

Evaluation of proximate composition and biological activities of sompoi (*Acacia concinna*) leaves in Thailand

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ABSTRACT

Acacia concinna leaves are beneficial as medicinal for treating many symptoms and vegetable plants in Thai cuisine. The objective of the study was to analyze proximate compositions and micronutrients value, as well as, to evaluate biological activities including, antioxidant and anticancer activities of *A. concinna* leaves in Thailand. Proximate composition and micronutrients value were determined relative to the Association of Official Analytical Chemists standard methodology. Antioxidant capacity was examined following oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assay. Anticancer activity was determined following resazurin microplate assay. Proximate composition of *A. concinna* leaves including crude protein and dietary fiber constituted 6.73 ± 0.03 g and 9.15 ± 0.03 g/100 g sample. Energy constituted 113.00 ± 3.61 kcal/100 g sample. Its leaves also had the highest micronutrients value on calcium, with constituted 321.38 ± 4.29 mg/100 g sample. *A. concinna* leaves hold antioxidant capacity with ORAC and FRAP values of 20407.55 ± 74.23 μ moles and 1016.38 ± 29.29 μ moles TE/100 g sample, respectively. Its ethanolic extract holds slightly anticancer effect against tested cancerous cell lines. *A. concinna* can be a healthy alternative source of nutrients with good antioxidant capacity and slightly anticancer properties.

Key words: *Acacia concinna*, anticancer, antioxidant, micronutrients, proximate, soap pod

INTRODUCTION

Acacia concinna (Soap pod), known as "Sompoi," belongs to the family Fabaceae, and widely grows throughout Southern Asia.^[1,2] In Thailand, sompoi is medicinal and vegetable

plants. Its pods are used in festivals for paying respect to seniors.^[2] Sompoi contains a lot of powerful chemical constituents, most of its parts contain several saponins, especially pods, barks, and leaves. However, the major chemical constituent in sompoi leaves is tartaric acid. It also contains oxalic, citric, succinic, ascorbic acid, and other constituents.^[1] These chemical constituents are considered medicinal properties to treat symptoms including malarial fever, flatulence, jaundice, and mild laxative.^[1,3]

Recently, the consumption of vegetables is increasing, because the human's body cannot synthesize some nutrients.

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Basic nutritional requirements are needed to maintain body functions.^[4] Macronutrients including carbohydrate, fat, and protein, are the energy-yielding nutrients which require large amounts per day. Micronutrients, including retinol (Vitamin A), thiamine (Vitamin B1), riboflavin (Vitamin B2), ascorbic acid (Vitamin C), calcium, sodium, and iron are not energy-yielding nutrients. They require small amounts per day. Micronutrients promote the release of energy and regulate body functions.^[5,6] As a leafy vegetable, sompoi leaves were used in Thai cuisine for a long time, consumed as a part of the main dish for mild sour taste; however, there is little information on their nutritional compositions.

Natural antioxidants are highly concentrated in fruits and vegetables. Their antioxidant substances, including polyphenols and other compounds, have antioxidant properties, which delay or prevent oxidation of oxidizable substrates.^[7,8] Consequently, natural antioxidants help to prevent oxidative stress-related diseases.^[7] This study used oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assay to evaluate the radical chain-breaking antioxidant activity^[9] and to evaluate the reducing power of antioxidants^[8] in sompoi leaves.

Cancer is the world's biggest problem. Cancerous cells can affect any part of the body.^[10] It is important to find medicinal plants with the anticancer property. Resazurin microplate (REMA) assay has been widely used to measure or detect surviving various

mammalian cell lines, both normal and cancerous cell lines.^[11] This study used REMA assay to evaluate the anticancer property, which had cytotoxicity effect against cancerous cell lines.

Our purpose was to investigate proximate compositions and micronutrients value, as well as, to analyze the biological activities including antioxidant and anticancer activities of *A. concinna* leaves in Thailand.

