International Food Research Journal 21(3): 1045-1052 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Phytochemicals of Thai local edible herbs

[1]Sirithon Siriamornpun, [2*]Chodsana Sriket and [3]Pornpimol Sriket

[1]Research Unit of Process and Product Development of Functional Foods, Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Kuntarawichai, Mahasarakham 44150, Thailand
[2]Program in Food Science and Technology, Faculty of Agriculture, Ubon Ratchathani Rajabhat University, Ubon Ratchathani, 34000, Thailand
[3]Program in Food Business and Nutrition, Faculty of Agriculture, Ubon Ratchathani Rajabhat University, Ubon Ratchathani, 34000, Thailand

Article history

Received: 18 October 2013
Received in revised form: 10 December 2013
Accepted: 13 December 2013

Keywords

Edible herbs
Volatile compounds
Phenolic compounds
Fatty acids

Abstract

Volatile compounds, phenolic acids, phenolic compounds and fatty acids concentration from Thai local edible herbs were studied. *Piper aurantuacum* showed the highest content of 7-hydroxy-5,6,7,8-tetrahydroind (67%). Total phenolic compound (TPC) was highest in *Limnophila aromatic* (19 mg GAE/g) compared with other samples. The main phenolic acids (hydrocinnamic acids) in these herbs were ferulic acid, sinapic acid and protocatechuic acid. Ferulic acid was the major hydrocinnamic acid derivative, ranging from 2 to 359 mg/g, followed by sinapic acid (16 to 225 mg/g) and syringic acid (1 to 41 mg/g). Polyunsaturated fatty acid (PUFA) was the most predominant fatty acid found in analyzed sample, followed by saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA). Four PUFAs; 18:3n-3, 18:2n-6, 18:3n-6 and 20:4n-6 were detected in the samples. These results provided useful information as potential sources of bioactive components for consumers and public health workers.

Introduction

Local herbs have been used as flavoring, seasoning, coloring agents and food preservatives as well as medicinal plants and folk medicines for a thousand years, especially in Thailand, China, India and many other southeastern Asian countries (Shan *et al.*, 2005). Edible herbs, vegetables, fruits, and medicinal herbs are known to have a variety of antioxidant properties. The antioxidant activities could be obtained from leave, roots, rhizome, flowers, fruits, seeds and bark (Balange and Benjakul, 2009). Herbs have also been recognized as antimicrobial, anti-inflammatory, anti-mutagenic, anti-carcinogenic potential (Ceylan and Fung, 2004). Local edible herbs always contribute in Thai dishes, especially in dietary cultures where local edible herbs are used regularly (Carlsen *et al*., 2010). Flavor is usually the result of many volatile and nonvolatile components presented in herbs, possessing diverse chemical and physicochemical properties (Gramatina *et al*., 2010). Whereas the nonvolatile compounds contribute mainly to the taste, the volatile ones influence both taste and aroma. A vast array of compounds may be responsible for the aroma of the food products, such as alcohols, aldehydes, esters, dicarbonyls, short to medium chain free fatty acids, methyl ketones, lactones, phenolic compounds and sulphur compounds (Urbach, 1997). Phenolic compounds have strong *in vitro* and *in vivo* antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions and chelate metals (Shahidi and Naczk, 2004). Increased consumption of phenolic compounds has been correlated with a reduced risk of cardiovascular disease and certain cancers (Shahidi, 2000; Barreira *et al*., 2008). Fatty acids play a major role as an energy source, affect cellular membrane structure and improve resistance to stress (Kamler *et al*., 2008). Polyunsaturated fatty acids (PUFAs) were found in some vegetables including watercress, mint, parsley, spinach, chinese cabbage, brussels sprouts, bok choy, cobs lettuce, broccoli and Chinese broccoli (Youdim *et al.*, 2000; Pereiar and Sinclair, 2001). Dietary intake of unsaturated fatty acids has been shown to reduce the risk of cardiovascular disease (CVD) and possibly the incidence of some cancers and diabetes among other conditions (Erasto *et al.*, 2007). The demand for these commodities has grown in recent years as a consequence of continually increasing consumption of ready-to-eat foods, which include local herbs as ingredients in Thai cuisine (Douglas *et al.*, 2005). The selected local edible herbs were used in various dishes such as Thai curry and spicy soups with local style cooking in Northeastern Thailand. However, the knowledge about volatile compounds, phenolic compounds and fatty acids of local edible herbs consumed in Northeastern Thailand is scared. Therefore, the aim of this research

*Corresponding author.
Email: *psriket@hotmail.com*
Tel: 66 45 352000; Fax: 66 45 352088

was to investigate the volatile compounds, phenolic compounds and fatty acids of eight Thai local herbs from Northeastern Thailand. The receiving results could be useful information for consumers and public health workers.

