in a desiccator and then weighed to determine the percentage of ash.

Fat: The fat content was determined by directly extracting the sample with petroleum ether in an intermittent Soxhlet extractor (Soxhlet Extractor Gerhadt, Germany) for 4 h. The residue in round bottom flask after solvent removal represents the fat content of the sample.

Crude protein: The crude protein content of the samples was estimated by macro-Kjeldahl method, in which the sample was digested with a known quantity of acid. The digested material was distilled after the addition of alkali. The ammonia released was collected in a 4% boric acid solution. The resultant boric acid solution which contained the ammonia released was titrated with 0.1 N HCl volumetric solution.

Crude fiber: Two grams of sample was put in a 250-mL conical flask, added with 200 mL of 1.25% sulfuric acid solution and heated for 30 min. After that it was filtered and washed with water until traces of acid could not be detected by pH paper. The undigested material was transferred into a 250-mL conical flask, added with 200 mL of 1.25% NaOH solution, and heated again for 30 min. The residue was filtered using vacuum filter and washed with water until traces of base was not detected. The residue was transferred into a weighed crucible, dried for 12 h at 120°C and weighed. After that the crucible was placed into a muffle oven at 550°C for 12 h and weighed. The fiber content was calculated using the difference between the dry residue weight and the ash residued weight.

Mineral Analysis:

The pulps were ashed at 550°C overnight and the ash was dissolved in

concentrated nitric acid and filtered, diluted to 50 mL with deionized water and the absorbance of the samples was read directly on the AAS.

Determination of minerals:

Working standard solutions of calcium (Ca), sodium, (Na), potassium (K), chromium (Cr) and iron (Fe) were prepared from stock standard solution (1000 ppm), in 2 N HNO_3 and absorbance was noted for standard solution of each element and samples using atomic absorption spectrophotometer (AAS).

Statistical analysis:

One way analysis of variance and Turkey's test was performed to determined differences in nutrient and mineral content of *H. anthelminthicus* pulps of six different samples. The significance level less than 0.05 was accepted for all comparisons SPSS statistics, version 16.0 for Windows was used for the statistical analysis.

Vitamins Analysis:

Pulp samples were cleaned and cut it into small pieces. The samples were weighed and placed in a freeze dry machine overnight. Thereafter they were weighed, powdered and stored at -20°C.

Determination of water soluble vitamins:

The samples were extracted using the method modified from that described by Eitenmiller, Ronald R[9]. In brief, fifty grams of samples were added with 50 mL of 0.1 N HCl and homogenized. The extract was filtered with a filter paper and kept in amber bottle. The filtrate was filtered with a 0.45-µm nylon syringe filter before injecting to HPLC. The HPLC was set at a wavelength of 270 nm and a flow rate of 1 mL/min. The ODS-2Hypersil HPLC C18 (4.6x250mm, 5 µm) column was used and the mobile phase was a mixture of 10 mM sodium 1-octanesulfonate, pH 3.4 with triethylamine and methanol (70:30).

Determination of fat soluble vitamins:

The method of Kienen V, *et al* [10], with slightly modification, was used for the extraction of samples. In short, five grams of samples were placed in an erlenmeyer flask. A 0.5 g of ascorbic acid and 30 mL of potassium-ethanolic solution were added. The mixture was put in a shaker for 3 h. After that it was filtered to remove solid residue. The filtrate was transferred to a separatory funnel and extracted three times with 25 mL of hexane. The organic phase was washed with 25 mL of reverse osmosis water for 2 times. The extract was evaporated using a rotary evaporator at 40°C. The residue was kept in amber bottles. On the day of analysis the residue was dissolved in 1 ml of methanol, filtered with a 0.45-µm nylon syringe filter and injected to HPLC. The HPLC was set at a wavelength of 295 nm, and a flow rate of 1 mL/min. The ODS-2Hypersil HPLC C18 (4.6 x 250mm, 5 µm) column was used and the mobile phase was a mixture of methanol and hexane (90:10).

RESULTS AND DISCUSSION

The average content of carbohydrate, fat, protein, and crude fiber in H. anthelminthicus were 34.77, 22.67, 3.78 and 9.70 %, respectively (Table 1); meanwhile the average content of mineral, which are calcium, sodium, potassium, magnesium, chromium and iron were 60.92, 1.48, 573.39, 65.81, 0.49, and 1.98 mg/100 gram, respectively (Table 2). The content of water-soluble vitamins: vitamin B1, vitamin B2 and vitamin B12 in H. anthelminthicus pulp were 0, 0.123-7.491 and 0-0.275 µg/g of dry weight, respectively (Table 3). For fat-soluble vitamins: the content of vitamin A, vitamin D and vitamin E in H. anthelminthicus pulp were 0-0.029, 1.049-3.580, 14.617-58.334 µg/g of dry weight, respectively. (Table 4).