MATERIALS AND METHODS

Sample collection

A. concinna was collected from Chiang Rai Province, Thailand, in June 2020, then authenticated by a botanist at Queen Sirikit Botanic Garden (QSBG), Chiang Mai Province, Thailand. Voucher specimen was deposited at QSBG, with voucher specimen number QBG136623, as shown in Figure 1. The plant drawing of *Acacia concinna* was shown in Figure 2.^[12]

Sample extraction

The leaves of *A. concinna* were dried in a hot air oven at 50°C. Dried leaves were finely ground to powdered, then exhaustively extracted in a Soxhlet extractor with ethanol solvent. The solvent was filtered, then evaporated in a rotary evaporator.

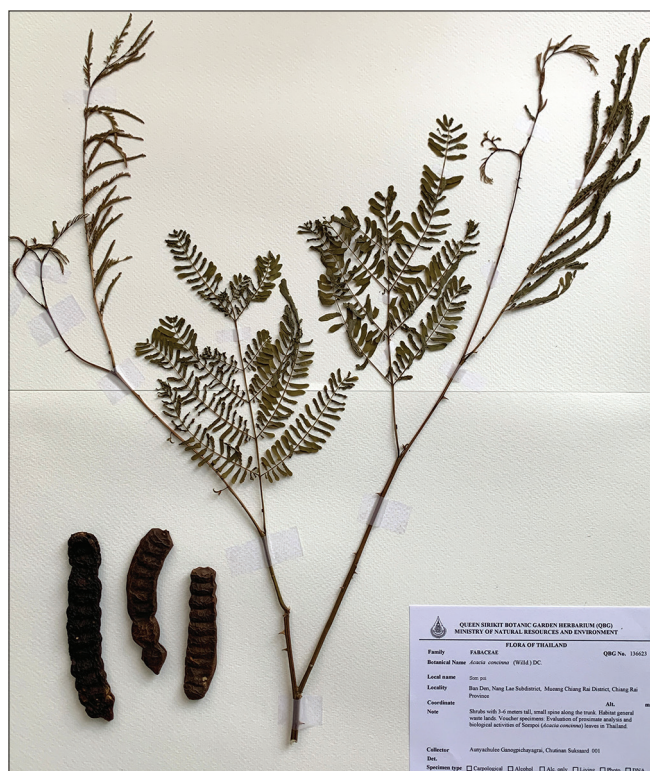


Figure 1: Herbarium Specimen of *Acacia concinna*

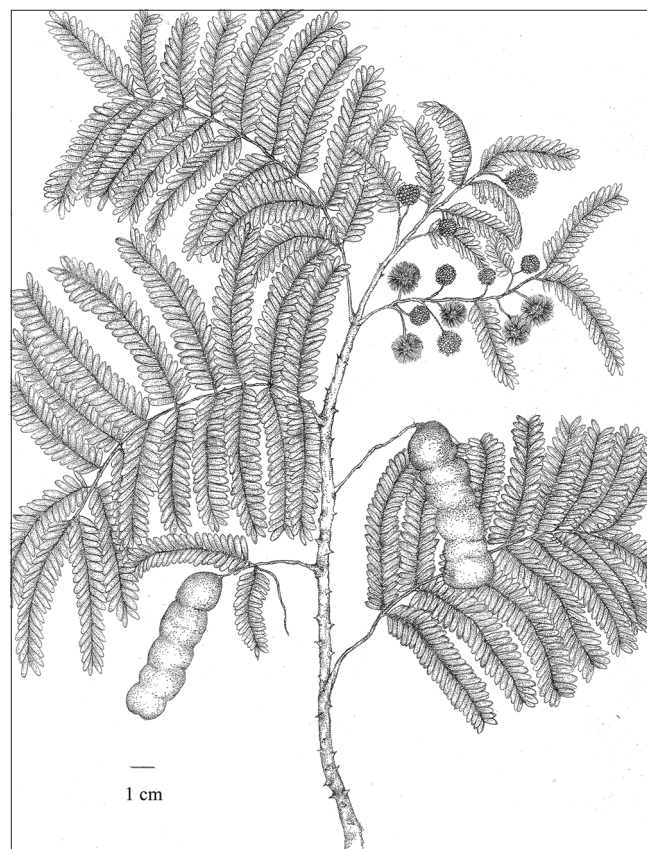


Figure 2: *Acacia concinna*^[12]

