ORIGINAL ARTICLE

Proximate composition, vitamin and mineral composition, antioxidant capacity, and anticancer activity of *Acanthopanax trifoliatus*

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ABSTRACT

Acanthopanax trifoliatus has been used as both traditional plant food and medicinal plant in Thailand. This study aimed to evaluate proximate, vitamin, and mineral compositions of *A. trifoliatus* leaf samples together with antioxidant and anticancer activities of ethanolic leaf extract of *A. trifoliatus*. For leaf samples, quantitative determination of proximate composition was evaluated comprising moisture, crude protein, total fat, total carbohydrate, dietary fiber, ash, as well as energy. Quantitative determination of vitamin and mineral composition including Vitamin A, Vitamin B1, Vitamin B2, Vitamin C, calcium, sodium, and iron was also assessed. For ethanolic leaf extract, antioxidant activity was investigated using oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) methods. Anticancer activity was determined against human ductal, bronchogenic, liver, gastric, and colon cancer cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. Proximate composition of *A. trifoliatus* was found to be 74.62 ± 0.38, 5.01 ± 0.05, 0.95 ± 0.04, 16.47 ± 0.40, 8.54 ± 0.06, and 2.95 ± 0.04 g/100 g sample, respectively, and energy was found to be 94.48 ± 1.30 kcal/100 g sample. Vitamin and mineral composition was found to be 428.47 ± 3.00 μg/100 g sample, 0.41 ± 0.01, 0.17 ± 0.00, 11.95 ± 0.86, 675.35 ± 46.57, 13.46 ± 0.95, and 4.79 ± 0.15 mg/100 g sample, respectively. *A. trifoliatus* ethanolic leaf extract revealed antioxidant capacity with ORAC value of 9057.29 ± 43.08 μmol TE/100 g and FRAP value of 1230.88 ± 19.51 μmol TE/100 g. Its extract also showed cytotoxic potential against all tested cancer cell lines. *A. trifoliatus* leaf is a good source of essential nutrients, which had antioxidant and anticancer potential.

Key words: Anticancer, antioxidant, mineral, nutrition, proximate, vitamin

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INTRODUCTION

Phak-pam (*Acanthopanax trifoliatus*), a medium-sized shrub that commonly distributes in Asia, belongs to the *Araliaceae* family. It has been used as both traditional plant food and medicinal plant.[1-6] As plant food, people of Thailand consume Phak-pam as a vegetable.

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As a medicinal plant, Phak-pam has been reported as ginseng-like plant.[5] It also possessed pharmacological effects, for instance, anti-inflammatory, antioxidant, and anticancer properties.[1-6] Plant food provides nutrients that help maintain the body functions. Nowadays, nutritional information is increasingly concern for many reasons, for example, to meet nutritional requirements or to prevent nutritional deficiency disease.[7,8] Nutrients can be divided into two groups based on the requirement in amounts per day: macronutrients include proteins, fats, and carbohydrates; in opposition, micronutrients include vitamins and minerals.[8,9]

Medicinal plant is also used to promote good health. Plant and its compounds have biological capability, such as antioxidant and anticancer.[10] Some bioactive compounds in the food plant has a natural antioxidant capacity, especially polyphenols, to scavenge free radicals.[3,10,11] Oxygen radical absorbance capacity (ORAC) measures the effectiveness of chain-breaking antioxidants, while ferric reducing antioxidant power (FRAP) measures the action of the electron donation from antioxidants (the reducing capacity of antioxidants).[10-14] Cancer is a disease differentiated by the uncontrolled growth and spread of abnormal cells.[15,16] 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay can be used for estimating the quantitative of antiproliferative and anticancer potential.[15] There is a necessity to find drug or medicinal plant with antioxidant and anticancer capability. This study covered evaluations of proximate, vitamin, and mineral compositions of leaf samples together with antioxidant capacity and anticancer activity of ethanolic leaf extract of *A. trifoliatus*.

MATERIALS AND METHODS

Materials and chemicals

A. trifoliatus leaf samples were collected in Thailand. They were authenticated at Queen Sirikit Botanic Garden, Thailand. Voucher specimens were deposited at School of Integrative Medicine, Mae Fah Luang University, Thailand. Leaf samples were washed and then dried at 50°C. The dried leaf samples were stored at room temperature. For antioxidant and anticancer activities, the dried leaf samples were pulverized and exhaustively extracted using Soxhlet apparatus. The extract was filtered, evaporated, and then stored at −20°C. All chemicals were analytical grade.

