

Antioxidant Activity and Phenolic Content of *Acanthopanax trifoliatum* and *Toddalia asiatica*

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ABSTRACT

Decoction and 75% ethanolic extracts from the leaves of *Acanthopanax trifoliatum* (AT) and *Toddalia asiatica* (TA) were spectrophotometrically examined for antioxidant activity and phenolics content. Phytochemical characteristics of the decoction extracts were also investigated by thin layer chromatography (TLC).

The leaf decoction extract of AT significantly exhibited the highest *in vitro* antioxidant activity determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay and the thiobarbituric acid reactive substances (TBARS) method. TA decoction and 75% ethanolic extracts significantly contained the highest amount of total phenolics and total flavonoids content, respectively. Analysis of the decoction extracts of AT and TA by TLC showed specific fingerprints composed of chlorogenic acid and rutin.

Key words: *Acanthopanax trifoliatum*, *Toddalia asiatica*, antioxidant activity, phenolic content

INTRODUCTION

Vegetables, which have been reported to have tonic or rejuvenation properties, have recently gained more attention as potential sources of bioactive extracts, as well as being a dietary supplement to support human well-being. Not only do they could they promote some related biological activities, such as antioxidant, anti-inflammatory or cancer chemopreventive properties, but they are also edible, which tends to be beneficial to safe and practical health-support applications.

In Thailand, there are two vegetable species that are commonly known as “phak-paem”, namely *Acanthopanax trifoliatum* (phak-paem) and *Toddalia asiatica* (phak-paem paa). These plants have some similarities in botanical characteristics, especially their palmate, compound leaves with

prickles (Figure 1). Moreover, both phak-paem species have been ethnomedically used for tonic purposes and their young leaves and shoots are popularly consumed as vegetables (Chi, 1997; Loi, 2000).

Acanthopanax trifoliatum (L.) Merr. is a shrub in the Araliaceae (ginseng family). It is used in the folk medicines of Southeast Asia as a drug with ginseng-like activity (Perry and Metzger, 1981a, Bucci, 2000). A decoction of the leaves and young shoots is used to treat tuberculosis and lung hemorrhages and as a tonic to improve general weakness (Perry and Metzger, 1981a; Chi, 1997; Loi, 2000). The preparation is believed to help in dispersing extravasated blood from bruises, relieving partial paralysis and is applied to ulcers and contusions (Perry and Metzger, 1981b; Bucci, 2000). There are not many phytochemical reports

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Figure 1 Botanical characteristics of *Acanthopanax trifoliatum* and *Toddalia asiatica*;
 A: *Acanthopanax trifoliatum*; (1) stem and leaves; (2) fruits.
 B: *Toddalia asiatica*; (1) stem, leaves and fruits; (2) fruits.
 C: Comparison of compound leaves; AT (left) and TA (right).

regarding the leaves of *A. trifoliatum*. However, some chemical constituents were separated and identified as terpenes, including continentalic acid, acantrifolic acid and the flavonoid, nevadensin (Du and Gao, 1992; Kiem *et al.*, 2004; Phuong *et al.*, 2006). *Toddalia asiatica* (L.) Lam. is a climber shrub that always occurs in forests near rivers or streams, which is reflected in its Thai name, “phak-paem paa”, which means “phak-paem that grows in the forest”. This plant grows well in clay soils and is widespread in Africa, Asia and Madagascar (Watt and Breyer-Brandwijk, 1962). The root bark is used medicinally as a tonic and for the treatment of stomach ailments (Watt and Breyer-Brandwijk, 1962), while the leaves are used in patients having lung diseases or rheumatism and for the treatment of fever, asthma and respiratory diseases (Watt and Breyer-Brandwijk, 1962; Gurib-Fakim *et al.*, 1997). A report about the phytochemistry of the leaves of *T. aculeata* showed that they contained some alkaloids (Jain *et al.*, 2006).

Even though the young leaves and shoots of these plants are popularly consumed as tonic vegetables in traditional cuisines, there are no reports that provide information on or compare the antioxidant activity of extracts from the edible parts of *A. trifoliatum* and *T. asiatica*. Furthermore, there is no information on the quantitative analysis of their active components, especially the phenolics content, which has been reported to be related to antioxidant activity (Hosein and Zinab, 2007). Therefore, this study was conducted to investigate the free radical scavenging activity and inhibitory effect to the lipid peroxidation of rat brain homogenate of extracts from the leaves of *A. trifoliatum* and *T. asiatica*. The extracts were obtained from two different methods of extraction, which were decoction and 75% ethanolic refluxing, the method that was officially described in Chinese pharmacopoeia for tonic preparation of Siberian ginseng (*Acanthopanax senticosus*) (Huang, 1999). In addition, the total phenolics and flavonoids content of these extracts were measured

and the chemical constituents of the leaf extract were studied by chromatographic techniques.