Proximate analysis and micronutrients value

Determination of proximate analysis

Proximate analysis was determined according to the Association of Official Analytical Chemists (AOAC) standard method.^[13] Crude protein was examined following AOAC 992.23, Kjeldahl method,^[13] then calculated as follows:

Crude protein = %Total nitrogen x Conversion factor of 6.25

Dietary fiber was determined following AOAC 985.29, enzymatic-gravimetric method,^[13] then calculated as follows:

Dietary fiber

$$= \frac{\left(\begin{array}{l} \text{Sample residue} - \text{Protein residue}^* \\ - \text{Ash residue}^{**} - \text{blank} \end{array} \right) \times 100}{\text{Weight of sample}}$$

*Protein residue was examined following the Kjeldahl method

** Ash residue was examined by incinerated at 525°C for 5 h, then weighted.

The energy was determined by calculated as follows:^[13]

Crude protein = (%Total fat x 9)+ (% Protein x 4)+ (% Carbohydrate x 4)

Determination of micronutrients value

Micronutrient value was evaluated. Retinol was determined following Speek *et al.*^[14] using high-performance liquid chromatography (HPLC) with detection wavelength at 445 nm, then calculated as follows:^[14]

Retinol equivalents

$$= \frac{1}{6}(\text{Betacarotene}) + \frac{1}{12}(\text{remaining carotenoids})$$

Thiamine and riboflavin were evaluated following AOAC 942.23 and 970.65 guidelines,^[13] using HPLC with detection wavelength at 445 nm. Ascorbic acid was evaluated following Odriozola-Serrano *et al.*^[15] using HPLC with detection wavelength at 245 nm. Calcium and sodium were evaluated following AOAC 985.35 guideline,^[13] using an atomic absorption spectrophotometer (ASS), with detection wavelength at 425 and 590 nm. Iron was evaluated following AOAC 984.27 guideline,^[13] using ASS, with detection wavelength at 250 nm.^[13]

Antioxidant activities

Determination of antioxidant capacity

The antioxidant capacity was determined following the ORAC assay, as mentioned by Ou *et al.*^[16] 0.5 g of *A. concinna* was added to 20 ml acetone/water (50:50) solvent, then shaken

with 400 rpm at 25°C for 1 h, followed by centrifuged at 10000 rpm, for 30 min. The radical chain-breaking antioxidant activity was determined by liquid chromatography–mass spectrometry, using fluorescein which contained hydroxyl group (3', 6'-dihydroxyspiro (isobenzofuran-1[3H],9'-[9H] xanthen]-3-one), with a fluorescein detection at 493 and 515 nm, respectively.

For FRAP assay, the sample was determined as mentioned by Benzie and Strain.^[8] The mixture of 25 ml of acetate buffer (300 mmol/l; pH 3.6), 2.5 ml of TPTZ (10 mmol/l) in HCl (40 mmol/l), and 2.5 ml of FeCl₃•6H₂O (20 mmol/l) were prepared. Added 100 ppm of *A. concinna* in ethanol. The sample was shaken at 25°C. The ferric ions reduction (Fe³⁺ + TPTZ) to ferrous ions (Fe²⁺) was examined by detected ferrous form (Fe²⁺), changed to a blue color using HPLC with an absorbance at 593 nm.

Anticancer activity

Cell cultures

Human hepatocarcinoma, breast cancer, lung cancer, and colon adenocarcinoma were obtained from the National Center for Genetic Engineering and Biotechnology, Pathum Thani province, Thailand.

