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Phenolic Constituents and Antioxidant Properties of some Thai Plants

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1. Introduction

Thai plants have been used as medicines for many centuries because they contain active phytochemicals including phenolic compounds. These components function as antibiotics, help to make cell walls impermeable to gas and water, act as structural materials to give plants stability and provide protection against ultraviolet (UV) light. Hence, plants in the tropical zone including Thailand contain a high concentration of phenolic compounds formed as secondary metabolites in plants (Shahidi & Naczk, 2003).

Several parts of edible plants from tropical and subtropical climates are known to contain many phenolic compounds which are receiving increasing interest from consumers for several reasons (Leong & Shui, 2002). Epidemiological studies have suggested that relationships exist between the consumption of phenolic-rich foods or beverages and the prevention of diseases such as cancer, stroke, coronary heart desease and others. This association has been partially explained by the fact that phenolic compounds retard oxidative modification of low density lipoproteins (LDL), which is implicated in the initiation of arteriosclerosis. More recently, alternative mechanisms have been proposed for the role of antioxidants in reducing the incidence of cardiovascular disease, besides that of the simple protection of LDL from reactive oxygen species (ROS)-induced damage. Several phenolic antioxidants significantly affect cellular responses to different stimuli, including cytokines and growth factors. Although many papers have reported studies on the composition and antioxidant activity of phenolic compounds in tropical edible plants (Auddy et al., 2003; Nũnez sellés et al., 2002; Habsah et al., 2000), information about the phenolics and antioxidant potential of Thai edible plant species is limited compared to the broad biodiversity of edible plants grown. Moreover, many studies have been reported in the Thai language, and are not available to English speaking scientists.

The aim of this chapter is to provide a critical review of the composition and antioxidant properties of phenolic compounds of some Thai plants. In addition, factors affecting extraction of these components from Thai plants are reported.

2. Phenolic compounds of some Thai plants

Plant materials contain many phytochemicals including compounds with antioxidant activity, which are mostly phenolic in structure(Johnson, 2001). Compounds with

antioxidant activity are mainly phenolic acids, flavonoids and polyphenols (Dillard & German, 2000). Phenolic acids such as caffeic acid and gallic acid are widely distributed in the plant kingdom. The most widespread and diverse phenolics are the flavonoids which have the same C15 (C6-C3-C6) skeleton and retard oxidationof a variety of easily oxidizable compounds (Zheng & Wang, 2001). Flavonoids include catechins, proanthocyanins, anthocyanidins, flavones, flavonols and their glycosides (Ho, 1992). Flavonoids are ubiquitous in plants, since almost all plant tissues are able to synthesize flavonoids. Among the most widely distributed are the flavonols quercetin and rutin.

Investigations have increased considerably in recent years in order to find natural plant antioxidants to replace synthetic compounds whose use is being restricted due to possible side effects such as carcinogenecity (Zeng & Wang, 2001). Many papers have reported that phenolic plant constituents provide protection against oxidation (Amarowicz et al., 2003; Pokorny, 2001). Phenolic substances inhibit propagation of the oxidation chain reactions due to their resonance stabilized free-radical forms (Lindsay, 1996).

Phenolic compounds possess one or more aromatic rings bearing two or more hydroxyl groups (Ho, 1992). They are closely associated with the sensory and nutritional quality of fresh and processed foods. In general, the leaves, flowers, fruits and other living tissues of the plant contain glycosides, woody tissues contain aglycones, and seeds may contain either (Huang & Ferraro, 1992).

Agricultural based manufacturers in Thailand produce and export many fruit and vegetable products. These Thai plants are widely distributed throughout the tropics particularly in Southeast Asia. Many researchers have shown that several parts of tropical and subtropical plants contain large amounts of natural phenolic phytochemicals, such as flavonoids (Leong & Shui, 2002; Kähkonen et al., 1999; Demo et al., 1998). Hence, there is a potential for Thai plants to be used as sources of phenolic antioxidants and commercial extracts could be prepared from numerous available raw plant materials. Cost, simplicity and safety should be considered in the development of an acceptable extraction procedure (Pokorny & Korczak, 2001).

Due to the diversity and complexity of natural mixtures of phenolic compounds in plant extracts, it is rather difficult to characterize every phenolic compound. Each plant generally contains different mixtures of phenolic compounds. The Folin-Ciocalteu method is a rapid, widely used assay to determine the total concentration of phenolic compounds. It is known that different phenolic compounds vary in their responses in the Folin-Ciocalteu method. Many researchers have reported the total phenolic content of Thai plants as shown in Table 1.

The data clearly indicate that some of these plants are rich in natural phenolic compounds. Many plants with a high phenolic content have an astringent taste (for leaves) or strong colors (for flowers and fruits) due to flavonoid components. Proanthocyanidins contribute astringency to plants and other flavonoids contribute red or violet colors (anthocyanins) or yellow colors (flavonols). Higher total phenolic and flavonoid contents have been found in seeds compared to other tissues. Typically, leaf photosynthesis products including essential nutrients such as sucrose are translocated from leaves to fruits and seeds which are the food storage organs of the plants (Salisbury & Ross, 1992). This leads to a concentration of phenolic compounds in seeds. Thai plant samples (Table 1) may be classified into two groups with high and low contents of polyphenolic phytochemicals, Samples having

Scientific name	Common name	Plant part	Moisture Content (%)	Total phenolics (mg GAE/g db plant)¥	Total flavonoids (mg RE/g db plant)	Total carotenes (mg%)	Total xanthophyll (mg%)	Tannin (mg% of tannic acid equivalent)
		Herb and vegetable						
Acacia pennata	Acacia leaf	Young leaves	-	121.00 [a]	-	1.27	1.59	11.1
Acanthopanax trifoliatum		Leaves	-	275.00[a]	-	2.54	3.17	57.30
Allium ascalonicum Linn.¥	Onion	Flower	94.70	55.70	20.20	-	-	-
Artemisia dubia Wall. ex DC. (Syn. *A. vulgaris* L. var. *indica* Maxim.)		Stem and leaves	-	14.24	-	-	-	-
Aspidistra sutepensis K. Larsen		Flower	-	5.06	-	-	-	-
Azadirachta indeca A. Juss Var. siamensis valeton¥		Flower	77.20	40.30	29.80	-	-	-
Basella alba Linn.¥	Ceylon spinash	Leaves	93.5	15.50	6.2	-	-	-
Bidens bipinnata , L.		Stem and leaves	-	34.18	-	-	-	-
Bidens pilosa Linn.		Stem and leaves	-	24.62	-	-	-	-
Buddieia asiatica Lour	Rachawadi pa	Stem and leaves	-	19.17	-	-	-	-
Cassia siamea Britt.¥,	Thai copper pod	Flower	74.8	51.50	24.8	-	-	-
		Leaves	-	384.00 [a]	-	1.92	1.59	110.00
Careya sphaerica Roxb.¥	Tummy wood	Young leaves and Leaves	75.3	54.50	20.5	-	-	-
Centella asiatica Linn.¥,	Pennywort	Leaves	86.6	12.40	10.6	12.80	10.60	24.30
Cratoxylum formosum Dyer.¥		Young leaves and Leaves	85.5	63.40	25.5	-	-	-
Coccinia grandis	Ivy gourd	Leaves	-	74.70 [a]	-	1.94	2.65	17.70
Coleus amboinicus	Country borage	Leaves	-	54.80	-	2.54	4.24	24.30
Commelina diffusa Burm.f.		Stem and leaves	-	19.73	-	-	-	-
Conyza sumatrensis (Retz.) Walker		Stem and leaves	-	15.66	-	-	-	-
Coriandrum sativum	Coriander	Leaves	-	33.0 [a]	-	2.52	1.05	24.30
Cucurbita moschata	Pumpkin	Leaves	-	87.8 [a]	-	1.92	1.59	4.48
Cuscuta australis R. Br.		Stem	-	33.21	-	-	-	-
Diplazium esculentum (Retz.) Sw.		Stem and leaves	-	19.48	-	-	-	-
Dolichandrone serrulata (DC.) Seem.		Flower	-	13.25	-	-	-	-
Dregea volubilis		Leaves	-	100.00[a]	-	6.14	1.07	17.70
Embelia ribes Burm.f.		Leaves	-	57.89	-	-	-	-
Embelia sessiliflora Kurtz		Leaves	-	65.08	-	-	-	-
Erythrina crista Galli.¥	Coral tree	Leaves	81.7	67.50	20.2	-	-	-
Gymnema inodorum		Leaves	-	188.00[a]	-	1.31	1.07	11.1
Hydrocharis dubia (Bl.) Back.*	Frogs bit	Bud	95.0	20.40	8.9	-	-	-