Materials and Methods

Local edible herbs

Eight local edible herbs namely; kaffir lime (*Citrus hystrix* DC.), indian mulberry (*Morinda citrifolia* Linn.), finger grass (*Limnophila aromatica* Merr.), variegatum (*Piper aurantuacum*), purple velvet plant (*Gynura divaricata* DC.), mulberry (*Morus alba* Linn.), Thai copper pod (*Cassia siamea* Britt.) and asiatic pennywort (*Centella asiatica* Linn.) Urban) were analyzed. The fresh leaves were obtained from three representative markets in the Ubon Ratchathani province during August to November, 2012. At each market, 5 kg samples were sampled from three representative outlets. Single composite samples for each representative market, were prepared by combining about 1000 g of homogenized single sample of the same vegetable variety from three representative outlets and then homogenizing again to obtain a uniform single composite sample. Samples were washed and drained. All samples were freeze-dried before analysis. Analyses were conducted in triplicate (n = 3).

Determination of volatile compounds

The samples were ground and 0.2 g was put in vials. The vials were sealed with an aluminium–rubber septum (Supelco, Bellefonte, PA, USA) and analyzed by the headspace sampling technique (Barcarolo and Casson, 1997). GC-MS analysis was carried out using a GC-2010 chromatograph coupled to a GC/MS-QP2010 (Shimadzu, Japan). Samples were analyzed on a fused-silica capillary column Rtx-5Ms (5% diphenyl 95% dimethyl polysiloxane, 30 mm length, 0.25 mm internal diameter, 0.25 µm film thickness; Restek, U.S.) and Rtx-5 (5% diphenyl 95% dimethyl polysiloxane, 30 mm length, 0.25 mm internal diameter, 0.25 µm film thicknesses; Restek, U.S.). Carrier gas, helium; constant pressure, 134.2 kPa; injector temperature, 250°C; split ratio, 1:5; temperature program, 80 to 250°C at 10°C /min then held isothermal (2 min) at 250°C; ion source temperature, 200°C; transfer line temperature, 250°C; ionization energy, 70 eV; electron ionization mass spectra were acquired over the mass range 35-550 u. Identification of the components was performed by comparing the mass spectra with those on record in the Wiley G 1035 A library (Díaz-Maroto *et al*., 2002).

Determination of total phenolic content

The extracts prepared from the sample (1 g) were extracted for 2 hr with 10 mL of 80% ethanol at room temperature on an orbital shaker set at 180 rpm. The mixture was centrifuged at 1400 x g for 20 min using a refrigerated centrifuge (Beckman Coulter, Avanti J-E Centrifuge, CA, USA) and the supernatant was decanted into a 30 mL vial. The pellet was re-extracted under identical conditions. Supernatant was combined and used for total phenolics contents. Total phenolics content was determined using Folin–Ciocalteu reagent as followed by Abu-Bakar *et al.* (2009) as adapted from Velioglu *et al*. (1998). Briefly, 300 µL of extract was mixed with 2.25 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min; 2.25 mL of sodium carbonate (60 g/L) solution was added to the mixture. After 90 min at room temperature, absorbance was measured at 725 nm using spectrophotometer. Results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g).

Identification and quantification of phenolic compounds

Phenolics extraction

The phenolic compounds in the samples were extracted using a modification of the procedure described by Bengoechea *et al.* (1997) as adapted from Uzelac *et al.* (2005). Each sample (5 g) was mixed with 50 mL of methanol/HCl (100:1, v/v) which contained 2% tertbutylhydroquinone, in inert atmosphere (N_2) during 12 h at 35°C in the dark. The extract was then centrifuged at 4000 rpm/min, and the supernatant was evaporated to dryness under reduced pressure (35–40°C). The residue was redissolved in 25 mL of water/ethanol (80:20, v/v) and extracted four times with 25 mL of ethyl acetate. The organic fractions were combined, dried for 30–40 min with anhydrous sodium sulfate, filtered through the Whatman-40 filter, and evaporated to dryness under vacuum (35–40°C). The residue was redissolved in 5 mL of methanol/ water (50:50, v/v) and filtered through a 0.45 µm filter before injection (20 µl) into the HPLC aperture. Samples were analyzed in triplicate.