Determination of anticancer activity

Leaves samples ethanolic extract was determined following REMA assay, as mentioned by O'Brien *et al.*^[11] Cancerous cell lines were diluted to a density of 7.5 × 10⁴ cells/ml. Either 5 µl of *A. concinna* extract or dimethyl sulfoxide or ellipticine was added, then incubated at 37°C for 72 h, with a 5% CO₂. A volume of 12.5 µl of resazurin (62.5 µg/ml) was added, then incubated at 37°C for 4 h. Cancerous cell proliferation was determined using a microplate reader, with an absorbance at 530 nm and 590 nm, respectively.

Statistical analysis

All of the determinations were done in triplicates. The results were presented as mean ± standard deviation.

RESULTS

Proximate analysis

In this study, proximate analysis, including crude protein and dietary fiber instituted 6.73 ± 0.03 g, and 9.15 ± 0.03 g/100 g samples. Energy constituted 113.00 ± 3.61 kcal/100 g sample [Table 1].

Micronutrients value including retinol (Vitamin A), thiamine (Vitamin B1), riboflavin (Vitamin B2), and ascorbic acid (Vitamin C) constituted 615.44 ± 1.46 µg, 0.05 ± 0.01 mg, 0.20 ± 0.01 mg, and 18.96 ± 0.54 mg/100 g sample, respectively. Mineral content including calcium, sodium, and iron was instituted at 321.38 ± 4.29 mg, 16.87 ± 2.13 mg, and 4.10 ± 0.04 mg/100 g sample, respectively [Table 2].

Table 1: The proximate analysis of *Acacia concinna* leaves

Proximate analysis	Contents (per 100 g sample)
Crude protein (g)	6.73±0.03
Dietary fiber (g)	9.15±0.03
Energy (kcal)	113.00±3.61

Table 2: Micronutrients value of *Acacia concinna* leaves

Micronutrients value	Contents (per 100 g sample)
Retinol as beta-carotene (µg)	615.44±1.46
Thiamine (Vitamin B1) (mg)	0.05±0.01
Riboflavin (Vitamin B2) (mg)	0.20±0.01
Ascorbic acid (Vitamin C) (mg)	18.96±0.54
Calcium (mg)	321.38±4.29
Sodium (mg)	16.87±2.13
Iron (mg)	4.10±0.04

Table 3: Antioxidant activities of *Acacia concinna* leaves

Parameter	Antioxidant activities (per 100 g sample)
Oxygen radical absorbance capacity (µmol TE)	20407.55±74.23
Ferric reducing antioxidant power (µmol TE)	1016.38±29.29

TE: Trolox equivalent

Table 4: Anticancer activity of *Acacia concinna* leaves

Cancerous cell lines	Cytotoxicity (%)	Activity
Human hepatocarcinoma (Hep-G2)	11.05±0.22	Low cytotoxic
Breast cancer (MCF-7)	18.98±0.73	Low cytotoxic
Lung cancer (NCI-H187)	16.78±0.77	Low cytotoxic
Colon adenocarcinoma (Caco2)	23.98±0.13	Low cytotoxic

Antioxidant activities

ORAC value was instituted 20407.55 ± 74.23 µmoles TE/100 g sample, whereas FRAP value instituted 1016.38 ± 29.29 µmoles TE/100 g sample [Table 3].

Anticancer activity

Leaves samples ethanolic extract, at 50 µg/ml, showed low cytotoxic effects against human hepatocarcinoma, breast cancer, lung cancer, and colon adenocarcinoma, with percentage cytotoxicity ranging from 11.05 ± 0.22% to 23.98 ± 0.13%, with IC₅₀ values >50 µg/ml [Table 4].