Scientific name	Common name	Plant part	Moisture Content (%)	Total phenolics (mg GAE/g db plant)¥	Total flavonoids (mg RE/g db plant)	Total carotenes (mg%)	Total xanthophyll (mg%)	Tannin (mg% of tannic acid equivalent)
Lasia spinosa (Linn.) Thw.¥		Leaves	94.8	6.40	4.4	-	-	-
Leucaena glauca Benth¥	Lead tree	Young leaves and Leaves	79.9	51.20	22.3	-	-	-
Limnocharis flava Buch.¥		Leaves	94.7	5.40	3.7	-	-	-
Macropanax dispermus		Leaves	-	651.00[a]	-	3.89	1.06	37.4
Marsdenia glabra		Leaves	-	51.50[a]	-	8.92	7.42	4.47
Melicope pteleifolia (Champ.ex Benth.)		Hartley Leaves	-	40.31	-	-	-	-
Mentha arvensis	Japanese mint	Leaves	-	70.0[a]	-	4.48	26.5	21.0
Mentha cordifolia	Kitchen mint	Leaves	-	280.00[a]	-	2.58	4.24	73.7
Micromelum minutum Wight & Arn		Stem and leaves	-	61.15	-	-	-	-
Momordica charantia Linn.*,	Balsum pear	Bud and Leaves	87.0	50.90	21.6	1.31	0.54	4.48
-Musa spiantum Linn.¥	Banana	Flower	92.8	45.30	20.3	-	-	-
Neptunia oleracea	Water cress	Leaves	-	104.00[a]	-	3.18	1.06	21.00
Ocimum americanum	Hairy basil	Leaves	-	43.6[a]	-	5.12	9.52	11.10
Ocimum basilicum Linn.¥,	Sweet basil	Leaves	89.8	50.50	15.3	10.80	13.30	30.90
Ocimum basilicum cultivar Purple Petra[c]		Leaves	-	3.40 (by fresh weight)	-	-	-	-
Ocimum sanctum Linn.¥,	Holy basil	Young leaves and Leaves	87.6	41.90	12.6	5.13	3.18	40.80
Oenanthe stolonifera	Chenese celery	Leaves	-	329.00[a]	-	3.83	14.8	34.2
Orthosiphon grandiflorus	Cat's whisker	Leaves	-	145.00[a]	-	3.20	25.40	30.90
Piper retrofractum	Long pepper	Flower	-	57.5[a]	-	1.28	5.31	7.78
Polycia fruticosa		Leaves	-	46.30[a]	-	2.52	2.13	24.30
Sauropus androgynus Linn.¥		Young leaves and Leaves	89.9	11.50	10.4	-	-	-
Schima wallichii (DC.) Korth.		Leaves	-	206.10	-	-	-	-
Sechium edule	Chayote	Leaves	-	66.1[a]	-	3.83	2.13	4.48
Sesbania grandiflora Desv.¥	Cork wood	Flower	91.1	58.60	13.1	-	-	-
Spondias pinnata Kurz.¥	Hog plum	Young leaves and Leaves	76.4	42.60	14.8	-	-	-
Suaeda maritima		Red mature Leaves	-	38.60	-	-	-	-
		Green young Leaves	-	66.90	-	-	-	-
		Green flower	-	59.30	-	-	-	-
Syzygium gratum (Wight) S.N.Mitra var. gratum¥		Young leaves and Leaves	79.6	57.30	23.6	-	-	-
Tamarindus indica	Tamarind	Young leaves	-	121.00[a]	-	0.64	1.05	77.00

Scientific name	Common name	Plant part	Moisture Content (%)	Total phenolics (mg GAE/g db plant)¥	Total flavonoids (mg RE/g db plant)	Total c (m
Telosma minor	Tonkin jasmine	Flowers	-	98.40 [a]	-	1
Vaccinium sprengelii (G.Don) Sleum.		Leaves	-	95.42	-	
		Berries and fruits				
Amomum krervanh	Siam cardamon	Fruit	-	46.30 [a]	-	1
Capsicum frutescus Linn.¥	Chilli pepper	Fruit	85.4	40.30	13.3	
Eugenia siamensis Craib.¥	Jambolan plum	Fruit	85.1	82.40	44.3	
Euginia malaccenses Linn.¥	Malay apple	Fruit	93.7	69.20	28.7	
Momordica charantia Linn.¥	Balsam pear	Fruit	75.9	50.90	21.6	
Phyllanthus emblica¥	Indian gooseberry	Fruit	82.6	69.10	23.4	
Spondias pinnata Kurz.¥	Hog plum	Fruit	77.3	47.20	12.6	
		Berry and fruit seeds				
Antidesma velutinum Tulas*		Seed	38.4	123.30	50.3	
Cleistocalyx operculatus var. paniala (Roxb.)*		Seed	55.1	173.60	44.2	
Eugenia siamensis Craib.*	Jambolan Plum	Seed	50.3	180.50	50.4	
Leucaena glauca Benth. *	Leadtree	Seed	76.5	20.40	5.3	
Nephelium lappaceum Linn.¥	Rambutan	Seed	36.3	43.50	13.3	
Parkia speciosa Hassk.¥		Seed	70.7	51.90	20.3	
Piper nigrum Linn.¥	Pepper	Seed	72.5	53.10	22.8	
Tamarindus indica Linn¥	Tamarind	Seed	49.5	40.70	23.2	
		Chewing plants				
Acacia catechu (L.F) Wild.*	Black catechu	Bark	16.3	177.70	41.8	
Areca catechu Linn.*	Betel nut	Whole fruit	90.2	52.50	12.6	
		Kernel	91.2	137.30	42.8	
Cassia fistula Linn.*	Golden shower	Stem core	11.4	103.60	25.4	
Piper betel Linn*	Betel leaf	Leaf	82.6	57.50	14.9	

Source: Adapted from ¥Maisuthisakul et al. (2008); *Maisuthisakul et al. (2007a); €Lee & Scagel (2009); ✪ Chuena
Areekul (2009); ⌗Chanwitheesuk, Teerawutgulrag & Rakariyatham (2005)

- means not reported or not determined.

[a] The data were calculated as mg% of pyrocatechol equivalent.

Table 1. Ranges of some phenolic constituents in some Thai plants

relatively high concentrations of phenolic phytochemicals (more than 100 mg GAE/g dry weight of material) included seeds of *Antidesma velutinum* Tulas, *Cleistocalyx operculatus* var. paniala (Roxb.), *Eugenia siamensis* Craib., bark of *Acacia catechu* (L.F) Wild., kernel of *Areca catechu* Linn. and stem of *Cassia fistula* Linn. Chanwitheesuk et al. (2005) reported the total phenolic content of Thai plants using Folin Denis reagent and pyrocatechol as reference, with data calculated as mg% of pyrocatechol equivalents. For comparison, the total phenolic content of Leucaena was reported as 405 mg% (Chanwitheesuk et al., 2005) and 51.20 mg GAE/g db plant (Maisuthisakul et al., 2008). The data showed that the different reference compound and the units used required a conversion factor of around eight to convert the values (Table 1).

Quantitative determination of individual flavonoid glycosides is difficult because most standards are not commercially available. Hence, the total flavonoid content determined using a colorimetric method (Bonvehí et al., 2001) is commonly used for flavonoid evaluation. Generally, the calculation of the total flavonoid content is quoted in units of mg rutin equivalent of flavonoid compounds in one gram of plant extract based on the dry weight of the original plant sample. It is well known that flavonoids possess antioxidant properties both in vitro and in vivo. The flavonoids contain a number of phenolic hydroxyl groups attached to aromatic ring structures, which confer the antioxidant activity. Flavonoids were found in leaves, seeds and fruits and were good antioxidants (Bonvehí et al., 2001). The data clearly indicate that some plants in Thailand are rich in natural antioxidant flavonoids. Some data in Table 1 were used to find a relationship between total phenolic and flavonoid content. The flavonoid content correlates moderately with the phenolic content as shown in Fig. 1.

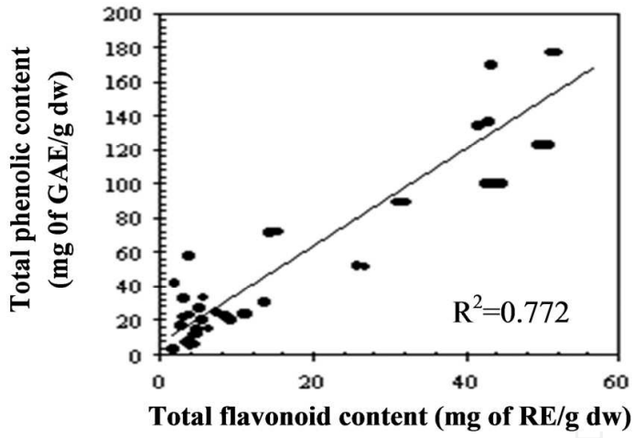


Fig. 1. Relationship between total phenolic and total flavonoid content in Thai plant extracts.

Chanwitheesuk et al. (2005) reported the total carotene, total xanthophyll and tannin contents of edible plants grown in Thailand as shown in Table 1. Total carotenes and total xanthophylls were determined according to Helrich (1990). Tannin contents were determined colorimetrically using the Folin-Dennis reagent according to Helrich (1990).

Identification of the phenolic components in Thai plants has not commonly been reported because it is expensive and standards required for identification are often not available. Hence, this chapter will report the phenolic profile of only four of the Thai plants

investigated; Piper betel Linn, *Careya sphaerica*, *Cratoxylum formosum* Dyer., and *Leucaena leucocephala* de Wit.

Piper betel Linn.; which is commonly known as Betel leaf, is chewed with Betel nut and lime by some people in Asia. Its Thai name is different in different areas, for instance, Plu-Cheen, Se-ke, Bul-plao-yuan, she-ke (South), Pu (North-West). The leaves are chewed alone or with other plant materials including the areca nut (Areca catechu Linn.) and lime. Many researchers have focused on the red lime betel quid in the past few years. A little information about the Betel leaf was found. The Betel leaf itself has a spicy taste and yields an essential oil widely used as a medicine. In Thailand, it is used to treat bruises, heal urticaria, cure ringworm and joint pain as well as relieving toothache. It is also used in cough and mucus remedies and infusions to cure indigestion, as a topical cure for constipation, as a decongestant and as an aid to lactation. The characteristics and chemical composition of 100 grams of Betel leaves are 44 kcal for energy, 85-90 g of water, 3-3.5 g of protein, 2.3 g of fiber, 0.63-0.89 mg of nicotinic acid, 0.005-0.01 g of vitamin C, 1.9-2.9 mg of Vitamin A, 10-70 µg of thiamine, 1.9-30 µg of riboflavin, 0.1-1.3 g of tannin, 0.05-0.6 g of phosphorus, 1.1-4.6 g of potassium, 0.2-0.5 g of calcium, 0.005-0.007 g of iron, 3.4 µg of iodine (Guha, 2006).