CONCLUSION

This study demonstrated that pulps of *H. anthelminthicus* cultivated in

Table 1. Proximate analysis of *Hydnocarpus anthelminthicus* pulps of six different samples

Sample	Content (mg/100 g)					
	Moisture	**Ash**	**Crude fat**	**Crude Protein**	**Crude fiber**	**Carbohydrate**
Khonkaen	17.43±0.05^{d}	5.73±0.04^{c}	22.32±0.57^{c}	7.24±0.16^{a}	8.53±0.19^{d}	38.75±0.63^{b}
Chanthaburi	24.51±0.10^{bc}	5.43±0.05^{d}	26.15±0.23^{a}	4.07±0.24^{b}	9.15±0.26^{c}	30.69±0.37^{cd}
Rayong	32.91±0.14^{a}	6.30±0.06^{a}	19.09±0.16^{d}	2.27±0.12^{d}	9.47±0.09^{c}	29.96±0.39^{d}
Mahasarakham	24.75±0.04^{b}	4.55±0.11^{e}	25.83±0.25^{a}	3.37±0.10^{c}	12.75±0.25^{a}	28.75±0.22^{e}
Phranakhonsiayutthaya	24.38±0.08^{c}	5.49±0.09^{d}	24.70±0.35^{b}	4.09±0.18^{b}	10.35±0.22^{b}	30.99±0.14^{c}
Bangkok	16.90±0.11^{e}	6.02±0.03^{b}	17.96±0.23^{e}	1.64±0.26^{e}	7.99±0.15^{d}	49.49±0.15^{a}
mean±SD	23.48±5.87	5.59±0.60	22.67±3.5	3.78±1.95	9.70±1.69	34.77± 7.55

Values were presented in mean±SD (n=3) followed by different letters imply the significant different ($p<0.05$) between values in the same column.

Table 2. Mineral Composition of *H. anthelminthicus* pulps of six different samples

Sample	Content (mg/100g)					
	Potassium	**Calcium**	**Iron**	**Sodium**	**Magnesium**	**Chromium**
Khonkaen	518.90±1.34^{c}	45.95±0.11^{d}	1.86±0.03^{b}	1.57±0.03^{b}	85.75±1.11^{a}	0.19±0.04^{b}
Chanthaburi	680.21±1.15^{b}	47.40±0.34^{d}	0.91±0.10^{bc}	1.00±0.14^{c}	86.58±0.83^{a}	0.21±0.06^{b}
Rayong	423.95±2.03^{d}	62.98±1.03^{b}	1.01±0.11^{b}	0.82±0.05^{c}	72.18±1.03^{b}	0.19±0.03^{b}
Mahasarakham	440.62±2.32^{d}	51.99±1.16^{c}	1.20±0.13^{b}	1.00±0.18^{c}	47.79±0.55^{d}	0.29±0.05^{b}
Phranakhonsiay utthaya	675.05±1.09^{b}	122.47±1.39^{a}	5.23±0.69^{a}	3.30±0.25^{a}	54.76±0.46^{c}	0.37±0.03^{b}
Bangkok	721.67±3.04^{a}	34.72±1.04^{e}	1.77±0.25^{b}	1.20±0.16^{c}	47.79±0.52^{d}	1.67±0.25^{a}
mean±SD	573.39±123.63	60.92±29.61	1.98±1.63	1.48±0.88	65.81±17.03	0.49±0.56

Values were presented in mean±SD (n=3) followed by different letters imply the significant different ($p<0.05$) between values in the same column.

Table 3. Water soluble analysis of *Hydnocarpus anthelminthicus* pulps of four different samples

Sample	Content (µg/g of dried weight pulp)		
	Vitamin B1	**Vitamin B2**	**Vitamin B12**
Bangkok	ND	0.123±0.082	0.061±0.002
Phranakhonsiayutthaya	ND	5.769±0.296	ND
Amnatcharoen	ND	4.047±0.044	0.275±0.029
Khonkaen	ND	7.491±0.088	0.241±0.003

ND = Not detected

Table 4. Fat soluble analysis of *Hydnocarpus anthelminthicus* pulps of four different samples

Sample	Content (µg/g of dried weight pulp)		
	Vitamin A	**Vitamin D**	**Vitamin E**
Bangkok	ND	1.482±0.001	51.12±0.032
Phranakhonsiayutthaya	ND	1.049±0.029	14.617±0.330
Amnatcharoen	ND	3.580±0.007	30.215±0.072
Khonkaen	0.029±0.001	2.662±0.008	58.334±0.133

ND = Not detected

Thailand are a good source of minerals. The high concentration of calcium, potassium and magnesium make it a potential pulp source food that is suitable for fortification of foods. These plant organs might be explored as a viable supplement and a ready source of dietary minerals in human food.

The results of present analysis revealed that *H. anthelminthicus* pulps indigenous to different agro-climatic regions of Thailand contained an appreciable amount of nutrients and might be used as a good supplement for some nutrients such as fiber and minerals.

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