HPLC–DAD system for analysis of phenolic compounds

RP-HPLC system for analysis of phenolic compounds HPLC analysis was performed using

Shimadzu LC-20AC pumps, SPD-M20A with diode array detector and chromatographic separations were performed on a LUNA C-18 column (4.6 x 250 mm i.d., 5 µm). The composition of solvents and the gradient elution conditions used were described previously by Bengoechea *et al*. (1997); Schieber *et al.* (2001) and Butsat *et al*. (2009) with some modifications. The mobile phase consisted of purified water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5 to 9% solvent B; from 5 to 15 min, 9% solvent B; from 15 to 22 min, linear gradient from 9 to 11% solvent B; from 22 to 38 min, linear gradient from 11 to 18% solvent B; from 38 to 43 min, from 18 to 23% solvent B; from 43 to 44 min, from 23 to 90% solvent B; from 44 to 45 min, linear gradient from 90 to 80% solvent B; from 45 to 55 min, isocratic at 80% solvent B; from 55 to 60 min, linear gradient from 80 to 5% solvent B and a re-equilibration period of 5 min with 5% solvent B used between individual runs. Operating conditions were as follows: column temperature, 38°C, injection volume, 20 µL, and UV-diode array detection at 280 nm (hydroxybenzoic acids), 320 nm (hydroxycinnamic acids) and 370 nm (flavonols) at a flow-rate of 0.8 mL / min. Spectra were recorded from 200 to 600 nm. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds and were detected using an external standard method. Standards namely: gallic, ferulic, phydroxybenzoic, protocatechuic, *p*-coumaric, caffeic, syringic, sinapic, chlorogenic and vanillic acids were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO).

Determination of lipid and chlorophyll extractions

Lipids and chlorophyll were extracted according to the method of Bligh and Dyer (1959). Approximately 5 g of well-ground samples was extracted with 50 mL of chloroform–methanol (2:1, v/v) containing 10 mg/L of butylated hydroxytoluene and 0.1 mg/mL of nanodecanoic acid (C19:0, Sigma) as an internal standard. Then, the samples were stored in a fume hood overnight. Each sample was filtered and transferred into a separate funnel and added with 15 mL of 0.9% sodium chloride. The samples were shaken well to allow the phases to separate; the lower phase was then evaporated and transferred into a 10 mL volumetric flask.

Determination of fatty acid analysis

Fatty acid methyl esters (FAMEs) of the total lipid extract were prepared by transesterification in H_2SO_4 (0.9 mol/L in methanol). Before injection into the gas chromatography, the FAMEs were filtered by Sep-pak silica column (Alltech Associates, Inc., Deerfield, IL). Samples (1 mL) were analyzed quantitatively using a Shimadzu model GC-2014 system (Shimadzu, Tokyo, Japan) fitted with flame ionization detection eluted with H_2 at 30 ± 1 mL/min, with a split ratio of 1:17. A fused silica capillary column (30 m x 0.25 mm, 25 µm film thickness; DB-Wax, USA) was used. The injector and detector were maintained at 250°C. Nitrogen was used as a carrier gas and temperature programming was from 150°C (hold 5 min) to 230°C at 15°C /min, then to 170°C (hold 10 min) at 10°C /min, then to 200°C (hold 3 min) at 5°C /min and then to 230°C (hold 2 min) at 15°C /min. Fatty acids were identified by comparison with standard mixtures of FAME (nanodecanoic acid) running the same method (Yang *et al.*, 2006). The emergent peaks were identified by comparing their retention time with internal standard fatty acid nanodecanoic acid (C19:0). Lipid content, fatty acid composition and concentration were calculated as the following formulas (Raksakantong *et al.*, 2010).

