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Acanthopanax trifoliatus, a Potential Adaptogenic Thai Vegetable for Health Supplement

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1. Introduction

Nowadays, people are struggling with stress, either from environmental pollutions and daily life routines of urgency and competition. From the risk of exposure to toxic or pathogenic substances from various routes, our bodies have to adapt and maintain a systemic balance for physiological functions. Obtaining the supplements or substances that promote good health is the recommended choice for people in this century. This is the beginning of the word “adaptogens” or "balancing material" that will help the body adjust and increase the tolerance to physical, emotional and environmental stresses as well as promote the metabolic system and homeostasis of the body.

An adaptogen was previously defined as a substance that had to; show some nonspecific effect, such as increasing bodily resistance to physically, chemically, or biologically noxious agents or factors; had a normalizing influence on a pathologic state, independent of the nature of that state; and was innocuous and not disturb body function at a normal level (Lasarev, 1947). Panossian and Wagner (2005) suggested that the adaptogenic substance from plants was a substance that increased the ability of an organism to adapt in various factors in the environment and to prevent damage caused by such factors. Some plants have been used for adaptogenic purposes as shown in Table 1.

Panossian (2003) suggested that most of the active phytochemicals separated from the adaptogenic plants were in 3 main chemical groups; phenolic compounds such as phenylpropanoids, phenylethane derivatives and lignans; tetracyclic triterpenes; and unsaturated trihydroxy or epoxy fatty acids. Chemical structures of some adaptogenic compounds are shown in Figure 1.

To demonstrate the adaptogenic effects of medicinal plants, related pharmacological activity determinations were studied including *in vivo* effects for decreasing the stress, promote physical performance, anabolic efficacy, and brain metabolism. Some *in vitro* studies were also conducted such as antioxidative and anti-inflammatory effect experiments. Adaptogenic plants can promote anti-stress effects via several mechanisms including

increasing of body temperature, improvements of body function, enhancing of cognitive abilities, promoting of locomotor and exploration activities, and moderating the emotional behavior (Wagner et al., 1994).

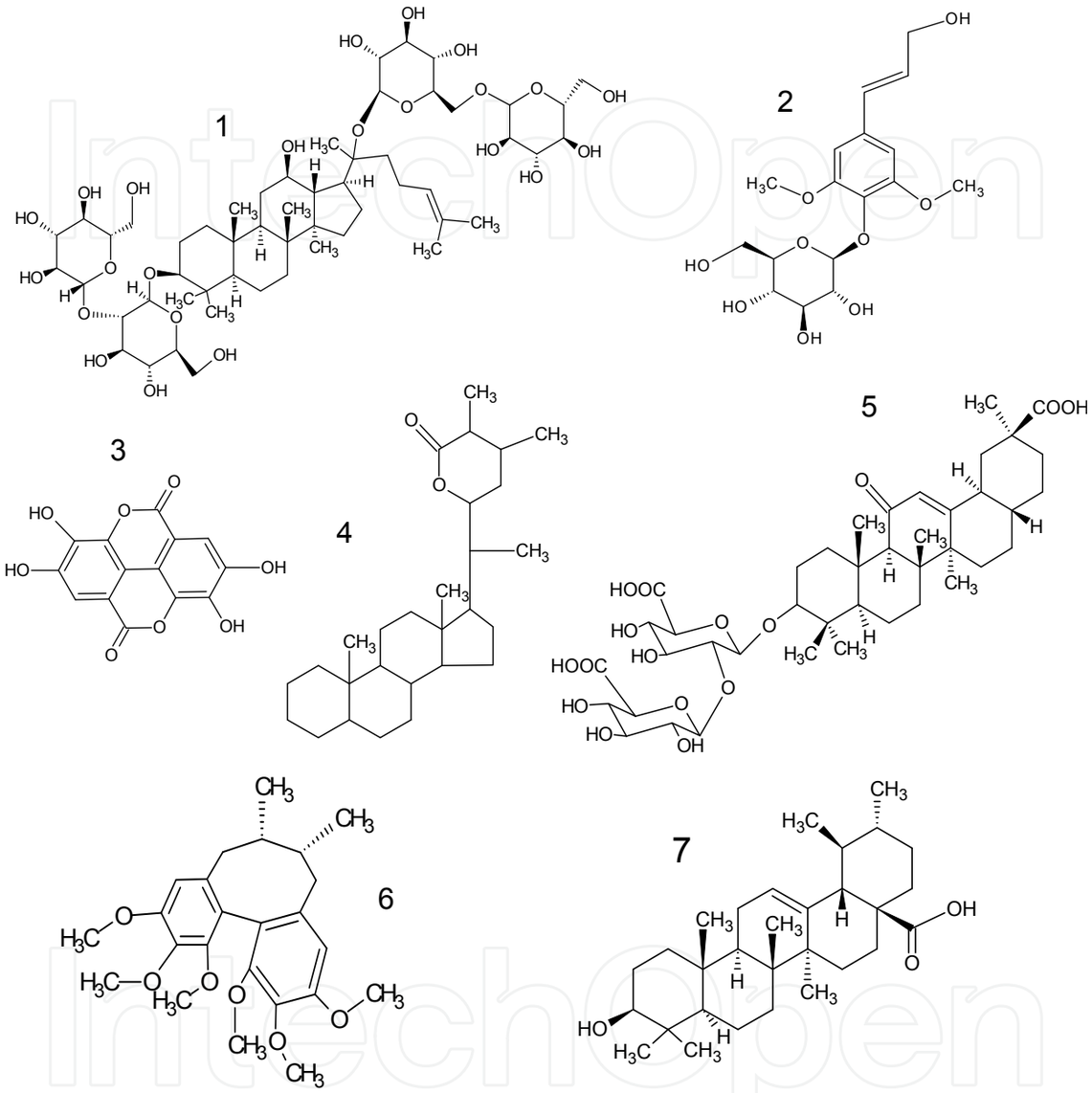


Fig. 1 Adaptogenic compounds; 1= ginsenoside Rb1, 2= eleutheroside B, 3= ellagic acid, 4= withanolide, 5= glycyrrhizin, 6= schisandrin, 7= ursolic acid.