Other biological activities described for the essential oil include antifungal, antiseptic and anthelmintic effects (Evans et al., 1984). It was reported that Betel leaf was rich in carotenes (80 IU/g fresh wt.) and phenolics. Data on the phenolic compounds of this plant have been reported for chavicol (Amonkar, et al., 1986), chavibetol, chavibetol acetate (Rimando, et al., 1986) and eugenol (Nagabhushan, et al., 1989). The major bioactive phenolic compoundsin Thai Betel leaf extracted with ethyl acetate were found to be relatively low in polarity. They are chavicol and two-unknown compounds which show higher polarity than chavicol (Maisuthisakul, 2008). The chemical structures of phenolic compounds found in betel leaf are shown in Fig. 2.

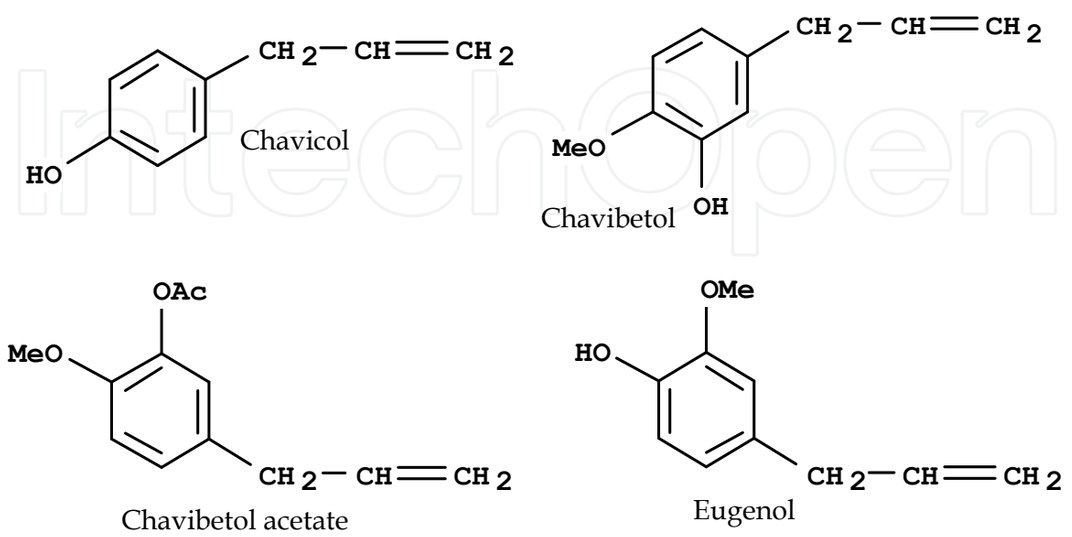


Fig. 2. Some phenolic compounds found in *Piper betel* Linn.

Careya sphaerica Roxb.; This plant is also known as *Careya arborea* Roxb. This plant is normally consumed fresh and mostly found in the North – East of Thailand. Its name is different in different areas, for instance, Kradon, Phak–Kradon, Kradonbok, Kradonkhon (North – East), Khui (Khanchanaburee), Phuk-Pui (North), Puikradon (South), Pui - khao (Chiangmai). Thai people traditionally eat shoots, young leaves and young flowers of this plant. It tastes a little astringent due to the phenolic compounds present. The harvesting season of Kradonbok is during March to May of each year. Kradonbok trees are planted commercially in Sakolnakhon, Kalasin, Yasothorn, Mahasarakham, and Bureerum which are provinces in the North – East of Thailand. Kradonbok has some health benefits such as the use of Kradonbok leaf for healing a wound and flowers for remedying a cough. The characteristics and chemical composition of 100 grams of Kradonbok leaves are 83 Kcal for energy, 1.9 g of fiber, 13 mg of calcium, 18 mg of phosphorus, 17 mg of iron, 3958 IU of riboflavin, 1.8 mg of niacin and 126 mg of vitamin C (Nutrition division, 1992).

There have been few reports about the phenolic compounds of *Careya sphaerica* Roxb. during the last 30 years. Gupta et al. (1975) reported that the phenolic consituents present in the leaf extracts when extracted with petroleum ether at room temperature were lupeol, hexacosanol ($C_{26}H_{54}O$), α-spinosterol ($C_{29}H_{48}O$), teraxerol ($C_{30}H_{50}O$), β-sitosterol ($C_{29}H_{50}O$), quercetin ($C_{15}H_{10}O_7$), taraceryl acetate ($C_{32}H_{52}O_2$) and ellagic acid ($C_{14}H_6O_8$). Careaborin, β-amyrin, careyagenolide, maslinic acid and α-hydroxyursolic acid were also found in the leaves (Das & Mahato, 1982 Das & Mahato, 1982; Talapatra et al., 1981). The chemical structures of some componentsextracted from *Careya sphaerica* Roxb.leaf are shown in Fig. 3.

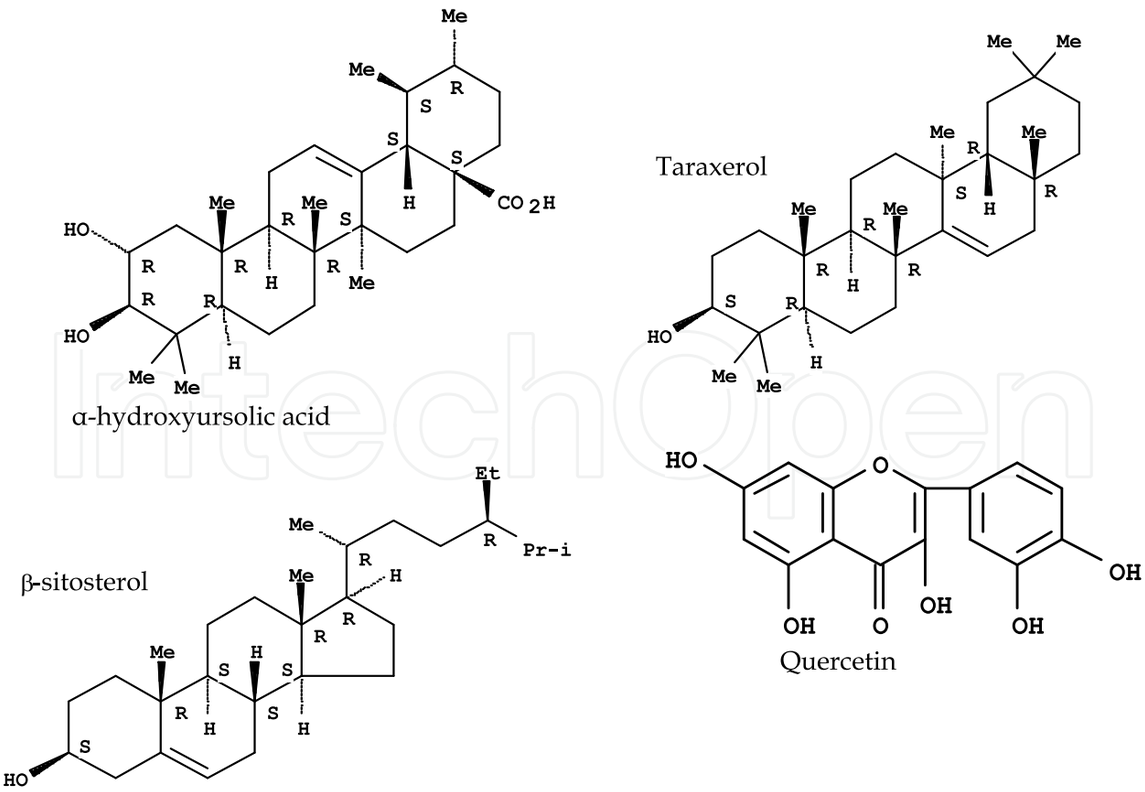
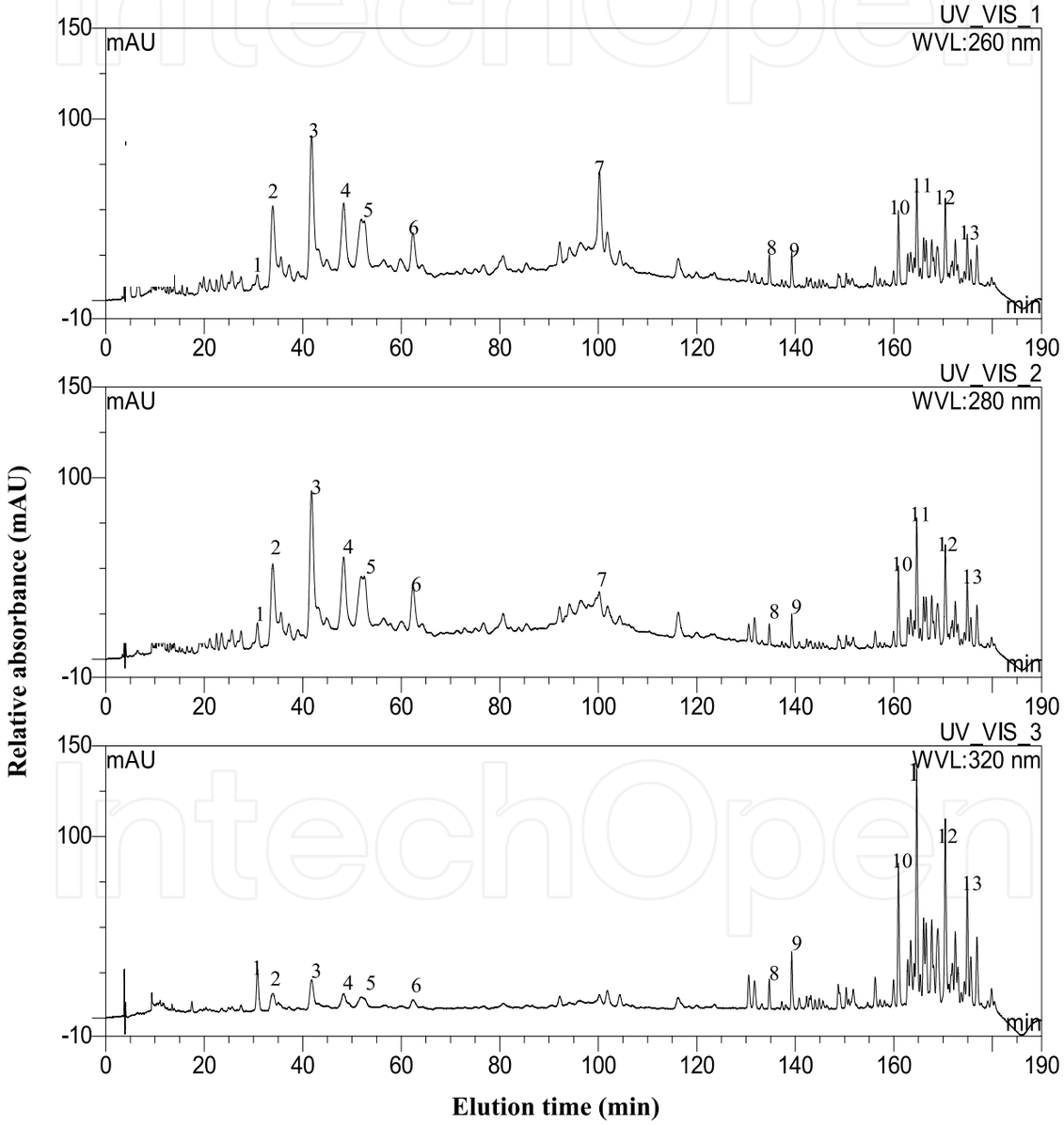