MATERIALS AND METHODS

In January 2008, leaves of *Acanthopanax trifoliatum* (AT) were collected from Sunpathong district, Chiang Mai province, Thailand, while those of *Toddalia asiatica* (TA) were collected from Pong district, Phayao province. The plant samples were identified by Mr Winai Somprasong, Botanist, Bangkok Herbarium, Department of Agriculture. The voucher specimens for AT (BK63947) and TA (BK63948) were deposited at the same herbarium.

Preparation of plant extracts

Decoction

The leaves of AT and TA were separately cleaned, dried in a hot air oven (60°C) for 6 h and powdered using an electronic mill (20 mesh sieve). The leaf powders were separately boiled with distilled water (1:10 w/v) for 3 h before filtration. The filtrates were separately taken to dryness by lyophilization to yield dried leaf decoction extracts of AT and TA (ATD and TAD, respectively).

Refluxing with 75% ethanol

The leaf powders of AT and TA were separately refluxed (80°C) with 75% ethanol for 3 h before filtration. The filtrates were collected and evaporated under reduced pressure to yield ethanolic leaf extracts of AT and TA (ATR and TAR, respectively).

Determination of antioxidant activity by DPPH scavenging method

The free radical scavenging effect of AT and TA extracts as well as standard ascorbic acid, chlorogenic acid and rutin corresponding to the quenching ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH) were determined, as described by Yamasaki *et al.* (1994). Each sample was assayed in triplicate and the average EC₅₀ value was calculated.

Inhibition of lipid peroxidation of rat brain homogenate by thiobarbituric acid reactive substances (TBARS) assay

Preparation of rat brain homogenate :

Rats weighing 200-250 g were sacrificed by carbon monoxide sacrificed and their whole brains were dissected out and homogenized using an ART-MICCRA D-8 homogenizer (Germany). A 100 mg/ml suspension containing ice-cold phosphate buffer (0.1 M, pH 7.4) was used as a source of polyunsaturated fatty acids for determining lipid peroxidation. The tissue homogenate was centrifuged at 800× g for 15 min at 4°C and the supernatant was divided into 1 ml aliquots and preserved at -80°C until used.

TBARS assay : Modified from Auddy *et al.* (2003) and Singh *et al.* (2007), 50 µL of rat brain homogenate and 25 µl of various concentrations (2-60 µg/ml) of plant extracts, or standard trolox (water-soluble vitamin E derivative), dissolved in phosphate buffer were used. In the control experiment, volume adjustments were carried out using the phosphate buffer. Peroxidation was initiated by adding 30 µl of 0.2 mM ferric chloride. After incubation in a shaking water bath at 37°C for 40 min, the reactions were stopped by adding 350 µl of 20% (v/v) acetic acid followed by 600 µl of 0.5% TBA in 20% (v/v) acetic acid (pH 3.5). The mixtures were incubated at 85°C for 60 min. After cooling, 50 µl of 10% SDS was added, centrifuged at 3,000× g for 10 min and the absorbance of the supernatant determined at 532 nm (Ohakawa *et al.*, 1979) using a microplate reader (Tecan, USA). The EC₅₀ values for the plant extracts were determined from a calibration curve. All experiments were done in triplicate. Results were reported as means of EC₅₀ ± standard deviation.

Determination of total phenolic content

Using the method modified from Naithani *et al.* (2005), plant extract solutions were oxidized with Folin-Ciocalteu reagent and the reactions were neutralized by sodium carbonate

solution. The absorbance of the resulting blue-colored solution was measured at 765 nm after 120 min. Each sample was duplicated. The total phenolics content was expressed as g chlorogenic acid equivalent in 100 g extract (g% CAE).

Determination of total flavonoid content

The total flavonoids content was investigated using the colorimetric method previously described (Meda *et al.*, 2005). Plant extract solutions were reacted with aluminum chloride solution in the same volume. Absorbances were read at 415 nm after 10 min and the flavonoids content was expressed as g rutin equivalent in 100 g of plant extracts (g% RE).

Phytochemical analysis by thin layer chromatography

Thin layer chromatography of AT and TA leaf decoction extracts was performed on TLC pre-coated silica gel 60 GF₂₅₄ plates (10 × 10 cm) using two different solvent systems, ethyl acetate-acetic acid-formic acid-water (137:11:11:26) and *n*-butanol-acetic acid-water (4:1:5), as solvent A and B, respectively. TLC plates were detected using 10% sulfuric acid, 10% ferric chloride and natural products-polyethylene glycol (NP-PEG) spray reagents.

Statistical analysis

All data are reported as mean ± standard deviation of triplicates in the antioxidant activity determinations. An independent sample t-test was used to compare means and the least significant difference at *P* < 0.05 was calculated. All analyses were performed using SPSS for Windows, version 16.0 (SPSS, Inc., USA).