Free radicals and reactive oxygen species are some chemical species that have odd number of electrons which have high reactivities and are capable to cause reversibly or irreversibly oxidative damages to compounds of all biochemical classes including nucleic acids, proteins and free amino acids, lipids and lipoproteins, carbohydrates and connective tissue macromolecules (Halliwell and Gutteridge, 1999). Oxidative stress is considered some importance for many ailments and pathologies including cardiovascular diseases, cancers, rheumatoid arthritis and Alzheimer's disease (Cross, 1987). Inflammation and oxidative

Common name	Scientific name	Family	Part used
American ginseng	*Panax quinquefolius*	Araliaceae	Root
Indian gooseberry	*Emblica officinalis*	Euphorbiaceae	Fruit
Ashwagandha	*Withania somnifera*	Solanaceae	Root
Korean ginseng	*Panax ginseng*	Araliaceae	Root
Siberian ginseng	*Eleutherococcus senticosus*	Araliaceae	Root, stem bark
Indian tinospora	*Tinospora cordifolia*	Menispermaceae	Root, stem
Holy basil	*Ocimum sanctum*	Lamiaceae	Whole plant
Jiaogulan	*Gynostemma pentaphyllum*	Cucurbitaceae	Whole plant
Licorice	*Glycyrrhiza glabra*	Fabaceae	Root
Reishi	*Ganoderma lucidum*	Ganodermataceae	Mushroom
Schisandra	*Schisandra chinensis*	Schisandraceae	Fruit, seed

Table 1. Reported adaptogenic plants (Winston and Maimes, 2007).

stress are key components of the pathology of chronic neurodegenerative conditions, in particular Alzheimer's disease, Down syndrome, multiple sclerosis and dementia (Auroma et al., 2003). Previous studies indicated that oxidative injury is present in the brains of patients with Alzheimer's disease may play important role in the development of the disease (Grundman and Delaney, 2002, Pratico and Delantly, 2000, Rottkamp et al., 2000, Smith et al., 2000). Excessive lipid peroxidation and increasing of malondialdehyde concentrations have been found in the patients' brains of Alzheimer's disease (Grundman and Delaney, 2002). From ethnomedical intellectual, various plants have been used to enhance cognitive function and to relieve other symptoms associated with Alzheimer's disease. The effects of traditional herbal drugs may not only be relevant in managing the cognitive decline that can be associated with general aging but may also be relevant in the treatment of specific cognitive disorders such as Alzheimer's disease. Therefore, plants with anti-aging or memory enhancing activities can be considered for potential efficacy in cognitive dysfunction including conditions that feature dementia. The activities of plants that show the effects in relation to cognitive disorders are including anti-cholinesterase (anti-AChE), anti-inflammation and antioxidant (Howes and Houghton, 2003).

Health promotion by Thai wisdom has been conducted from the Thai traditional practice in prevention, diagnosis, and treatment of imbalancing in human bodies and minds. The process is accompanied with folk medicine which herbs and natural products in each region all over the country are main constituents. The knowledge and medical procedures have been transferred from generation to generation until nowadays. In Thai traditional medicine, herbs with adaptogenic or antioxidative effects should relate to plants that exhibit detoxification, blood purifying, jaundice curing, hepatoprotection, tonic and haematonic, aphrodisiac, anti-fatigue, nourishment and longevity promotion (Faculty of Pharmacy, Mahidol University, 1998).

Eleven indigenous plant species traditionally used as ginseng-like agents or adaptogens were collected from the north and northeastern parts of Thailand and evaluated for adaptogenic-related properties including antioxidant and anti-anxiety activities as well as total phenolic and total flavonoid contents. Along with the rhizome extract of *Smilax corbularia*, leaf decoction extract of *Acanthopanax trifoliatus* (*A. trifoliatus*) exhibited strong antioxidant activity with high amount of phenolic and flavonoid contents (Sithisarn et al., 2010). The results suggested that *A. trifoliatus* is an interesting plant that could promote

significantly pharmacological activities related to adaptogenic properties, which supported the ethnomedical uses of this plant.