Fig. 3. Some phytochemicals extracted from *Careya sphaerica* Roxb.

The HPLC chromatogram of the Kradonbok extract is shown in Fig. 4. The ethanol extract was dissolved in methanol and passed through a Sep-Pak C18 cartridge (Waters, Milford, MA.). The C-18 cartridge was first conditioned by suction with 1 column volume of methanol followed by 2 column volumes of a 3% HCl solution (v/v) in HPLC grade water. The cartridge was not allowed to dry out during conditioning. The aqueous extract was then transferred to the cartridge. The cartridge bed was then rinsed with HCl (3%, 5 mL) and air-dried under vacuum for ~10 min. Phytochemicals were eluted with HPLC grade methanol. Samples were filtered through a 0.20 mm Millipore filter (type HA) into a 2 mL autosampler



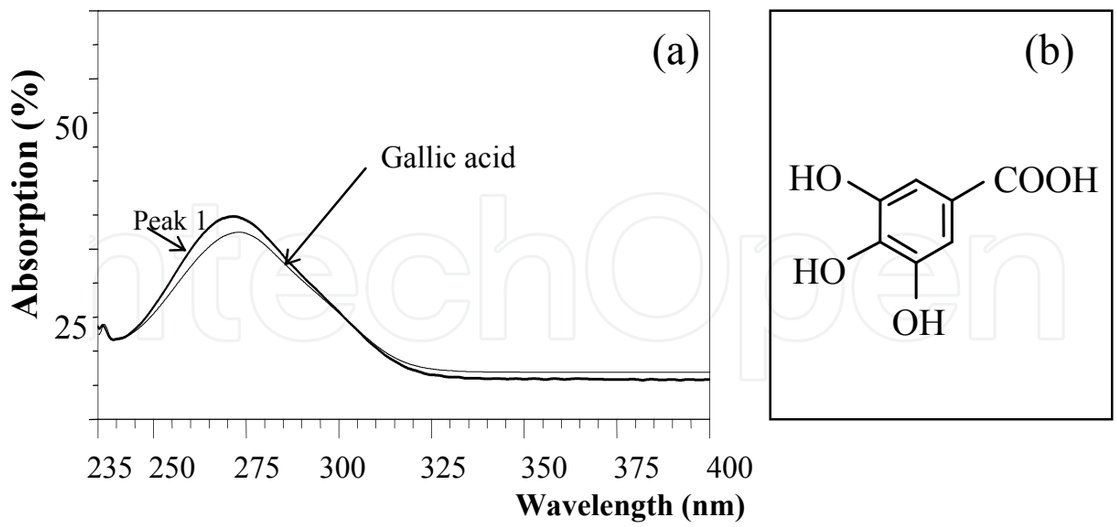
Source: Maisuthisakul (2007)

Fig. 4. HPLC chromatogram for the extract from *Careya sphaerica* Roxb. (Kradonbok) detected at 260, 280 and 320 nm.

vial for subsequent analysis by HPLC. The solution (10 mL) was injected into the HPLC and analyzed according to the following conditions: column, Synergi Hydro RP column (150 × 4.6 mm id., 4mm, Phenomenex), fitted with a Allsphere ODS-2 guard column (10 × 4.6 mm id., Alltech). The HPLC system was equipped with diode array detector (Dionex PDA 100 photodiode array, USA) controlled by Chromeleon software version 6.60 Build 1428 (Dionex Corporation, Sunnyvale, USA). Chromatograms were recorded at 260, 280 and 320 nm.

Thirteen main peaks were detected in Thai Tummy wood leaf extracted with ethanol and the components corresponding to peaks 1, 2, 3, 4, 5, and 6 eluted at 11.06, 33.91, 41.79, 48.35, 51.91 and 62.36 min in the more hydrophilic region (short retention time). The other main components eluted at 100.19 min (peak 7), 134.71 min (peak 8), 139.27 min (peak 9), 160.93 min (peak 10), 164.63 min (peak 11), 170.44 min (peak 12) and 174.90 min (peak 13) in the more hydrophobic region (long retention time).

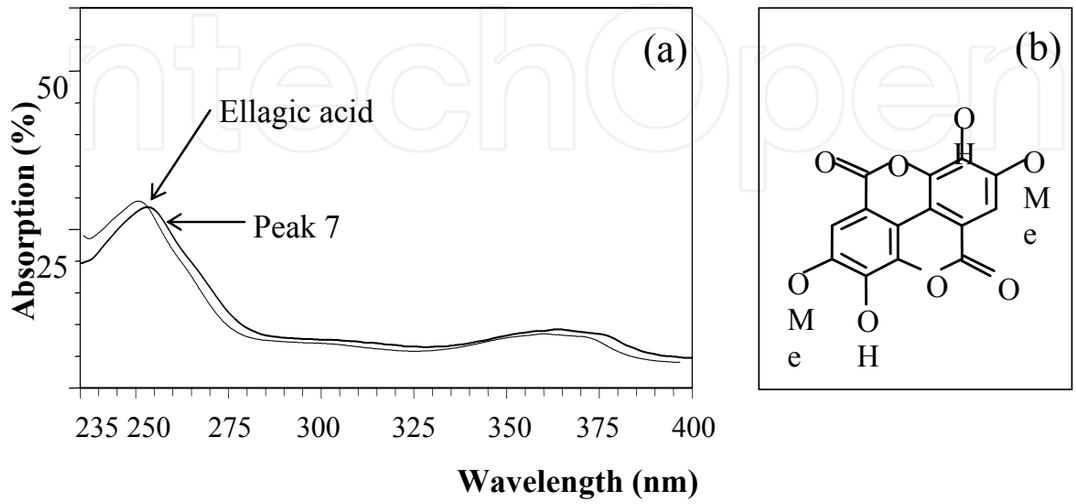
The identity and purity of peak 1 in the HPLC chromatogram of the extract of *Careya sphaerica* Roxb. was determined by comparison of the retention time and UV spectrum (Fig. 5) with that of pure gallic acid. The identification of peak 1 as gallic acid was confirmed by co-injection of gallic acid with the plant extract. HPLC-ESI-MS confirmed the identification of this compound (MS [M + H]+ at m/z 171, MS [M + Na]+ at m/z 193 and MS [M+4Na]+ at m/z 262), which is consistent with the molecular weight of 170.12 for gallic acid. Compound 7 was identified by comparison of the retention time and UV spectrum (Fig. 6) with that of pure ellagic acid. The identification of peak 7 as ellagic acid was confirmed by co-injection of ellagic acid with the plant extract. HPLC-ESI-MS confirmed the identification of this compound (MS [M + H]+ at m/z 303, MS [M + Na]+ at m/z 325), confirming the molecular weight of 302.19 for ellagic acid. Talapatra et al. (1981) reported that ellagic acid was isolated from the leaves of *Careya arborea*, which is the synonym of *Careya sphaerica* Roxb.



Source: Maisuthisakul (2007)

Fig. 5. UV spectrum of (a) peaks 1 of *Careya sphaerica* Roxb. (Kradonbok) extract, gallic acid and (b) structure of gallic acid.

The known antioxidant components 1 (gallic acid) and 7 (ellagic acid) were present at 0.93 % and 2.37 % of the extract. These concentrations were calculated according to the peak areas from the HPLC chromatogram. The thirteen phenolic compounds represented about 56.16% of the total phenolic compounds of Kradonbok extract.



Source: Maisuthisakul (2007)

Fig. 6. UV spectrum of (a) peak 7 of *Careya sphaerica* Roxb. (Kradonbok) extract, ellagic acid and (b) structure of ellagic acid.