Fatty acid composition = area under each peak/ total areas of all fatty acids appeared in the chromatogram x 100

Fatty acid concentration = (area under each peak/area of internal standard) x100x10/g sample

Statistical analysis

The experiments were run in triplicate determinations. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT) (Steel and Torrie, 1980). Statistical analyses were performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

Results and Discussion

Volatile compounds

The extraction of the volatile compounds from Thai local edible herbs was carried out following the headspace sampling and analysis by means of coupled GC–MS method. Forty volatile compounds were identified in the leave samples. There were significant differences among different varieties tested. *Piper aurantuacum* had the highest content of 7-hydroxy-5,6,7,8-tetrahydroind (67%) (Table 1), while Valeric aldehyde was the most dominant volatile compound in *Morinda citrifolia* Linn. (65%). Isoserine was found to be a volatile in *Cassia siamea* Britt. (16%) (Table 1). However, paeonol, (*E*)-carveol,

Table 1. Volatile compounds (% area) of Thai local edible herbs

Herbs	Volatile compounds
Cassia siamea Britt.	Cadinene (16.62%) Isoserine (16.07%) Carotol (15.56%) Furfural (10.01%) Oxymetholone (8.52%) Selina-6-en-4-ol (8.24%) Spathulenol (6.84%) Farnesene (5.71%) 1-heptadecene (5.67%) Spinacen (4.03%)
Centella asiatica Linn. Urban	3-Chloromethylfuran (53.39%) Tetradecanal (19.42%) Citronellal (11.82%) Octadecanal (7.27%)
Citrus hystrix DC.	Citronellal (53.76%) Propylenediamine (13.68%) Phytol (13.55%) Linalool (10.58%) Squalene (4.99%) Farnesene (3.44%)
Gynura divaricata DC.	Butanal (41.84%) Alpha-pinene (39.37%) Cadinene (11.02%) Stellasterol (7.78%)
Limnophila aromatica Merr.	Stereoisomer (54.28%) Cycloheptene (19.95%) Alpha-pinene (16.47%) Neodihydrocarveol (9.30%)
Morinda citrifolia Linn.	Valeric aldehyde (65.31%) Benzaldehyde (10.32%) Benzenacetaldehyde (10.25%) Palmitaldehyde (7.90%) Cyclohexanol (6.15%)
Morus alba Linn.	n-Pentanal (42.73%) 2,3,3-trimethyloctane (17.86%) 2,2,4-Trimethyl (13.62%) Columbin (10.16%) 1,3,7-octatriene (9.70%) Farnesol (5.93%)
Piper aurantuacum	7-hydroxy-5,6,7,8-tetrahydroind (67.35%) Cysteine (26.70%) 1,2,3-butanetriol (3.69%) Xexanol (1.16%) 2-propylheptanol (0.58%)

Table 2. Phenolic acids of Thai local edible herbs

Herb	Hydrobenzoic acids (mg/g DW)					Hydrocinnamic acids (mg/g DW)					Total phenolic acids
	GA	PCCA	p-HO	ChA	VA	CFA	SyA	p-CA	FA	SNA	
Cassia siamea Britt.	1.91±0.15^{fgh}	273.83±27.12^{a}	26.93±1.26^{fg}	83.48±2.45^{b}	ND	45.85±3.18^{c}	41.40±8.53^{c}	7.14±0.59^{g}	34.07±1.71^{d}	23.81±1.46^{f}	502.69±27.35
Centela asiatica Linn.	1.64±0.09^{gh}	1.52±0.10^{gh}	2.88±0.11^{e}	21.92±0.65^{b}	ND	2.38±0.09^{e}	10.87±0.96^{d}	2.53±0.06^{e}	60.76±9.45^{a}	19.01±1.12^{c}	123.51±7.48
Citrus hystrix DC.	2.39±0.45^{e}	2.92±0.41^{e}	ND	ND	8.96±0.68^{d}	3.33±0.48^{e}	24.08±0.34^{b}	8.29±0.32^{d}	18.85±0.79^{c}	31.73±1.36^{a}	100.55±5.60
Gynura divaricata subsp.	6.02±0.16^{d}	ND	ND	11.71±0.94^{b}	0.54±0.05^{g}	ND	8.75±0.08^{c}	1.44±0.06^{f}	5.39±0.02^{e}	18.96±0.09^{b}	52.81±2.38
Limnophila aromatica (Lam.) Merr.	1.83±0.20^{d}	ND	1.54±0.39^{d}	ND	ND	ND	2.11±0.07^{c}	ND	25.43±3.54^{b}	72.53±9.47^{a}	103.44±9.42
Morinda citrifolia Linn.	ND	2.72±0.28^{g}	3.15±0.59^{g}	ND	6.07±0.51^{c}	1.77±0.54^{f}	17.04±0.19^{b}	2.47±0.32^{e}	ND	225.44±10.60^{a}	258.66±11.46
Morus alba Linn.	1.76±0.06^{f}	1.44±0.06^{f}	ND	ND	5.15±0.11^{c}	ND	21.62±3.46^{b}	2.33±0.21^{e}	4.25±0.09^{d}	75.72±12.17^{a}	112.27±8.72
Piper sarmentosum Roxb.	2.85±0.10^{e}	3.71±0.15^{d}	ND	ND	ND	2.53±0.06^{e}	2.27±0.08^{e}	4.61±0.07^{c}	359.29±22.50^{a}	60.25±9.08^{b}	435.51±24.63