2. Botanical characteristics of *A. trifoliatus*

A. trifoliatus is a shrub that belongs to the family Araliaceae. Its taxonomic position is as follows:

Order	Apiales
Suborder	Apiineae
Family	Araliacea (ginseng family)
Genus	Acanthopanax (Eleutherococcus)
Species	trifoliatus

In Flora of China (2004), *A. trifoliatus* was described as a shrub, scandent or climber that usually reaches a height of 7 m. The branches are scattered with recurved prickles. The glabrous and prickly petioles are 2-6 cm long with the 2-8 mm long petiolules. The papery, adaxially glabrous or slightly setose on midvein and veins, secondary veins 5 or 6 pairs, base cuneate, margin serrulate, apex acute or acuminate leaves are 4-10 x 2-4.5 cm and the ovate, elliptic-ovate, or oblong leaflets which number up to 3 or 5. The inflorescence is terminal raceme, umbel or compound umbel which borne on leafy shoots and 3-10 umbels. The peduncles are 2-7 cm long with the 1-2 cm long pedicels. The glabrous calyx has 5 teeth. The ovary has 2 carpellates, the styles are united to middle. The fruit is globose and laterally compressed with the size of 3-4 mm. The flowering period is from August to November. The fruiting time is during September to December.



Fig. 2. *Acanthopanax trifoliatus*. 1= leaves and flowers, 2= roots, 3= whole plant.

Normally *A. trifoliatus* thrives in shrub fields, roadsides, forest margins, in valleys or on mountain slopes; below 1000 m in the East and 3200 m in the West part of range. This plant is widely distributed in India, Japan, the Philippines, Thailand, Vietnam, and many provinces of China including Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hunan, Hubei, Jiangsu, Jiangxi, Sichuan, Taiwan, Yunnan and Zhejiang. The photos of *A. trifoliatus* from Chiang Mai province, Thailand are shown in Figure 2.

3. Ethnomedical uses and previous reported biological activities of *A. trifoliatus*

The leaves of *A. trifoliatus* have been used as a tonic to improve general weakness, the leaves and young shoots are also used for treatments of tuberculosis, lung hemorrhages, bruises, ulcers and, contusion (Perry and Metzger, 1981a, Perry and Metzger, 1981b, Chi, 1997, Loi, 2000). The stem bark is used as antiflatulent agent and is used for treatments of emaciation and neurosis (Petelot, 1954). The root bark and stem bark are used in the treatments of rheumatism, lumbago, ostealgia and impotence (Nguyen and Doan, 1989). The bark was reported to be used as tonic, CNS stimulant, and memory enhancer (Nguyen and Doan, 1989). In Chinese medicine, this plant was used for the treatments of cold, cough, neuralgia and rheumatism (Duke and Ayensu, 1985). Moreover, young leaves and the shoots are popularly consumed in Northern Thai traditional cuisine as vegetables. Antioxidant and anti-inflammatory activities of the extracts from *A. trifoliatus* have been previously reported and suggested that young leaves provided the most active sample (Sithisarn and Jarikasem, 2009, Sithisarn et al., 2009). Anti-anxiety effects in animal models of leaf extract from *A. trifoliatus* were also reported (Sithisarn et al., 2010).

4. Chemical constituents of *A. trifoliatus*

Some chemical components including terpenoids, phenylpropanoids, phenolics, flavonoids, lactones and essential oils were separated and identified from various parts of *A. trifoliatus* as shown in Table 2.

5. Antioxidant activity of *A. trifoliatus*

The extracts from some parts of *A. trifoliatus* including leaves, stems, stem barks, roots and root barks with various extraction methods were tested for free radical scavenging activity using DPPH scavenging method and inhibitory effect to lipid peroxidation of rat brain homogenate by thiobarbituric acid reactive substances (TBARS) method. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with a deep violet color. When DPPH radical receives a proton from antioxidants, it converts to a colorless protonated DPPH molecule. After 15 minutes of DPPH scavenging reaction, the absorbance at 517 nm was determined then % of inhibition and EC_{50} value were calculated (Yamasaki et al., 1994). It was found that the leaf decoction extract, the root bark decoction and 75% ethanolic refluxing extracts and the stem bark 75% ethanolic refluxing extract showed high radical scavenging activity with EC_{50} values of 14.50 ± 1.04, 34.24 ± 5.01, 34.51 ± 2.74 and 37.85 ± 0.85 µg/ml, respectively (Sithisarn and Jarikasem, 2009). TBARS method is popular single assay for the measurement of lipid peroxidation. The sample under test is treated with thiobarbituric acid (TBA) at low pH, and a maximum absorption of pink chromogen is

Group of chemical	Chemical compounds (plant part)	Reference
Terpenoid	- acanthoic, continentalic, kaurenoic acids (root, stem, leaves)	Phuong, 2006 Kiem, 2004
	- acantrifoic acid A, acantrifoside C (leaves)	Kiem, 2004
	- acantrifoside D (stem bark)	Kiem, 2004
	- 16αH, 17-isovalerate-ent-kauran-19-oic acid, ent-Kaur-16-en-19-oic acid, ent-Primara-8(14), 15-dien-19-oic acid (stem bark)	Yook, 1998 Yook, 1999
	- acantrifoside A (leaves)	Kiem, 2003a
	- lupane triterpene glycoside (leaves) - 24-nor-11alpha-hydroxy-3-oxo-lup-20(29)-en-28-oic acid 28-*O*-alpha-L-rhamnopyranosyl-(1→4)-beta-D-glucopyranosyl-(1→6)-beta-D-glucopyranosyl ester (leaves)	Du, 1992
	- kaur-16-en-19-oic acid, taraxerol, taraxerol-acetate (leaves)	
Phenylpropanoid	- 1-β-D-glucopyranosyl-2,6-dimethoxy-4-propenylphenol, 1-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-2,6-dimethoxy-4-propenylphenol (stem bark)	Kiem, 2003b
Polyphenolic and flavonoid	- syringin, quercitrin (stem bark)	Kiem, 2003b
	- nevadensin (leaves)	Du, 1992
Lactone	- eleutheroside E, (2R,3R)-2,3-di-(3,4-methylenedioxybenzyl)-butyrolactone (stem bark)	Kiem, 2003b
Essential oil	- α-pinene, sabinene, terpinen-4-ol, β-pinene, *p*-cymene, carissone (leaves)	Muselli, 1999