Cratoxylum formosum **Dyer.**; The name of this plant differs in different local areas, for instance, Teawkon (Central), Teawdang (North) and Tao (South). Thai people traditionally eat shoots and young leaves of this plant. It tastes sour and a little astringent due to the phenolic phytochemicals present. The harvesting season of *Cratoxylum formosum* Dyer. is during March to May of each year. Trees of this plant are planted commercially in Sakolnakhon, Kalasin, Yasothorn, Mahasarakham, Bureerum, which are the provinces in North – East Thailand. The chemical composition per 100 grams of Teaw leaves which provides 58 Kcal for energy includes 1.5 g of fiber, 67 mg of calcium, 19 mg of phosphorus, 205 mg of iron, 4500 µg of β-carotene, 750 µg vitamin A as retinol, 10.04 mg of thiamin, 0.67 mg of riboflavin, 3.1 mg of niasin and 58 mg of vitamin C (Nutrition division, 1992).

Relatively little work has been done on the phytochemicals in *Cratoxylum* sp. The only studies concerning the phytochemistry of *Cratoxylum* sp. was published by Kitanov & Assenov (1988), and Kumar et al. (2004) who reported that the phenolic compounds in *Cratoxylum pruniflorum* Kurz were quercetin ($C_{15}H_{10}O_{12}$), hyperoside ($C_{21}H_{20}O_{12}$), 1,3,6,7-tetrahydroxyxanthone, mangiferin ($C_{19}H_{18}O_{11}$) and isomangiferin ($C_{19}H_{18}O_{12}$), (Kitanov & Assenov, 1988). The phenolic constituents in *Cratoxylum neriifolium* Kurz were biflavonol GB-2, pentahydroxyflavanone chromone and stigmasterol (Kumar et al., 2004). The chemical structures of some phytochemicals found in *Cratoxylum* sp. leaf are shown in Fig. 7.

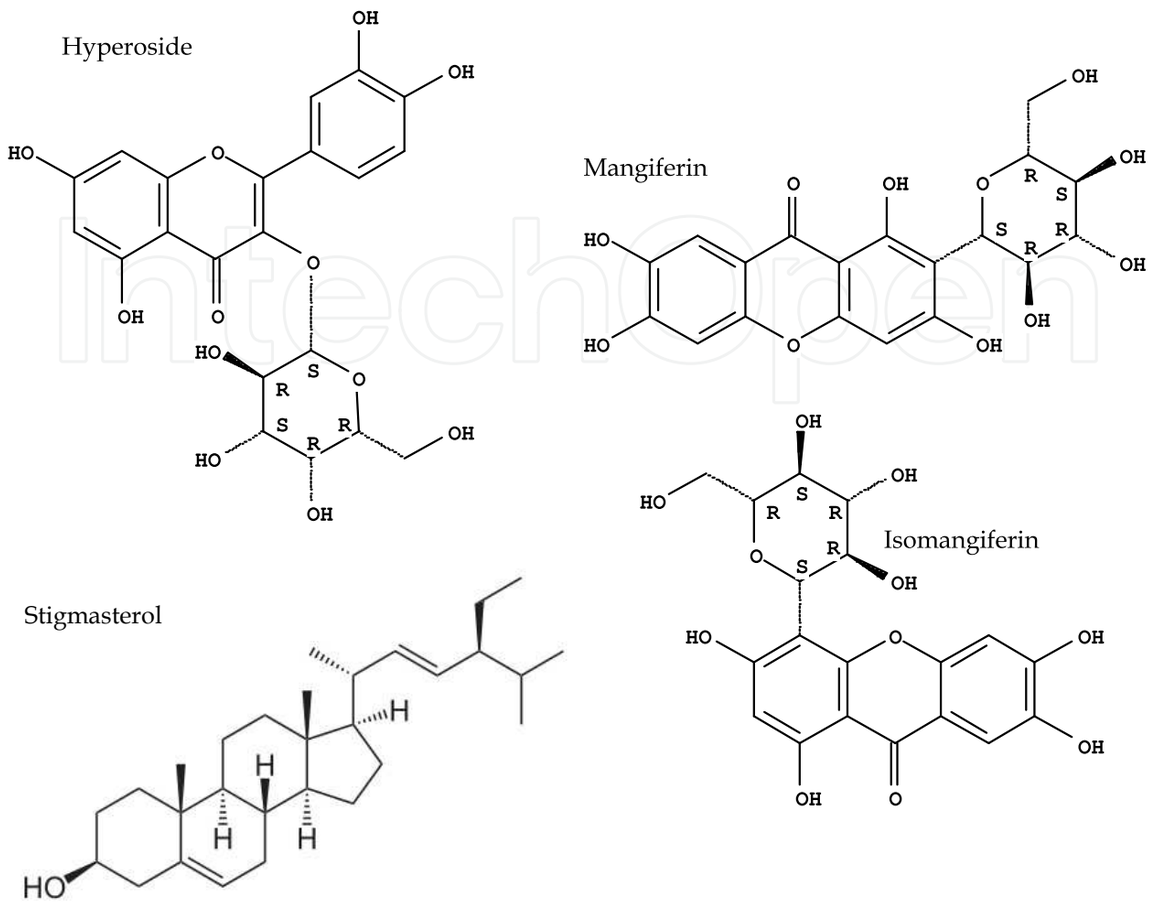
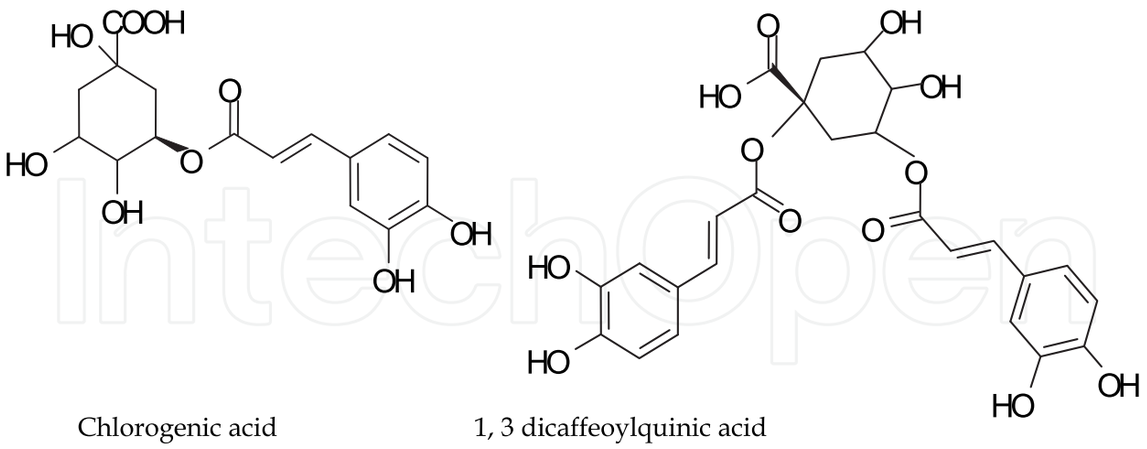


Fig. 7. Some phytochemicals found in *Cratoxylum* sp.

With regards to Thai plants, three active phenolic ingredients were chlorogenic acid, dicaffeoylquinic acid and ferulic acid hexose derivative (Maisuthisakul et al., 2007b). Chlorogenic acid was present at 60 % of the extract. Two minor components (dicaffeoylquinic acid and ferulic acid hexose derivative) were present at 7 % and 2 %, and other components that were present at lower concentrations were also detected. Some chemical structures of phenolic compounds found in Thai *Cratoxylum formosum* leaf are shown in Fig. 8.

Leucaena glauca **Benth**; the plant is found throughout Thailand in the settled areas at low and medium altitudes. It occurs widely and is abundant. Its name is different in different areas, for instance, Kratin-Thai (Central), Satorban (South), Katong and Kratin. Thai people traditionally eat young leaves and the young pod of this plant. The young leaf is found in all seasons, however, it is most abundant during March to May of each year. Kratin trees are planted commercially in Roi-ed, Amnat Charoen, Pichit, Nakhonsawan, Songkla, Krabi, Pattani and Trang. Kratin leaves contain leucine which can absorb selenium. The charcateristics and chemical composition of 100 grams of Kratin leaves are 62 Kcal for energy, 8.4 g of protein, 3.8 g of crude fiber, 137 mg of calcium, 11 mg of phosphorus, 9.2 g of iron, 7883 IU of total vitamin A, 0.33 mg of thiamin, 0.09 mg riboflavin, 1.7 mg of niacin and 8 mg of vitamin C (Nutrition division, 1992).



Chlorogenic acid 1, 3 dicaffeoylquinic acid

Fig. 8. Some phenolic compounds found in *Cratoxylum formosum* in Thailand.

Relatively little information on the phenolic constituents of *Leucaena glauca* Benth has been published during the last 30 years. Chen (1979) found foeniculin ($C_{14}H_{18}O$) and kaempferol-3-xyloside ($C_{20}H_{18}O_{10}$) in the leaves. Guaijaverin ($C_{20}H_{18}O_{11}$), juglania ($C_{20}H_{18}O_{10}$), kaempferol-3-O-β-xyloside and quercitrin ($C_{21}H_{20}O_{11}$) were also found in the leaves (Morita et al., 1977). The chemical structures of phenolic compounds found in Thai *Leucaena glauca* Benth leaf are shown in Fig. 9.