RESULTS AND DISCUSSION

This work was conducted following the work that reported on the evaluation of antioxidative effects (Becker *et al.*, 2004). The first step suggested was quantification and

identification of the phenolic compounds in the samples. The second step concerned quantification of the radical scavenging activity of the samples. Finally, evaluation of the ability of samples to inhibit lipid oxidation in relevant model systems (Madsen *et al.*, 1997; Becker *et al.*, 2004) was performed. In addition, phytochemical analysis of the chemical composition in aqueous extracts from the leaves of the two plant species was conducted by TLC.

Antioxidant activity determination of extracts from AT and TA

Table 1 indicates that all tested samples showed strong DPPH scavenging activity ($EC_{50} < 50 \mu\text{g/ml}$, Cervantes-Cervantes, 2005). However, ATD significantly ($P < 0.05$) exhibited the strongest activity with an EC_{50} value of $24.08 \pm 1.65 \mu\text{g/ml}$ while the other three samples showed similar activity. This extract also significantly showed the strongest inhibitory effect to lipid peroxidation of rat brain homogenate (EC_{50} value of $11.38 \pm 0.24 \mu\text{g/ml}$) being at least five times greater than the others.

Analysis of phenolic content

Extracts from the leaves of AT and TA contained phenolics and flavonoids in the range

10.14 ± 0.35 to $19.96 \pm 0.28 \text{ g\% CAE}$ and 0.78 ± 0.00 to $1.91 \pm 0.01 \text{ g\% RE}$, respectively. The TA decoction extract significantly contained the highest amount of phenolic compounds, while the 75% ethanolic extract of TA contained the highest flavonoids component. Considering the extraction methods, decoction with water yielded extracts with the higher phenolics content, while refluxing using 75% ethanol gave extracts with the higher flavonoids content.

The mechanism for DPPH scavenging activity was provided by the proton-donating ability of chemical components in the extract, while the inhibitory effect on lipid peroxidation could have come from synergistic mechanisms, including chelation of metal catalysts, scavenging of initiating radicals, chain breaking reactions and reductions in the concentration of reactive oxygen (Aruoma, 1994). This suggests that not only the amount of antioxidant components (such as phenolics and flavonoids), but also their chemical structures, their ratios in the extract and synergistic effects between them, could affect the antioxidant ability of each sample. This is supported by the results in Table 1, showing that ATD, which contained some phenolics and flavonoids contents had the strongest *in vitro* antioxidant activity, while TAD and TAR, which contained the highest

Table 1 Antioxidant activity and phenolics content of leaf extracts of *A. trifoliatum* and *T. asiatica*.

Sample	Yield (%w/w)	Antioxidant activity** (EC_{50} , $\mu\text{g/ml}$)		Phenolics content**	
		DPPH assay	TBARS method	Total phenolics ($\text{g\% CAE}$)	Total flavonoids ($\text{g\% RE}$)
ATD	46.56	24.08 ± 1.65^a	11.38 ± 0.24^a	14.21 ± 0.25^a	0.99 ± 0.00^a
ATR	25.70	41.82 ± 1.96^b	63.88 ± 2.60^b	10.14 ± 0.35^b	1.75 ± 0.02^b
TAD	33.07	41.81 ± 1.39^b	149.61 ± 4.98^c	19.96 ± 0.28^c	0.78 ± 0.00^c
TAR	18.91	40.52 ± 1.97^b	321.59 ± 3.45^d	14.29 ± 0.08^a	1.91 ± 0.01^d
Ascorbic acid*	-	3.05 ± 0.11	-	-	-
Chlorogenic* acid	-	18.87 ± 0.76	-	-	-
Rutin*	-	8.04 ± 0.10	-	-	-
Trolox*	-	-	$331.37 \pm 7.41 \times 10^{-3}$	-	-

* These were used as standards.

** different letters are significantly different ($P < 0.05$).

amounts of phenolics and flavonoids, respectively, showed lower activities. It has also been reported that antioxidant effects were related to various antioxidative components in plants, such as ascorbic acid, carotenoids, tocopherols, chlorophylls, and metal chelating compounds (Takamatsu *et al.*, 2003, Chanwitheesuk *et al.*, 2005).