Table 2. Reported chemical constituents in *A. trifoliatus.*

measured at 532 nm (Punchard, 1996). Inhibitory effects to lipid peroxidation of rat brain homogenate of various extracts from *A. trifoliatus* ranged from EC_{50} of 11.18 ± 2.60 to 75.35 ± 7.52 μg/ml. Root and leaf decoction extracts showed significantly higher effects (EC_{50} of 11.18 ± 2.60 and 16.11 ± 0.29 μg/ml, respectively) (Sithisarn and Jarikasem, 2009).

6. Anti-cholinesterase activity of *A. trifoliatus*

Cholinergic deficit was reported to associate with memory loss and the severity of Alzheimer's (Bierer, 1995). To restore cholinergic function in AD, many mechanisms are studied including stimulation of cholinergic receptors or prolonging the availability of acetylcholine (ACh) released into neuronal synaptic cleft by inhibiting ACh hydrolysis by acetylcholinesterase (AChE) which can be developed by the applications of proper AChE inhibitors. Extracts from several parts of *A. trifoliatus* were tested for AChE inhibitory activity using Ellman's colorimetric method in 96-well microplate. According to Vinutha et al. (2007), at the concentration of 100 μg/ml, most tested extracts exhibited moderate acetylcholinesterase inhibitory effect (30-50% inhibition). All root extracts including 95% ethanolic, decoction and 75% ethanolic extracts showed the highest effects among tested samples with AChE inhibition of 40.24 ± 3.56, 42.06 ± 5.29 and 47.41 ± 3.52 %, respectively. The results of inhibitory effect of *A. trifoliatus* extracts to AChE are shown in Table 3.

Sample	AChE inhibitory activity*
LM	$15.51 \pm 9.95^{a,e,g,h,i,j}$
LR	$22.82 \pm 8.71^{a,b,e,h,i,j}$
LD	$31.76 \pm 2.51^{c,g}$
SBM	$36.83 \pm 3.34^{b,c,d}$
SBR	22.51 ± 0.53^{e}
SBD	$25.78 \pm 1.92^{i,j}$
SM	$31.27 \pm 3.54^{a,c,j}$
SR	$16.98 \pm 4.36^{e,h}$
SD	15.37 ± 1.68^{h}
RBM	$36.26 \pm 4.09^{b,c,d}$
RBR	$36.30 \pm 1.50^{a,c,d}$
RBD	$20.65 \pm 3.14^{e,h,i}$
RM	$40.24 \pm 3.56^{b,d}$
RR	47.41 ± 3.52^{f}
RD	$42.06 \pm 5.29^{d,f}$
Galanthamine	Completely inhibit
Physostigmine	Completely inhibit

* different letters in the same column are significantly different (P<0.05)

Table 3. Acetylcholinesterase inhibitory effect of extracts and standard compounds at 100 µg/ml from various parts of *A. trifoliatus*. LM: leaf maceration, LR=leaf refluxing, LD=leaf decoction, SBM=stem bark maceration, SBR=stem bark refluxing, SBD=stem bark decoction, SM=stem maceration, SR=stem refluxing, SD=stem decoction, RBM= root bark maceration, RBR=root bark refluxing, RBD=root bark decoction, RM=root maceration, RR=root refluxing, RD=root decoction.

7. Anti-anxiety activity of *A. trifoliatus*

Adult male ICR mice with a weight range 30-35 g were used for determination of anti-anxiety effect of *A. trifoliatus* by light-dark task and hole-board test. Tested mice were received for corticosterone solution about 13 mg/kg per day via drinking water for 17 consecutive days. Then mice were tested for anxiety-like behavior in the light-dark task and hole-board test.

7.1 Light-dark task

Thirty mice were randomly divided into five groups of six mice. Each group of mice was orally administered with distilled water as a control group, phenobarbital (30 mg/kg) was used as a standard drug, and *A. trifoliatus* leaf decoction extract in 3 different doses of 500, 750 and 1000 mg/kg, respectively. Modified from Ardayfio and Kim (2006) and Krishna et al (2006), the apparatus consisted of a Plexiglas box with two compartments, one of which was illuminated with a white light while the other remained dark. One hour after drugs administration, each mouse was placed at one corner of the dark compartment, facing against of the light area. The time spent in illuminated and dark places, as well as the number of entries in each space, was recorded for 10 min. The administration of all 3 different doses of the extract in mice induced both significant increments (P<0.05) of the

number of entries and time spent by mice in light chamber of the light-dark apparatus (Sithisarn et al., 2010).

7.2 Hole-board test

Applied from Brown and Nemes (2008), the apparatus consisted of a square plastic plate with 16 holes, regularly spaced on the surface. Each hole contains sensors for detecting when animal dips the head in a hole. Thirty mice were randomly divided into five groups of six mice. Each group of mice was orally administrated with distilled water as a control group, phenobarbital (30 mg/kg) was used as a standard drug, and *A. trifoliatus* leaf extract in 3 different doses of 500, 750 and 1000 mg/kg, respectively. Forty five minutes after drug administration, each mouse was placed on the centre of the board and the number of head dips was automatically counted for 3 min. Administration of the leaf decoction extract of *A. trifoliatus* in the concentration of 1000 mg/kg in mice significantly (P<0.05) induced an increment of the number of head-dip of the animals, similar to the effect observed in phenobarbital treated group (Sithisarn et al., 2010).