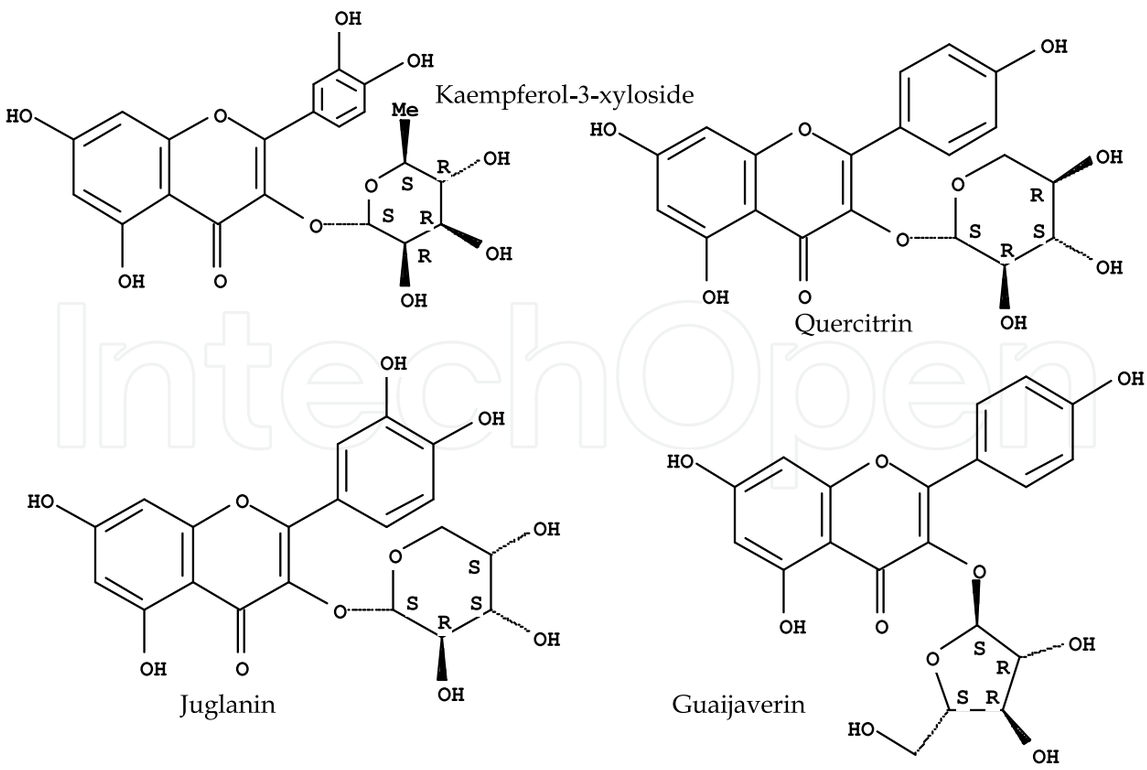


Fig. 9. Some phenolic compounds found in *Leucaena glauca* Benth

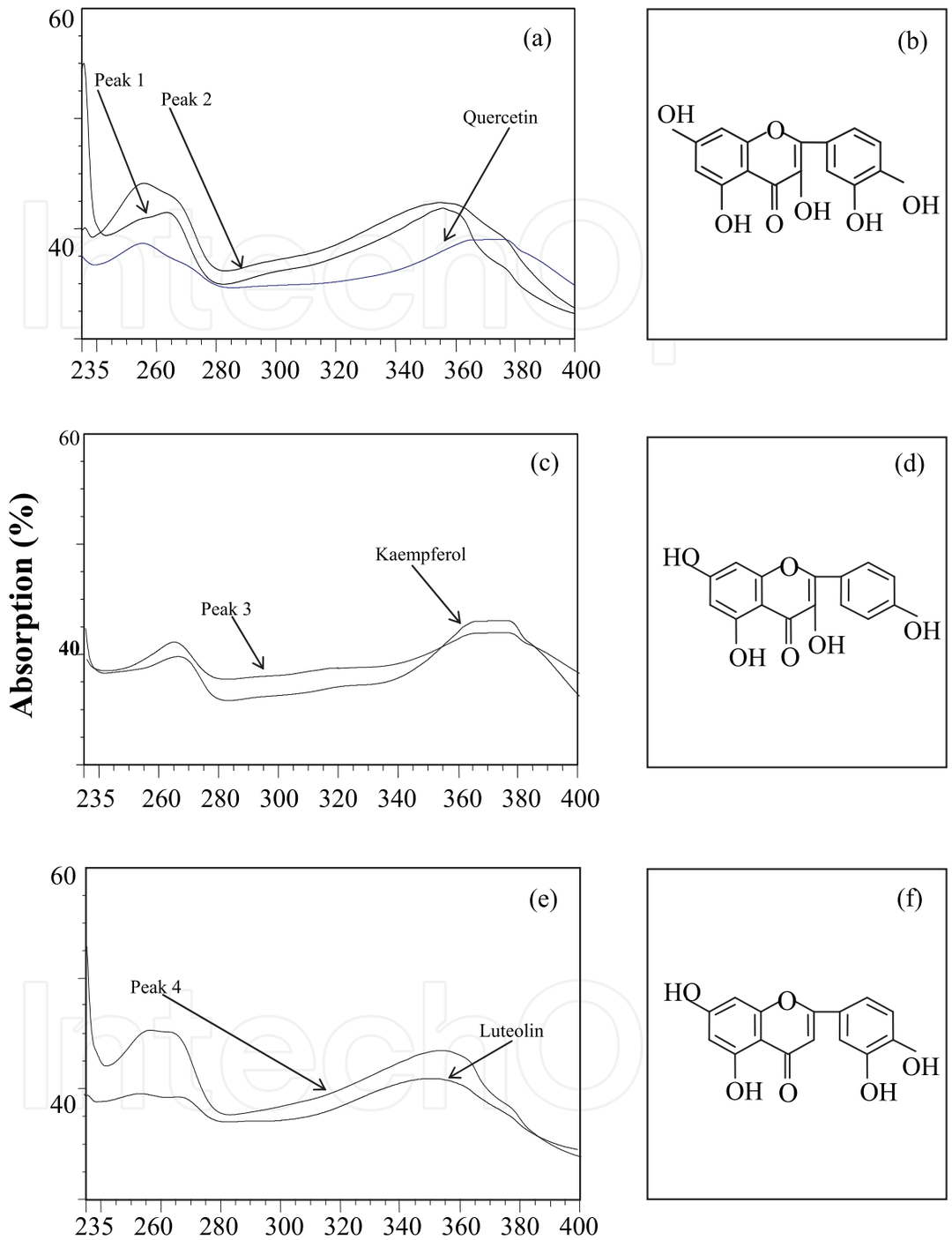
The phenolic components of Leucaena glauca Benth in the HPLC chromatogram appeared as 4 peaks which were present at 15.28%, 6.50%, 9.71% and 36.91%, respectively, and other components that were present at lower concentrations were also observed. These concentrations were calculated from each area by dividing by the total peak area from the HPLC chromatogram. The four phenolic compounds were about 68.4% of the total phenolic content of Leucaena glauca Benth extract. The compounds corresponding to peaks 1, 2, 3, and 4 had a similar UV spectrum to that of flavonoids such as quercetin, and kaempferol which are flavonols and luteolin which is a flavone (Fig. 10). The identification of each peak was confirmed by using comparisons of their UV spectra and LC-MS in both positive and negative mode in order to obtain more information on the structural features of the conjugated forms of the phenolic compounds.

3. Antioxidant properties of some Thai plants

In general, the antioxidant activity of plant extracts is associated with specific compounds or classes of compounds, such as flavones, flavonols and proanthocyanidins in plant materials native to the Mediterranean area (Skerget et al. , 2005), carotenoids (Stahl & Sies, 2003) and melatonin (Chen et al., 2003). Most of the antioxidant substances in plants are phenolic compounds. Phenolic compounds serve as oxidation terminators by scavenging radicals to form resonance stabilized radicals (Rice-Evans et al., 1997).

Although the antioxidant capacities are influenced by many factors, which cannot be fully described with a single method, the DPPH radical scavenging activity is the most commonly used method for assessment of antioxidant properties of natural products. The DPPH• assay overcomes the limitations of monitoring the activity of the numerous samples over a specified period of time. It is reproducible and strongly correlated with phenolic compounds (Maisuthisakul et al., 2007; Katalinic et al., 2006; Miliauskas et al., 2004; Matsuda et al., 2001). In addition the radical scavenging method had many benefits compared to the lipid oxidation method (Nuutila et al., 2002). Gallic acid, which has been used as a standard, has been reported to be the most abundant phenolic compound in plants (Witzell et al., 2003., Nuutila et al., 2002). The EC_{50} is defined as the amount of antioxidant required to cause a 50% reduction in the absorbance of DPPH. These values were changed to antiradical activity defined as $1/EC_{50}$, since this parameter increases with antioxidant activity. The antiradical activity of α-tocopherol was 0.67 (Maisuthisakul et al., 2008). Many researchers have reported the total phenolic content of Thai plants as shown in Table 2. The plants which had strong antiradical activity (Table 2) had high total phenolic and flavonoid contents (Table 1).

In Thailand, there are many plants which show antiradical activity higher than α-tocopherol such as *Careya sphaerica* Roxb., *Cratoxylum formosum* Dyer., *Erythrina crista* Galli., *Leucaena glauca* Benth, *Momordica charantia* Linn., *Ocimum basilicum* Linn., *Ocimum sanctum* Linn., *Syzygium gratum* (Wight) S.N.Mitra var. gratum, *Allium ascalonicum* Linn., *Azadirachta indeca* A. Juss Var. siamensis valeton, *Cassia siamea* Britt., *Musa spiantum* Linn., *Sesbania grandiflora* Desv. The antiradical activity of Thai fruits and fruit seeds were higher than that of α-tocopherol except for seeds of *Leucaena glauca* Benth (Table 2).