Proximate analysis

For quantitative determination of proximate composition of *A. trifoliatus* leaf samples, moisture was determined using hot air oven (Association of Official Analytical Chemists; AOAC 952.08, 2016),[17] dried leaf samples at 105°C, until constant weight. Crude protein was determined according to the Kjeldahl method (AOAC 992.23, 2016);[17] total nitrogen was multiplied by a protein factor of 6.25. Total fat was determined according to the acid hydrolysis method (AOAC 948.15, 2016),[17] using Soxhlet extractor at 60°C, until constant weight. Dietary fiber was determined according to the enzymatic gravimetric method (AOAC 985.29, 2016),[17] digested leaf samples with heat using alpha-amylase, protease, and amyloglucosidase at 60°C, respectively, then added ethanol to samples to precipitate fiber. Ash was determined according to gravimetric method (AOAC 930.30, 2016),[17] incinerated leaf samples at 550°C, until constant weight. Total carbohydrate was determined according to the difference method by calculation. Energy was determined by calculation based on the contents of carbohydrate, protein, and lipid multiplied by a factor of 4, 4, and 9 respectively, and then added the results together.

Mineral analysis

For quantitative determination of mineral composition of *A. trifoliatus* leaf samples, Vitamin A as alpha-carotene was determined using high-performance liquid chromatography (HPLC),[18] then measured absorbance at 445 nm, and calculated as follows:

Vitamin A = 1/6 (alpha-carotene) +1/12 (remaining carotenoids)

Vitamin C was determined using ultraviolet-HPLC methods[19] and then measured absorbance at 245 nm. Vitamin B1 and Vitamin B2 were determined by fluorometric method according to the AOAC 942.23 and 970.65, 2016,[17] respectively, and then measured emission at 365 nm and excitation at 435 nm. Calcium and sodium were determined by atomic absorption spectrophotometer (AAS) (AOAC 985.35, 2016),[17] dried leaf samples at 450°C then measured AAS absorbance at 425 and 590 nm, respectively. Iron was determined by AAS (AOAC 984.27, 2016)[17] and then measured absorbance at 250 nm.

Antioxidant analysis

For antioxidant capacity of *A. trifoliatus*, the ethanolic leaf extract was determined using ORAC, according to the method reported by Ou *et al*. (2001),[12] using fluorescein as the fluorescent probe. For FRAP, according to the method reported by Benzie and Strain (1996).[14]

Anticancer analysis

Cell cultures

The human cancer cell lines – ductal carcinoma (BT474, ATCC HTB-20), bronchogenic carcinoma (Chago K1, ATCC HTB-168TB), liver hepatoblastoma (Hep G2, ATCC HB-8065), gastric carcinoma (Kato III, ATCC HTB-103), and colon adenocarcinoma (SW 620, ATCC CCL-227) – were obtained from the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Thailand.

For anticancer activity of *A. trifoliatus* ethanolic leaf extract, cell viability was determined using MTT (Sigma-Aldrich,

USA) methods[15] with minor modifications. Each human cancer cell line in 198 µl of RPMI 1640 culture medium was incubated with 5% CO_2 atmosphere at 37°C for 24 h. Either 2 µl of *A. trifoliatus* leaf extract or dimethyl sulfoxide (DMSO) (as negative control) was added and then incubated at 37°C for 48 h. 10 µl of MTT solution (5 mg/ml) was added, then incubated at 37°C for 4 h, and removed all media. 150 µl of DMSO and 25 µl of glycine (0.1 mol/l) were added, mixed, and then measured absorbance at 540 nm.

Statistical analysis

For proximate composition, vitamin and mineral composition, and antioxidant capacity, the data were expressed as mean ± standard deviation (SD). All tests were carried out in triplicates. For anticancer activity, the data were expressed as mean ± SD. This test was carried out in quadruplicates.

RESULTS

Proximate analysis

Proximate composition of A.trifoliatus leaf samples including moisture, crude protein, total fat, total carbohydrate, dietary fiber, ash; as well as, energy were found to be 74.62±0.38, 5.01±0.05, 0.95±0.04, 16.47±0.40, 8.54±0.06, 2.95±0.04 g/100 g sample respectively, and energy was found to be 94.48±1.30 kcal/100 g sample.

Mineral analysis

Vitamin and mineral composition of *A.trifoliatus* leaf samples comprising vitamin A, vitamin B1, vitamin B2, vitamin C, calcium, sodium and iron were found to be 428.47±3.00 µg/100 g sample, 0.41±0.01, 0.17±0.00, 11.95±0.86, 675.35±46.57, 13.46±0.95 and 4.79±0.15 mg/100 g sample respectively.

Antioxidant analysis

A. trifoliatus ethanolic leaf extract showed antioxidant capacity with ORAC value of 9,057.29±43.08 µmoles TE/100 g and FRAP value of 1,230.88±19.51 µmoles TE/100 g.