8. Anti-inflammatory activity of *A. trifoliatus*

Leaf decoction extract from *A. trifoliatus* was tested for anti-inflammatory effect using rat paw edema model induced by carrageenan injection. Thirty adult male Wistar rats with a weight range of 180-200 g were randomly divided into five groups of six rats. Each group of rats was orally administrated with distilled water as a control group, indomethacin (20 mg/kg) was used as a standard drug, and *A. trifoliatus* leaf extract in 3 different doses of 100, 300 and 600 mg/kg, respectively. The method of edema induction described by Winters et al. (1987) was modified to induce inflammation in rats' paws. 1% carrageenan in 0.9% sodium chloride solution was injected into the right hind paw of each rat after 1 h of each treatment. The volumes of rat paw edema were determined using plethysmometer at 1, 2, and 3 h after edema induction. The percentage of edema inhibition was calculated (Palanichamy and Nagarajan, 1990). Two hours after edema induction, the extract showed dose-dependent inhibition and the extract at the dose of 600 mg/kg exhibited significantly anti-inflammatory activity (41% inhibition). While the standard drug, indomethacin in the dose of 20 mg/kg showed significant effects at both 2 h and 3 h with the percentages of inhibition of 35 and 26, respectively (Sithisarn et al., 2009).

9. Toxicity of *A. trifoliatus*

Acute toxicity of the decoction extract from the *A. trifoliatus* leaves collected from Sunpathong district, Chiang Mai province, of Thailand which contained total phenolic and total flavonoid of 16.26 and 1.31 g% CAE and g% RE was determined for an oral lethal dose in rats. Adult male Wistar rats with a weight range of 215-250 g and adult female Wistar rats with a weight range of 178-198 g were randomized into control and experimental groups. Each tested group of rats consisting of 5 males and 5 females was orally administered a dose of 2 g/kg of *A. trifoliatus* leaf decoction extract while the control animals were administered distilled water. The animals were observed for mortality or any signs of abnormalities periodically during the first 0.5, 1 and 3 h and once daily for 14 days thereafter. Clinical sign, morbidity and mortality of tested group was observed for 14 days and compared to the respective control group. Body weight of each animal was recorded on day 1, 8 and 15. The

position, shape, size and color of visceral organs, i.e., heart, kidneys, lungs, stomach, intestine, liver, pancreas and sex organ were visually observed for any signs of gross lesions.

Upon gross examinations of visceral organs, no abnormality sign was observed in all tested groups compared to the control. In addition, there was no significant difference in average body weights of treated and controlled animals. According to the common classification of chemicals (Auletta, 1995, Organization for Economic Co-operation and Development, 2001), the extract showed no sign of toxicity (LD_{50} > 2 g/kg body weight).

10. Phytochemistry of *A. trifoliatus*

Extracts of the leaves and roots of *A. trifoliatus* prepared by different methods of extraction were chromatographic analyzed by thin layer chromatography. Flavonoids and polyphenolic compounds were detected with natural product/polyethylene glycol (NP/PEG) spraying reagent which showed fluorescence bands under UV 366 nm. Thin layer chromatographic fingerprints of *A. trifoliatus* extracts are shown in Figure 3. From TLC, extracts from the roots and leaves of *A. trifoliatus* showed some chromatographic bands positive to NP/PEG spraying reagent under UV 366 nm suggested the presence of phenolic and flavonoid compounds. TLC of the root extracts from refluxing with 75% ethanol and decoction as shown in track number 3 and 5 revealed bright blue fluorescence band corresponded to chlorogenic acid while those of the leaf extracts as shown in track number 2 and 4, other than chlorogenic acid, also contained orange fluorescence band corresponded to rutin. The leaf 95% ethanol maceration extract as shown in track number 2 also positively showed some chromatographic bands to the spraying reagent while the root 95% ethanol maceration extract revealed very weak chromatographic bands as shown in track number 1.

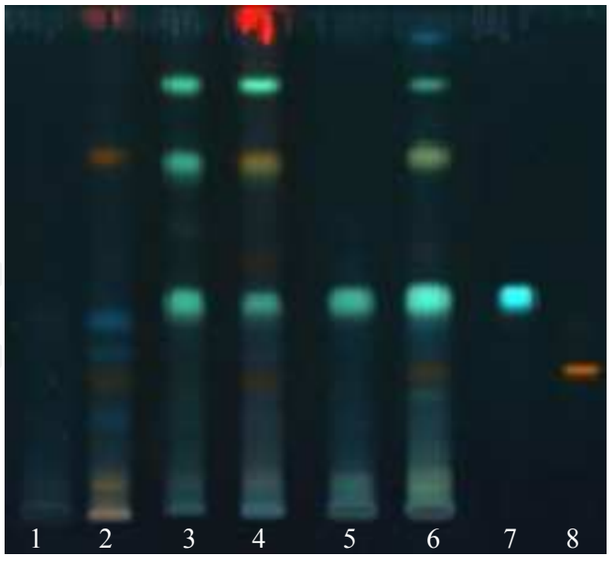


Fig. 3. TLC chromatogram of extracts from *A. trifoliatus*. Track : 1= root 95% maceration, 2= leaf 95% maceration, 3= root 75% refluxing, 4= leaf 75% refluxing, 5= root decoction, 6= leaf decoction, 7= standard chlorogenic acid, 8= standard quercetin, Stationary phase: silica gel GF254 Solvent system: ethyl acetate: acetic acid : formic acid : water 137: 11: 11: 26 Detection: NP-PEG spraying reagent / UV 366 nm.