Source: Maisuthisakul (2007)

Fig. 10. UV spectrum of (a) peaks 1, 2 of *Leucaena glauca* Benth (Kratin) extract, quercetin, (b) structure of quercetin, (c) UV spectrum of peaks 3 of Kratin extract, kaempferol, (d) structure of kaempferol, (e) UV spectrum of peaks 4 of Kratin extract, luteolin and (f) structure of luteolin.

Scientific name	Local name	Plant part	Antiradical activity (1/ EC_{50})
	Herb and vegetable		
Allium ascalonicum Linn.¥	Hom	Flower	2.6
Artemisia dubia Wall. ex DC. (Syn. *A. vulgaris* L. var. *indica* Maxim.)⌘	Hia	tem and leaves	0.19
Aspidistra sutepensis K. Larsen ⌘	Nang-laeo	Flower	0.06
Azadirachta indeca A. Juss Var. siamensis valeton¥	Sa-dao	Flower	1.2
Basella alba Linn.¥	Plang	Leaf	0.7
Bidens bipinnata , L.⌘	Ya-Puen-Laem Nok-Sai	Stem and leaves	1.04
Bidens pilosa Linn.⌘	Peen-nok-sai	Stem and leaves	1.22
Buddieia asiatica Lour ⌘	Ra-cha-wa-di-pa	Stem and leaves	0.26
Careya sphaerica Roxb.¥	Kra-don	Young leaf and leaf	2.3
Cassia siamea Britt.¥	Kee-lek	Flower	2.4
Centella asiatica Linn.¥	Bua-bok	Leaf	0.7
Cratoxylum formosum Dyer.¥	Tew	Young leaf and leaf	4.4
Commelina diffusa Burm.f.⌘	Plap	Stem and leaves	0.64
Conyza sumatrensis (Retz.) Walker ⌘	Ya-khamai	Stem and leaves	0.46
Cuscuta australis R. Br.⌘	Khruea-kham	Stem	1.08
Diplazium esculentum (Retz.) Sw.⌘	Kut-khao	Stem and leaves	0.63
Dolichandrone serrulata (DC.) Seem.⌘	Khae-pa	Flower	0.06
Embelia ribes Burm.f.⌘	Som-jee	Leaves	2.86
Embelia sessiliflora Kurtz ⌘	Som-kui	Leaves	5.0
Erythrina crista Galli.¥	Tong-lang	Leaf	3.1
Hydrocharis dubia (Bl.) Back.*	Tub-tao	Bud	0.82
Lasia spinosa (Linn.) Thw.¥	Nham	Leaf	0.1
Leucaena glauca Benth¥	Kra-tin	Young leaf and leaf	1.5
Limnocharis flava Buch.¥	Pai	Leaf	0.1
Melicope pteleifolia (Champ.ex Benth.)⌘	Sa- Riam -Dong	Hartley Leaves	0.18
Micromelum minutum Wight & Arn ⌘	Sa-mui	Stem and leaves	0.83
Momordica charantia Linn.*	Ma-ra-khee-nok	Bud and leaf	1.70
Musa spiantum Linn.¥	Hua-plee	Flower	1.8
Ocimum basilicum Linn.¥	Ho-ra-pa	Young leaf and leaf	1.80
Ocimum sanctum Linn.¥	Ka-prow	Young leaf and leaf	1.8
Sauropus androgynus Linn.¥	Whan-ban	Young leaf and leaf	0.7
Schima wallichii (DC.) Korth.⌘	Talo	Leaves	12.5
Sesbania grandiflora Desv.¥	Kae	Flower	1.7
Spondias pinnata Kurz.¥	Ma-kok	Young leaf and leaf	0.7
Stachytarpheta jamaicensis (L.) Vahl (*S.indica* Vahl)✡	Pun-ngu-keaw	Leaf Stem Root Inflorescence	0.016 0.018 0.018 0.020
Syzygium gratum (Wight) S.N.Mitra var. gratum¥	Mek	Young leaf and leaf	1.8
Tiliacora triandra (Colebr.) Diels ⌘	Ya-nang	Leaves	0.15
Vaccinium sprengelii (G.Don) Sleum.⌘	Som-pi	Leaves	1.89
	Berries and fruits		
Capsicum frutescus Linn.¥	Prik	Fruit	1.8

Scientific name	Local name	Plant part	Antiradical activity (1/ EC_{50})
Eugenia siamensis Craib.¥	Chom-pu-nam	Fruit	5.0
Euginia malaccenses Linn.¥	Chom-pu-ma-meaw	Fruit	2.2
Momordica charantia Linn.¥	Mara-khee-nok	Fruit	1.7
Phyllanthus emblica¥	Ma-kham-pom	Fruit	2.0
Spondias pinnata Kurz.¥	Ma-kok	Fruit	1.6
	Seeds		
Antidesma velutinum Tulas*	Ma-mao	Seed	14.28
Cleistocalyx operculatus var. paniala (Roxb.)*	Ma-kieng	Seed	11.11
Eugenia siamensis Craib.*	Chom-pu-nam	Seed	6.67
Leucaena glauca Benth. *	Kra-tin	Seed	0.14
Nephelium lappaceum Linn.¥	Ngo	Seed	2.2
Parkia speciosa Hassk.¥	Sa-tor	Seed	1.5
Piper nigrum Linn.¥	Prik-Thai-dum	Seed	3.0
Tamarindus indica Linn¥	Ma-kham	Seed	2.0
	Chewing plants		
Acacia catechu (L.F) Wild.*	See-sead	Bark	20.0
Areca catechu Linn.*	Mhak	Whole fruit	2.13
		Kernel	5.56
Cassia fistula Linn.*	Kaen-khun	Stem core	6.25
Piper betel Linn*	Bai-plu	Leaf	3.13

Source: Adapted from ¥Maisuthisakul et al. (2008); *Maisuthisakul et al., 2007a; €Lee & Scagel, 2009; ✪ Kerdchoechuen & Laohakunjit, 2010; ✡Ongard & Dara, 2010; ✂Phomkaivon & Areekul (2009)
- means not reported or not determined.

Table 2. Ranges of antiradical activity in some Thai plants

4. Factors affecting extraction of plant phenolics

Natural antioxidants are available from raw materials of variable composition. Both the content of active substances and the content of various other compounds may vary. The quality of natural extracts and their antioxidative activity depends not only on the quality of the original plant, date and storage, but also the extraction conditions which affect the plant phenolic compounds extracted (Moure et al., 2001).

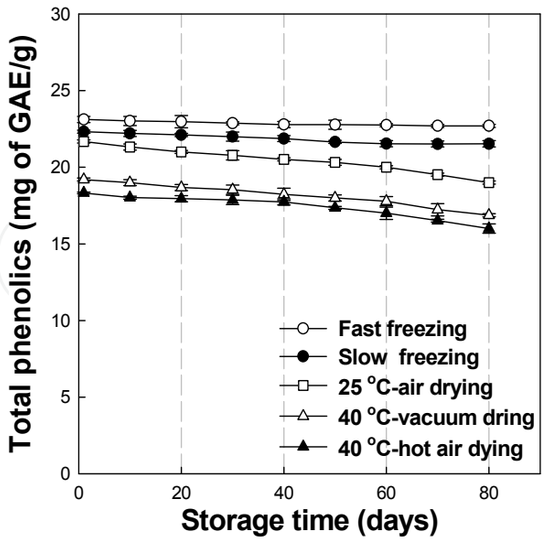
4.1 Sample preparation for storage

Sample preparation is required to keep samples for a certain period of time before analysis since even the total phenolic content was shown to decrease during storage compared with the fresh leaves. In addition, the availability of raw materials is usually limited to a harvesting season. The preliminary processing of plants is necessary for storage.

Temperature is the major factor influencing changes in antioxidant activity during storage (Moure et al., 2001). From numerous publications, the preservative processes before storage vary such as drying citrus peel and seed in an oven at 40 ^{o}C and keeping at room temperature (Bocco et al., 1998) or drying Indian Laburnum by air at 25^{o}C and keeping at room temperature (Siddhuraju et al., 2002) or keeping berries in a still air freezer at -25 to -30^{o}C (Amakura et al., 2000).

Sample preparation methods gave significant effects on total phenolic content and antioxidant activity but had no marked effects on yield of the extract of *Careya sphaerica* or Kradonbok (Maisuthisakul & Pongsawatmanit, 2005). Freezing, especially fast freezing, for keeping the leaves until extraction gave a higher total phenolic content compared with those obtained from other drying methods and slow freezing (Fig. 11). The drying methods studied were (1) hot air drying by tray dryer at 40°C for 18 h with air velocity about 0.5 m/s; (2) vacuum drying by vacuum dryer at 40°C, 100 mmHg (EYELA, model VOS-300SD, Japan) for 10 h; (3) air drying at room temperature 25°C for 12 h with air velocity about 3.2 m/s. The rate of freezing affected the total phenolic content obtained because larger ice crystals grew during slower freezing which would damage plant cells and cause a loss of antioxidant activity. Enzymes from plant cells such as lipoxygenase can oxidize polyphenols (Akoh & Min, 1997). The total phenolic content obtained from air drying was higher than those obtained from vacuum drying and hot air drying because some phenolic components may be degraded by higher temperature (Moure et al., 2001). This effect also found in fresh Mulberry leaves, where the amount of flavonoids was higher in air-dried samples than that in oven-dried samples, probably due to decomposition after storage (Zhishen et al., 1999).