Phytochemical analysis by TLC

Decoction extracts of AT and TA exhibited higher antioxidant activity than the ethanolic extracts and were selected for TLC analysis with specific detection. From Figure 2, they both obviously contained components that corresponded to rutin and chlorogenic acid, which, with ferric chloride spray reagent, appeared as dark

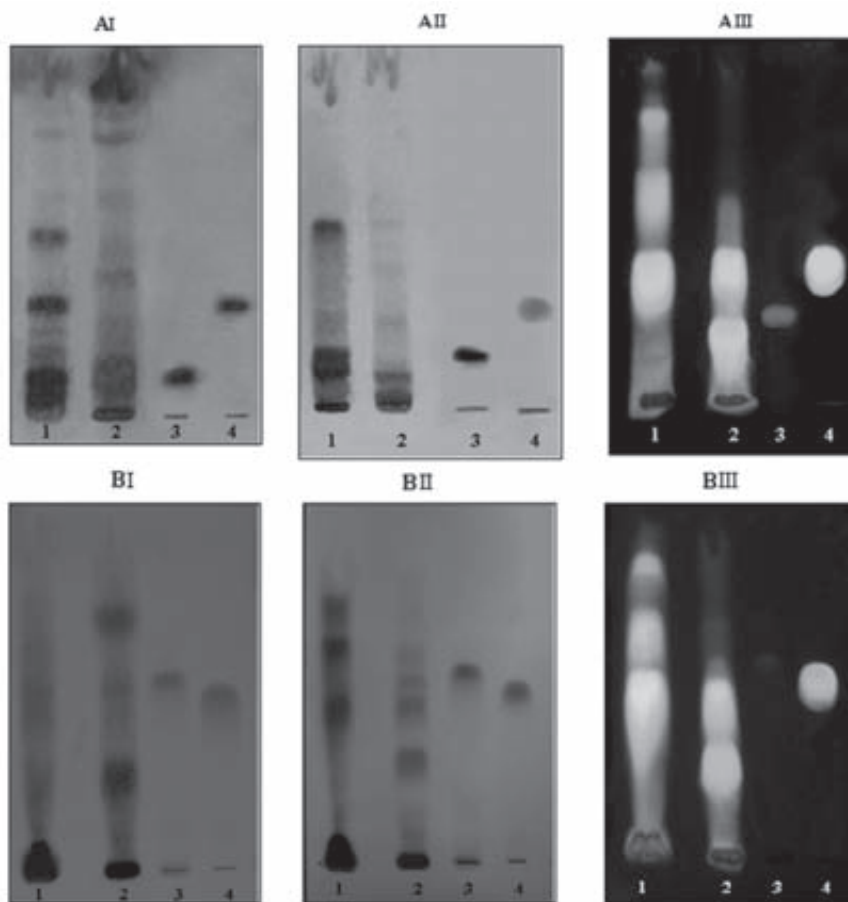


Figure 2 Thin layer chromatographic analysis of *A. trifoliatum* and *T. asiatica*.

1 = *A. trifoliatum* leaf extract

2 = *T. asiatica* leaf extract

3 = standard rutin

4 = standard chlorogenic acid

Solvent system: A = ethyl acetate-acetic acid-formic acid-water (137:11:11:26)

B = *n*-butanol-acetic acid-water (4:1:5)

Spray reagent: I = 10% H₂SO₄ spray reagent

II = 10% FeCl₃ spray reagent

III = NP-PEG spray reagent under UV 366 nm

green spots, and deep orange and bright blue fluorescence spots, respectively after detection by NP-PEG spray reagent under UV 366 nm. These two compounds showed strong radical scavenging effects as (Table 1), with EC_{50} values of 8.04 ± 0.10 and 18.87 ± 0.76 mg/ml, respectively. There were also other spots that appeared as positive to these spraying reagents, especially in TAD. Detection by various spray reagents, including NP-PEG, ferric chloride and sulfuric acid spray reagents, showed different TLC patterns between ATD and TAD. This suggested that ATD and TAD had their specific TLC fingerprints and contained at least two components that possessed antioxidant activity. Further separation and identification of other antioxidant components should be conducted.

CONCLUSION

Leaf decoction extract of AT exhibited strong free radical scavenging activity and an inhibitory effect to lipid peroxidation of rat brain homogenate. Analysis of the phenolics and flavonoids content showed that all extracts contained high amounts of phenolic and flavonoid compounds. Chromatographic analysis of decoction extracts of the two plant species showed specific phytochemical characteristics. They both contained some phenolics and flavonoids, including chlorogenic acid and rutin, which were previously reported to exhibit some biological activity, including antioxidant and anti-inflammatory effects (Kozlov *et al.*, 1994, Jung *et al.*, 1999, Guardia *et al.*, 2001, Yonathan *et al.*, 2006, Nakajima *et al.*, 2007). The antioxidant activities of extracts from AT and TA observed in this study confirmed the ethnomedical uses of these plants as tonics, which could also support the consumption of the young leaves of these two phak-paem species as vegetables to promote good health. It is believed that the current research was the first to report on a comparison of the

antioxidant activities and phytochemical characteristics of AT and TA leaves, with detection of the presence of rutin and chlorogenic acid.

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