10.1 Total phenolic and total flavonoid contents

Phenolics and flavonoids are known to be strong antioxidants especially through a free radical scavenging mechanism (Tyrrell, 1992). Total phenolic and total flavonoid contents in plant extracts can be determined colorimetically using specific reagents such as Folin-Ciocalteu and aluminium chloride reagents, respectively. Extracts from various parts of *A. trifoliatus* were determined for total phenolic content using Folin-Ciocalteu reagent (Dasgupta and De, 2007) and showed high concentration of phenolic compounds especially 75% refluxing extracts from stem barks and stems and root bark decoction extract (21.79 ± 0.23, 19.17 ± 0.58 and 20.74 ± 0.44 g chlorogenic acid equivalent (CAE) in 100 g extract (Sithisarn and Jarikasem, 2009). Total flavonoid content of extracts was determined using the method adapted by Meda et al. (2005). Aluminium chloride solution was mixed with the same volume of the sample solution. Absorption readings at 415 nm were taken and total flavonoid content was calculated as g rutin equivalent (RE)/100 g extract. Among tested extracts, leaf 95% ethanolic maceration extracts contained the highest amounts of flavonoids (9.61 ± 0.52 g% RE) (Sithisarn and Jarikasem, 2009).

10.2 High performance liquid chromatography

Leaf decoction extract of *A. trifoliatus* was subjected for HPLC-DAD and HPLC-MS analysis, six main peaks were separated by column chromatographic technique and were identified by their mass spectra and UV absorption spectra as chlorogenic acid, 3,5-di-*O*-caffeolyquinic acid, rutin, isoquercetin, 4,5-di-*O*-caffeolyquinic acid and quercitrin, respectively (Sithisarn and Jarikasem, 2009). MS chromatogram and HPLC chromatogram of these compounds are shown in Figure 4. Chemical structures are shown in Figure 5.

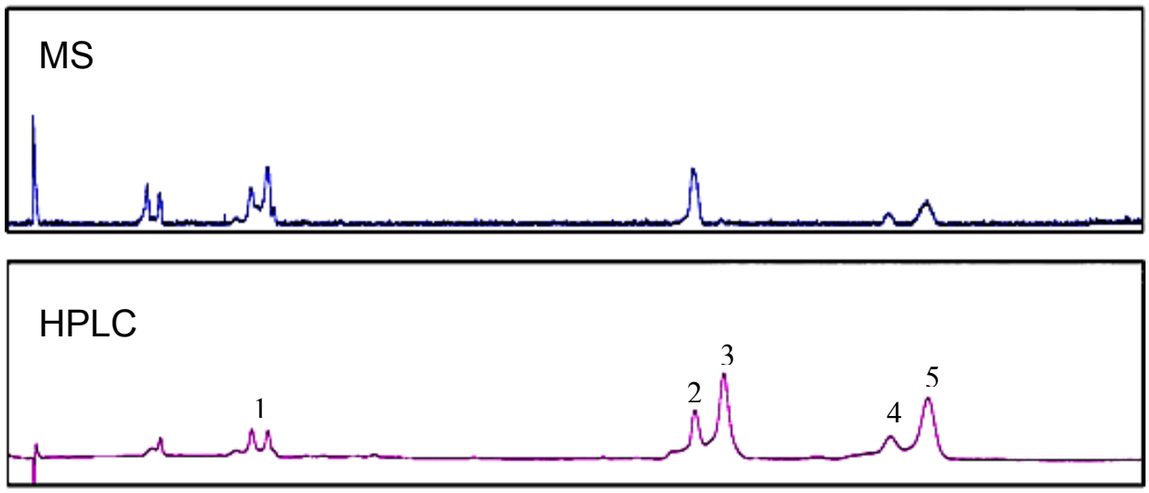


Fig. 4. HPLC chromatogram (UV 310 nm) and MS chromatogram of *n*-butanol fraction of leaf extract from *A. trifoliatus* (Sithisarn et al., 2008). No. of peak (t_R, min); 1= chlorogenic acid (16.6), 2= 3,5-di-*O*-caffeolyquinic acid (47.2), 3= rutin and isoquercetin (49.1), 4= 4,5-di-*O*-caffeolyquinic acid (60.7), 5= quercitrin (63.3).