Normally, temperature affects the compounds' stability due to chemical and enzymatic degradation. These mechanisms were reported as mainly responsible for a reduction in phenolic content (Larrauri et al., 1997). Maisuthisakul & Pongsawatmanit also reported that the reduction in antioxidant activity was higher than that expected from the reduction in phenolic contents, probably due to the synergistic effect of natural phenolics (Moure et al., 2001). In addition, phenolics can react with other plant components, and prolonged exposure at moderate temperatures can also cause phenolic degradation. Therefore, sample preparation conditions including temperature and time before storage should be controlled.



Source: Adapted from Maisuthisakul & Pongsawatmanit (2005)

Fig. 11. Total phenolic content of the dried extracts from *Careya sphaerica* leaves obtained by various sample preparation methods.

4.2 Extraction conditions

Sovent effect; solvent extraction is more frequently used for isolation of antioxidants and both extraction yield and antioxidant activity of extracts are strongly dependent on the solvent, due to the different antioxidant potential of compounds with different polarity (Marinova & Yanishlieva, 1997). Apolar solvents are among the most common solvents for removing polyphenols from water. Ethyl acetate and diethyl ether have been used for extraction of low molecular weight phenols from oak wood (Fernández de Simón et al., 1996). Ethanol and water are the most widely employed solvents for reasons of lack of toxicity and abundance, respectively.

With regards to Thai plants, Maisuthisakul (2007) reported that the solvent used had significant effects on antioxidant activity, total phenolic content, yield and partition coefficient of Thai betel leaf extract ($p < 0.05$). The antioxidant activity assessed by DPPH and ABTS radicals was stronger with less polar solvents (Table 3). The results showed that the DPPH activity of the extract obtained with ethyl acetate was significantly higher than that obtained with the other solvents. Total phenolic content confirmed this finding. The extract with a solvent which has higher polarity was found to contain rather small amounts of phenolic compounds. The EC50 and TEAC value of α- tocopherol was also measured and gave values of 14.95±0.23 µg.mL-1 and 2.30±0.03 mmol of Trolox/g sample, respectively. Betel leaf extracted with ethyl acetate (Table 3) had a weak antioxidant activity compared to α- tocopherol. The antioxidant activity and total phenolic contents were significantly different from various solvent extractions. The antioxidant activity values were consistent with those of Chen et al. (2001).

The effectiveness of phenolic antioxidants is often dependent on their polarity. Decker (1998) used the term "antioxidant paradox" to describe how polar antioxidants are most effective in bulk lipids while nonpolar antioxidants are most effective in dispersed lipids. The polarity of the Betel leaf extract was assessed by determination of the oil-water partition coefficient by HPLC. The oil-water partition coefficient was calculated by summing the areas of the three phenolic peaks in the HPLC chromatogram. The oil-water partition coefficient of the Betel leaf extract from ethyl acetate was significantly different from those extracted with other solvents as shown in Table 4.

Solvent used	DPPH activity (EC_{50}, µg.mL^{-1})	TEAC (mmol Trolox/g sample)	Total phenolic content (mg GAE/g sample)
Methanol	36.65 ± 2.60^{a}	2.01± 0.06^{c}	49.89 ± 0.21^{d}
Ethanol	33.85 ± 2.81^{ab}	2.12± 0.03^{bc}	50.38 ± 0.08^{c}
Acetone	30.09 ± 1.21^{b}	2.21± 0.04^{ab}	53.28 ± 0.19^{b}
Ethyl acetate	17.04 ± 0.51^{c}	2.34± 0.06^{a}	55.35 ± 0.14^{a}

Note: ¥ Data followed by different letters within each column are significantly different according to Duncan's multiple range tests at $P < 0.05$. Data were represented as means from three replicate measurements.
Source: Maisuthisakul (2007)

Table 3. Antioxidant activity and total phenolic content of Betel leaf extracted by different solvents¥

Solvent used	Partition coefficient of solvent#	Partition coefficient of extract
Methanol	-0.77	2.02± 0.01[a]
Ethanol	-0.32	2.09± 0.02[ab]
Acetone	-0.24	2.15± 0.01[b]
Ethyl acetate	0.66	2.31± 0.03[c]

Note: ¥ Data followed by different letters within each column are significantly different according to Duncan's multiple range test at $P < 0.05$. Data were represented as means from three replicate measurements.
Data obtained from literature review.
Source: Maisuthisakul (2007)

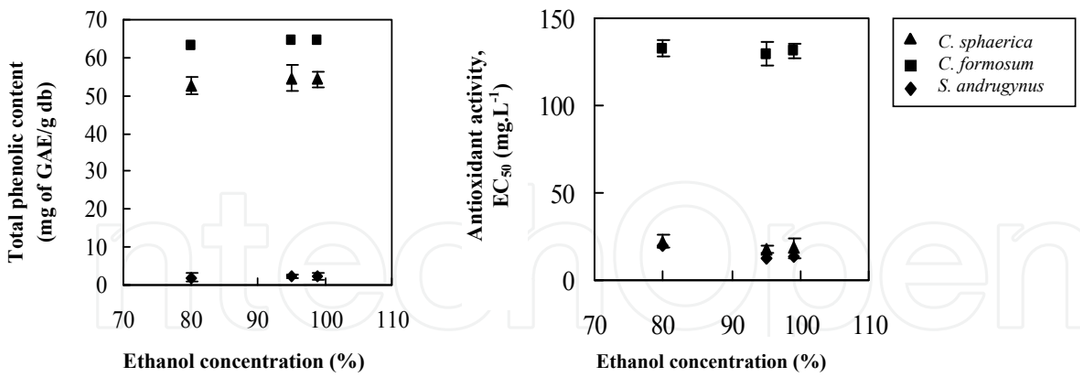
Table 4. Partition coefficient of solvent used and Betel leaf extract extracted by different solvents¥

The phenolic compounds in Betel leaf from the literature include low polarity compounds such as chavicol (log P = 2.50±0.21), chavibetol (log P = 2.30±0.23), chavibetol acetate (log P = 2.39±0.24), and eugenol (log P = 2.20±0.23). Normally, a frequently used descriptor for the estimation of the lipophilicity of phenolic compounds is the partition coefficient. The partition coefficients of Betel leaf extracts were those of low polarity compounds since the value is higher than 0 (Munishwar, et al., 1997) (Table 4). Less polar solvents showed higher extraction efficacy due to the low polarity of the phenolic compounds of Betel leaf extract. Compounds, which have a polarity similar to the solvent, are able to dissolve more than compounds with different polarity. It can be noted here that Betel leaf extract is rich in less polar phenolic compounds. The solvent used for extraction also affected the total phenolic content in the extracts.

The ratios of solvents used in mixed solvents affected the phenolic content extracted from some Thai plants. *Careya sphaerica* Roxb. (Kradonbok), *Cratoxylum formosum* Dyer. (Teaw) and *Sauropus andrugynus* Merr. (Phak whan ban) leaves were used to study the effect of ethanol concentration used in the extraction. The total phenolic content of plant extracts readily increased with increasing concentration of ethanol from 80% to 95%, but there was no significant difference between the extracts using ethanol concentrations of 95% and 99% for each plant. The effect of ethanol concentration on antioxidant activity and total phenolic content was similar (Fig. 12).

The inflorescence, leaves, stem and root of *Stachytarpheta indica* Vahl were extracted with three methanol and water solvent mixtures, namely water, 75% methanol and 50% methanol. The results showed that the leaf extract from 75% methanol had the highest antioxidant activity in both fresh and dry samples (Ongard & Dara, 2010).

Extraction temperature effect; the temperature of extraction affects the compounds' stability due to the decomposition of phenolic compounds. The effect of temperature has been studied in the extraction of anthocyanins. They were shown to be degraded since the visible spectrum showed both a reduction in the peak at 400-500 nm and reduction in the red color. The temperature during extraction can affect extractable compounds to different extents; boiling and resting increases the total phenol content extracted from *Quercus suber* cork (Conde et al., 1998). Milder extraction temperatures are desirable in those cases where some compounds can be degraded, e.g. carnosic acid (Ibañez et al., 1999).



Source: Adapted from Maisuthisakul (2007)

Fig. 12. Total phenolic contents and antioxidant activity of the extracts from *Careya sphaerica* Roxb., *Cratoxylum formosum* Dyer. and *Sauropus andrugynus* Merr. leaves obtained with various ethanol concentrations.

Other factors; such as extraction time, pH, the particle size of materials, and extraction methods were reported to affect the antioxidant activity and concentrations of phenolic compounds extracted. Sheabar & Neeman (1988) reported the maximum solubility of phenolic compounds from olive rape at pH 4 in the organic phase. The yield of extracted phenolics was correlated with plant cell wall breakdown. Particle size reduction significantly increased the antioxidant activity as a result of both increased extractability and enhanced enzymatic degradation of polysaccharides (Weinberg et al., 1999). Various process conditions (refluxing, shaking and ultrasonic extraction) also affected the concentrations of antioxidants in extracts from balm leaves (Herodež et al., 2003).

5. Conclusion

Many plants in Thailand show potential as a source of extracts rich in phenolic constituents and natural antioxidants. Phenolic compounds are the major antioxidants in plants. Moreover, practical aspects relevant to the use of this class of compounds need to be considered including extraction efficiency, availability of sufficient raw material, and toxicity or safety considerations. To utilize these significant sources of natural antioxidants, further characterization of the phenolic composition is needed.

6. Acknowledgement

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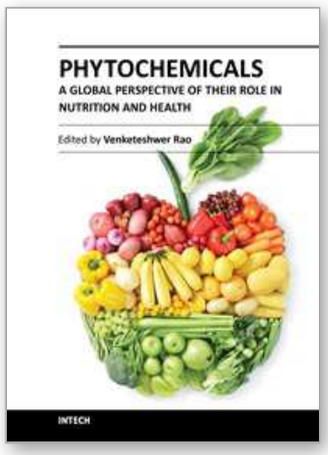

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