Anticancer analysis

A. trifoliatus ethanolic leaf extract possessed cytotoxic effects against all tested human cancer cell lines against gastric and colon human cancer cell lines with the IC50 of 72.9 and 73.4 µg/ml, respectively; against ductal, bronchogenic and liver human cancer cell lines with the IC50 at ≥100 µg/ml.

DISCUSSION

Proximate analysis is used for estimation of the quantitative of food and food substance including moisture, crude protein, total fat, total carbohydrate, and dietary fiber.[7,20,21] Proximate composition of *A. trifoliatus* leaf samples is shown in Table 1. Moisture content is the amount of loss on drying of water and volatile substances.[7,20,21] Moisture is sometime used for estimation of the qualitative of food. However, the amount of moisture content is one of the main factors in storage, due to the proliferation of microorganisms, such as fungi and mold.[7,20] When fresh *A. trifoliatus* leaf samples dried until constant weight, moisture content was found to be 74.62 ± 0.38 g/100 g sample. Crude protein is the amount of total nitrogen multiplied by protein factors. Total nitrogen consisted of protein nitrogen and a few nonprotein nitrogens. Different types of food had different protein factors.[7,20,21] In this study, *A. trifoliatus* leaf samples used protein factor of 6.25 to convert nitrogen to protein; then, crude protein in the leaf samples was found to be 5.01 ± 0.05 g/100 g sample. Total fat, ether extract, is the amount of fat including fatty acid, oil-soluble dyes, fat-soluble vitamins, and steroids.[7,20,21] Total fat of *A. trifoliatus* leaf samples was found to be 0.95 ± 0.04 g/100 g sample. Total carbohydrate is the amount of carbohydrate, which is one of the main components of structural materials in plants.[7,20,21] In this study, we calculated that based on difference method, the total carbohydrate of *A. trifoliatus* leaf samples was found to be 16.47 ± 0.40 g/100 g sample. Dietary fiber is the amount of total dietary fiber.[7,20,21] Dietary fiber of *A. trifoliatus* leaf samples was found to be 8.54 ± 0.06 g/100 g sample. Ash content is the amount of total mineral residue left after incinerated leaf samples until constant weight.[7,20,21] Ash content of *A. trifoliatus* leaf samples was found to be 2.95 ± 0.04 g/100 g sample. Protein, lipid, and carbohydrate each contribute to the total energy composition. Energy of *A. trifoliatus* leaf samples was found to be 94.48 ± 1.30 kcal/100 g sample.

Table 1: Proximate composition of *Acanthopanax trifoliatus* leaf samples (per 100 g sample)

Proximate analyses	Compositions*
Energy (kcal)	94.48±1.30
Moisture (g)	74.62±0.38
Crude protein (g)	5.01±0.05
Total fat (g)	0.95±0.04
Total carbohydrate (g)	16.47±0.40
Dietary fiber (g)	8.54±0.06
Ash (g)	2.95 ± 0.04

Vitamin and mineral analyses are used for estimation of the quantitative of mineral in food. Vitamins are arranged into two groups based on solubility including water-soluble vitamins and fat-soluble vitamins.[8,9,22,23] In this study, we determined the content of Vitamin A, fat-soluble vitamins, calculated as retinal equivalent (1 µg of retinol = 6 µg of alpha-carotene or 12 µg of mixed carotenoids), according to the WHO.[18,24] The content was found to be 428.47 ± 3.00 µg/100 g sample. Vitamin B1, Vitamin B2 and Vitamin C, and water-soluble vitamins were determined. Their contents were found to be 0.41 ± 0.01, 0.17 ± 0.00, and 11.95 ± 0.86 mg/100 g sample, respectively. Minerals

Table 2: Vitamin and mineral compositions of *Acanthopanax trifoliatus* leaves (per 100 g sample)

Mineral analyses	Compositions*
Vitamin A as alpha-carotene (µg)	428.47±3.00
Vitamin B1 (mg)	0.41±0.01
Vitamin B2 (mg)	0.17±0.00
Vitamin C (mg)	11.95±0.86
Calcium (mg)	675.35±46.57
Sodium (mg)	13.46±0.95
Iron (mg)	4.79 ± 0.15

Table 3: Antioxidant capacity of ethanolic leaf extract of *Acanthopanax trifoliatus*

Parameter	Antioxidant capacity*
ORAC (µmol TE)	9057.29±43.08
FRAP (µmol TE)	1230.88 ± 19.51

ORAC: Oxygen radical absorbance capacity, FRAP: Ferric reducing antioxidant power, µmol TE: Micromoles Trolox Equivalents

Table 4: Anticancer analysis of ethanolic leaf extract of *Acanthopanax trifoliatus*