11. Standardization of *A. trifoliatus*

Chemical and biological standardizations of plant extracts are also needed to be clarified before further studies in animal model or clinical trial. The major variables in plant geography are area, annual rain fall, ground water, types of soil, pH of soil, temperature, altitude, wind, light and local insecticides. These factors, especially rain fall and

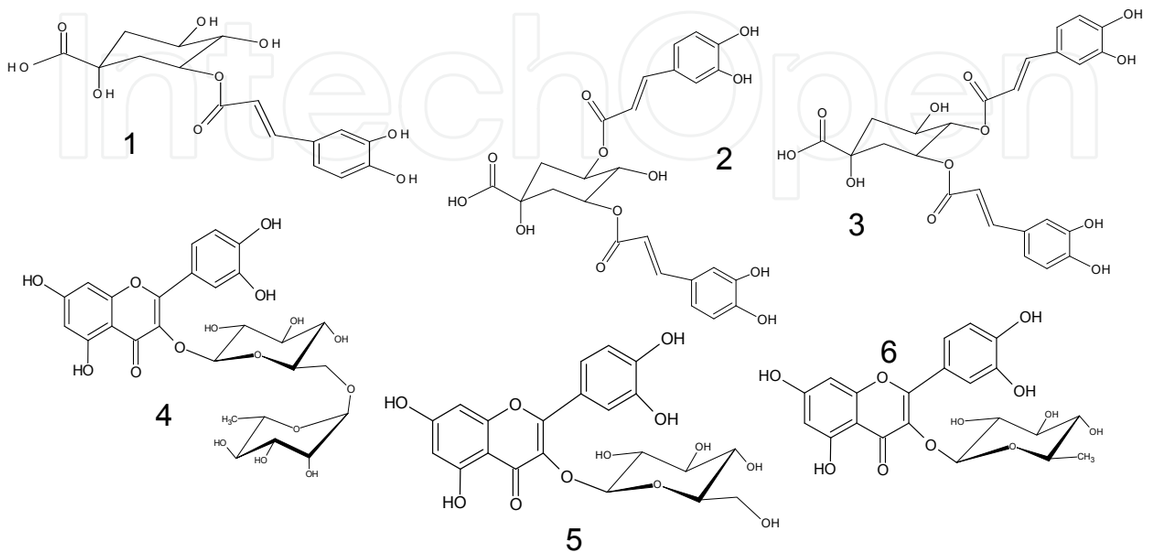


Fig. 5. Chemical structures of compounds from *n*-butanol fraction of leaf extract from *A. trifoliatus* : 1 = chlorogenic acid, 2 = 3,5-di-*O*-caffeolyquinic acid, 3 = 4,5-di-*O*-caffeolyquinic acid, 4 = rutin, 5 = isoquercetin, 6 = quercitrin.

temperature, could affect the biosynthesis of caffeoylquinic acid and flavonoid in *A. trifoliatus* (Sithisarn et al., 2011a). Chemical, physical and biological standardization of decoction extract of *A. trifoliatus* was set up by determination of antioxidant activity and quantitative analysis of the marker compositions of 11 *A. trifoliatus* leaf samples harvested in Chaing Mai province at different time intervals within a year. Using decoction, the most suitable extraction method for active *A. trifoliatus* leaf extract as previously reported (Sithisarn, and Jarikasem, 2009), it was found that samples collected in Winter and Fall contained significantly higher amount of total phenolic (12.72 – 14.66 g% CAE in dried extract) and total flavonoid (1.97 – 2.20 g% RE in dried extract) (Sithisarn et al., 2011b). HPLC analysis of marker components including monocaffeoylquinic acid (chlorogenic acid), dicaffeoylquinic acids (3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid), and flavonoid glycosides (rutin, isoquercetin and quercitrin) revealed that samples collected in January and November significantly contained high amount of phenolic and flavonoid contents suggesting the harvesting period of *A. trifoliatus* leaf samples during Winter or in low temperature condition (Sithisarn et al., 2011a). HPLC fingerprints of all extracts as shown in Figure 6 showed the similar chromatographic characteristics suggested that this HPLC fingerprint could be used for both quantitative and qualitative analysis of leaf extracts of *A. trifoliatus*.

Free radical scavenging activity of the collected extracts was studied by DPPH scavenging assay, it was found that most of the extracts showed strong effects (EC_{50} < 50 μg/ml)

(Cervantes-Cervantes, 2005). Samples which contained high amount of total phenolic and total flavonoid contents significantly exhibited the strongest activity. The high correlations of total phenolic and total flavonoid contents with DPPH scavenging activity were found to be 0.863 and 0.831 ($P < 0.05$), respectively. For the capacity to inhibit iron-induced lipid peroxidation, moderate correlations of 0.586 and 0.389 ($P < 0.05$) were found between the inhibition and total flavonoid and total phenolic contents, respectively (Sithisarn et al., 2011b).

An excess of water in medicinal plant materials will lead to microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. Therefore, limits for the amount of water should be set for plant materials. This is especially important for materials which absorb moisture easily or deteriorate quickly in the presence of water (World Health Organization, 1992). Extracts and the leaves of *A. trifoliatus* collected at 11 different times were investigated for loss on drying as mentioned in BP (2004). Loss on drying of plant samples ranged from 6.58 ± 0.13 to 15.06 ± 0.05 % while the extract contained the loss on drying amount of 8.28 ± 3.30 to 11.51 ± 0.52 %.