DISCUSSION

Vegetables substantively provide carbohydrates and protein with little or no fat.^[5] For carbohydrates, vegetables contain

different kinds of polysaccharides, both starch and fibers. Starch can be digested and then hydrolyzed to glucose. Fibers cannot be digested. However, fibers are crucial in reducing the risks of some diseases, i.e., heart diseases and type 2 diabetes; or enhancing the body's health such as preventing constipation.^[5,6] Total dietary fiber of *A. concinna* leaves constituted 9.15 ± 0.03 g/100 g sample. Vegetables also contain proteins, which play an important role in repairing damaged cells and tissues, maintaining hormonal activities, energy metabolism, and acid–base balancing in the human's body.^[5,6] Crude protein of *A. concinna* leaves constituted a 6.73 ± 0.03 g sample. The energy of *A. concinna* leaves was also analyzed and instituted 113.00 ± 3.61 kcal/100 g sample.

For vitamin analyses of *A. concinna* leaves, the fat-soluble Vitamin A enhances vision, involves protein synthesis and cell differentiation constituted 615.44 ± 1.46 µg/100 g sample. The water-soluble vitamins, Vitamin B1 supports the membranes of nerve cells, Vitamin B2 acts as a coenzyme in energy metabolism, and Vitamin C is an antioxidant, these contents were found to be 0.05 ± 0.01 mg, 0.20 ± 0.01 mg, and 18.96 ± 0.54 mg/100 g sample, respectively. For minerals analyses of *A. concinna* leaves, the major minerals including calcium, is crucial in bone structure, and sodium, which encourages fluid regulation and muscle contraction were analyzed. Their contents were constituted 321.38 ± 4.29 mg and 16.87 ± 2.13 mg/100 g samples. The trace mineral and iron, which enhances the transport of oxygen to cells, constituted a 4.10 ± 0.04 mg/100 g sample.

Vegetables as natural antioxidants can delay or inhibit oxidative cell damage and prevent degenerative diseases, caused by free radicals.^[7,17,18] This study evaluated antioxidant capacity using two different based assays, consisting of hydrogen atom transfer (HAT) and single-electron transfer. For HAT, the radical chain-breaking antioxidant capacity was examined, using the ORAC assay.^[16] Decreasing fluorescence intensity reflected the index of the free radical degree.^[19] *A. concinna* extract showed good antioxidant capacity. The ORAC value constituted 20407.55 ± 74.23 µmoles TE/100 g sample. For SET, the capability to transfer electrons to access the reducing power, ferric to ferrous ions reduction, of *A. concinna* leaves ethanolic extract, were examined through FRAP assay.^[8] *A. concinna* extract showed antioxidant capacity. The FRAP value constituted 1016.38 ± 29.29 µmoles TE/100 g sample. Nonetheless, the antioxidant capacities of *A. concinna* were done in different parts of this plant, especially pods. The previous study showed that *A. concinna* pods had been extracted by various techniques, including ethanolic, freeze-dried hydroethanolic, and spray-dried hydroethanolic. Among three different extracts, the pods' spray-dried hydroethanolic showed the greatest antioxidant capacity. The extract also inhibited lipid peroxidation.^[19]

For anticancer properties, the cytotoxicity against four selected cancerous cell lines of *A. concinna* leaves ethanolic

extract was measured following the REMA assay.^[11] Living cells can reduce blue nonfluorescent to pink-fluorescent dye; hence the reduction amount of resazurin is directly proportional to activate cells.^[11] *A. concinna* extract at the concentration of 50 µg/ml showed low cytotoxic effects against human hepatocarcinoma, breast cancer, lung cancer, and colon adenocarcinoma cell lines, ranging from 11.05% ± 0.22% to 23.98% ± 0.13% cytotoxicity, with IC₅₀ values >50 µg/ml.

CONCLUSION

A leafy vegetable, *A. concinna*, can be healthy alternative source of nutrients, both macronutrient and micronutrient. Its leaves had the highest macronutrient value on dietary fiber, and micronutrients value on calcium. *A. concinna* leaves possessed good antioxidant capacity, with slightly cytotoxic effects against cancerous cell lines including human hepatocarcinoma, breast cancer, lung cancer, and colon adenocarcinoma.

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Conflicts of interest

There are no conflicts of interest.

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