Cancer cell lines	IC_{50} (µg/ml)
Ductal carcinoma (µg/ml)	>100
Bronchogenic carcinoma (µg/ml)	>100
Liver hepatoblastoma (µg/ml)	>100
Gastric carcinoma (µg/ml)	72.9
Colon adenocarcinoma (µg/ml)	73.4

IC_{50}: Inhibitory concentration

perform an essential role as a catalyst of biochemical reaction in the plant. Minerals are grouped into two groups based on human diets in amounts. Macroelements or major minerals are required >100 mg, whereas microelements or trace minerals are required <100 mg.[8,9,22,23] In this study, we determined calcium and sodium, macroelements, and iron, a microelement. Calcium, sodium, and iron contents of *A. trifoliatus* leaf samples were found to be 675.35 ± 46.57, 13.46 ± 0.95, and 4.79 ± 0.15 mg/100 g sample, respectively. The vitamin and mineral compositions of *A. trifoliatus* leaf samples are shown in Table 2.

In this study, ethanolic leaf extract of *A. trifoliatus* had antioxidant capacity, which is shown in Table 3. ORAC quantified antioxidant capacity, measured fluorescence quenching, based on the production of peroxyl free radicals generated interacted with fluorescent probe.[11-13] *A. trifoliatus* leaves' extracts had an ORAC value of 9057.29 ± 43.08 µmol TE/100 g. FRAP quantified antioxidant power, measured directly reducing capacity of antioxidants (ferric to ferrous ion reduction).[11,14,25] Ethanolic leaf extract of *A. trifoliatus* had an FRAP value of 1230.88 ± 19.51 µmol TE/100 g.

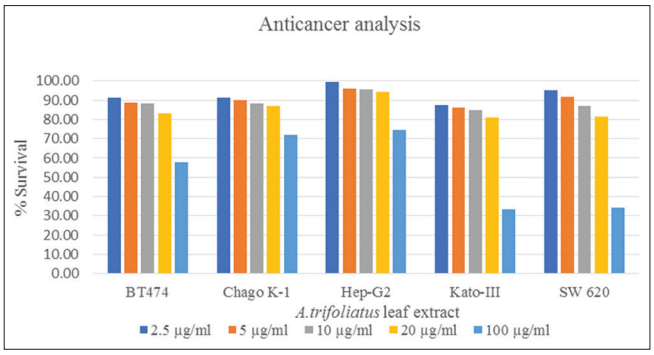

Figure 1: Inhibition of cancer cells growth by ethanolic *Acanthopanax trifoliatus* leaf extract

However, from previous study, *A. trifoliatus* leaf extracts had also been reported with strong antioxidant capacity. For DPPH assay, leaves extracts had antioxidant capacity with EC_{50} value of 100.81 ± 5.05 µg/ml (leaf maceration extract), 72.20 ± 9.61 µg/ml (leaf refluxing extract), and 14.50 ± 1.04 µg/ml (leaf decoction extract).[5] For FRAP assay, leaf extract had antioxidant capacity with 930 µmol Fe (II)/g extract.

From previous study, stem and leaf extracts from *A. trifoliatus* were found anticancer effects on human cancer cell lines, including prostate cancer (PC-3 cells), central nervous system tumor (SF-268), breast adenocarcinoma (MCF-7), hepatoma cell (Huh-7), liver hepatoblastoma (Hep-G2), lung cancer (A 549), nonsmall-cell lung carcinoma (NCL-H460) and fibrosarcoma cell (HT1080).[4,6,16] In this study, ethanolic leaf extract of *A. trifoliatus* also possessed cytotoxic activities against all tested cancer cell lines, especially gastric carcinoma (Kato-III) and colon adenocarcinoma (SW 620) with IC_{50} value of 72.9 and 73.4 µg/ml, respectively. However, its extract also had cytotoxic activities against ductal carcinoma (BT474), bronchogenic carcinoma (Chago K-1), and liver hepatoblastoma (Hep-G2) cell lines at high dose (IC_{50} >100 µg/ml). The anticancer analysis of ethanolic leaf extract of *A. trifoliatus* is shown in Table 4 and Figure 1.

CONCLUSION

A. trifoliatus leaf is a good source of essential nutrients including protein, carbohydrate, dietary fiber, and fat. It also contains health-promoting compounds including vitamins and minerals, especially calcium. Moreover, ethanolic *A. trifoliatus* leaf extract had antioxidant and anticancer potentials. For antioxidant capacity, ORAC value and FRAP value were found to be 9057.29 ± 43.08 and 1230.88 ± 19.51 µmol TE/100 g, respectively. For cytotoxic activity, its leaves' extract possessed cytotoxic activities against all tested cancer cell lines.

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Conflicts of interest

There are no conflicts of interest.

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