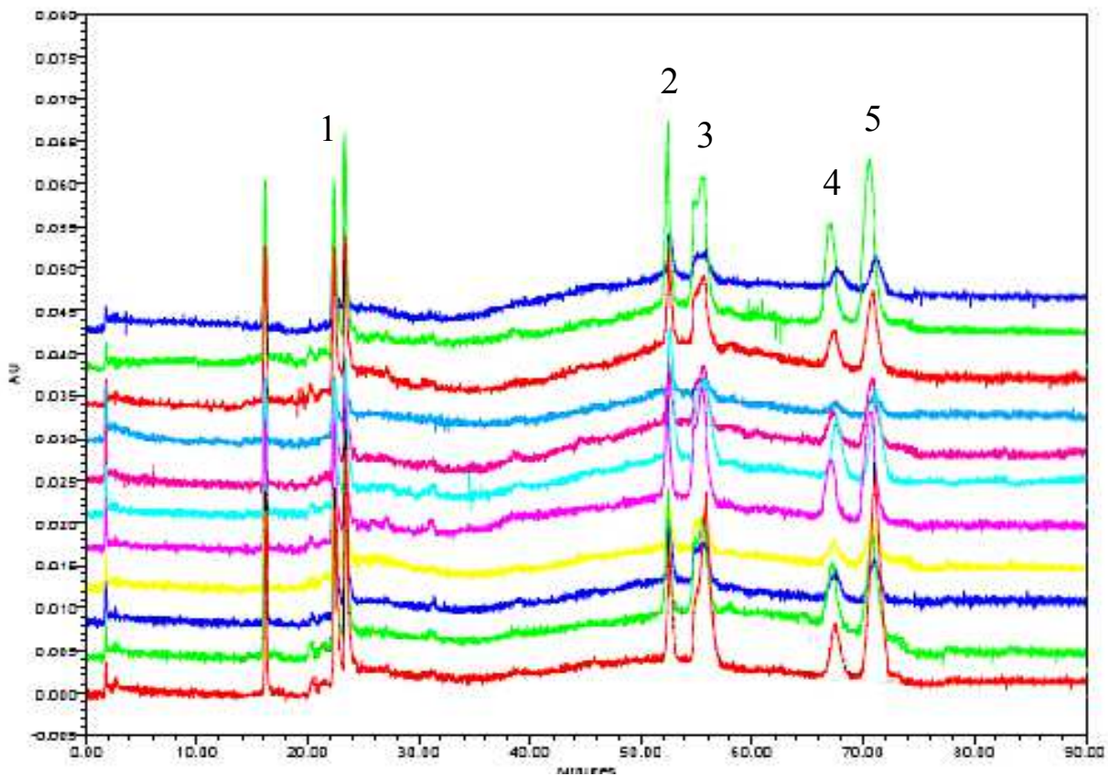


Fig. 6. HPLC chromatogram of leaf decoction extracts from *A. trifoliatus* collected from 11 different durations. No. of peak; 1= chlorogenic acid, 2= 3,5-di-*O*-caffeolyquinic acid, 3= rutin and isoquercetin, 4= 4,5-di-*O*-caffeolyquinic acid, 5= quercitrin.

12. Conclusion

As the mention of Teeguarden (1998), "an adaptogen is substance that helps bring the body into a state of harmony with its environment by introducing chemical, cellular, and

systematic balance. This harmonizing function reduces the effects of unfavorable conditions and stimulates the body's own immune and healing functions. These adaptogenic substances help the body to adapt to various stressful challenges presented by the environment and reduce the damage inflicted on the body. They tend to promote the body's own ability to cope successfully with stress, thus prolonging well-being", the effects of adaptogen could relate to some biological activities including antioxidative, anti-cholinesterase, anti-inflammatory and anti-anxiety, which associated with the excessive activity of stress system (Chrousos and Gold, 1992, Panossian, 2003). Several parts of *Acanthopanax trifoliatus*, a Thai traditional herb promoted *in vitro* antioxidant and anti-cholinesterase activities. Extracts from the leaves of this plant could also exhibit *in vivo* adaptogenic related biological effects including anti-inflammatory and anti-anxiety activities. Polyphenolics and flavonoid, phytochemicals that play important role in antioxidation, anti-inflammatory and adaptogenic actions in plants (Hoorn, 2003, Panossian and Wagner, 2005, Wagner et al., 1994) were found to available in high amount in *A. trifoliatus*, especially in the leaves and the roots. Active polyphenolics and flavonoid compounds were structurally identified. *A. trifoliatus* could then be considered as the potential plant that promote adaptogenic effects which conduced to the chemical, biological and physical standardization of the leaf extract of this plant. However, at present there is no *A. trifoliatus* product used for health or medicinal purposes in the marketplace, especially in Thailand. Since young leaves of *A. trifoliatus* have been traditionally consumed as vegetables, and the leaf extract of this plant showed various biological activities related to adaptogenic properties with no acute toxicity was found, therefore, it would be great opportunity to develop the effective, high quality and standardized health supplement or herbal medicine for adaptogenic related purposes in the near future.

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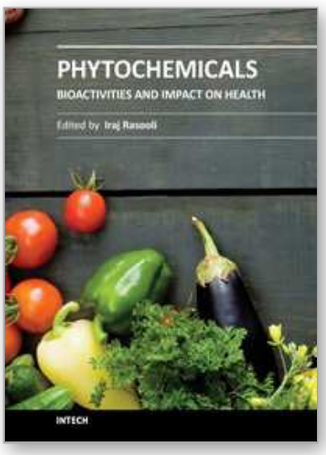
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Among the thousands of naturally occurring constituents so far identified in plants and exhibiting a long history of safe use, there are none that pose - or reasonably might be expected to pose - a significant risk to human health at current low levels of intake when used as flavoring substances. Due to their natural origin, environmental and genetic factors will influence the chemical composition of the plant essential oils. Factors such as species and subspecies, geographical location, harvest time, plant part used and method of isolation all affect chemical composition of the crude material separated from the plant. The screening of plant extracts and natural products for antioxidative and antimicrobial activity has revealed the potential of higher plants as a source of new agents, to serve the processing of natural products.

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