ND = not detectable
GA = Gallic acid; PCCA = Protocatechuic acid; p-HO = p-hydroxy benzoic acid; ChA = Chorogenic acid; VA = Vanilic acid; CFA = Caffic acid; SyA = Syringic acid; p-CA = p-Coumaric acid; FA = Ferulic acid; SNA = Sinapic acid.
*Means ± SD (n = 3).
Different superscripts in the same row indicate the significant differences ($p < 0.05$).

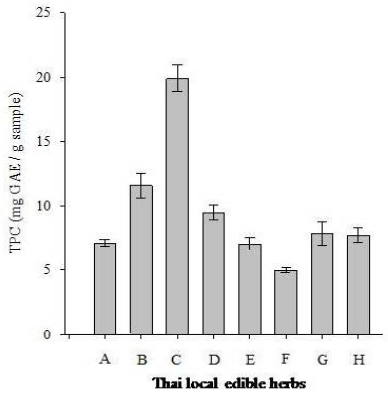

Figure 1. Total phenolic content (TPC) of Thai local edible herbs. *Citrus hystrix* DC., A; *Morinda citrifolia* Linn., B; *Limnophila aromatica* Merrill., C; *Piper aurantuacum*, D; *Gynura divaricata* DC., E; *Morus alba* Linn., F; *Cassia siamea* Britt., G; *Centella asiatica* (Linn) Urban, H.

(*E,E*)-2,4-octadienal, methyl salicylate, myrtanol and eugenol acetate were the major volatile compounds in *Paeoniae* Radix (*Paenia albiflora Pallas* var. trichocarpa Bunge) (Shim *et al*., 2009). The different types of volatile compounds in different herb species might be resulted in the various tastes and flavor of those herbs. Volatile compounds are responsible for plant flavor (Quintavalla and Vicini, 2002). Herbs have been used for providing humans with tastes in foods. In addition to taste, their beneficial health effects have also been widely attracted by food scientists.

Total phenolic content (TPC)

Ethanolic extracts obtained from the samples were evaluated for the presence of phenolic compounds. The samples were evaluated using the Folin-Ciocalteu assay, which was suggested as a fast and reliable method to quantify phenolics in foods (Konczak *et al*., 2010). TPC was determined in comparison with standard gallic acid and the results expressed in terms of mg gallic acid equivalent (GAE)/g DW. The levels of TPC in the evaluated local edible herbs varied significantly from 3 mg GAE/g DW in *Kaempferia parriflora* Wall. to 19 mg GAE/g DW in *Limnophila aromatic* (Lam.) Merr. (Fig. 1). The highest value of TPC was found in *Limnophila aromatica* Merrill., followed by *Morinda citrifolia* L. compared to other samples. However, high phenolic content was found in villous amomum, *Fructus amomi* (83.47 mg GAE/g) (Lu *et al*., 2011). The different phenolic content in various herb species might be different from cultivars. Phenolic compounds are the main bioactive compounds in fruits and vegetables (King and Young, 1999). Recently, phenolic compounds have been received considerable attention, due to their potential antioxidant activities and free-radical scavenging abilities, which potentially have beneficial implications in human health (Maqsood *et al*., 2013).

Identification of phenolic acids

RP-HPLC analysis was used to identify the phenolic compounds of the extracts, by comparison with standard compounds. Phenolic acids are hydroxylated derivatives of hydrobenzoic acid and hydrocinnamic acid, which often occur in plants as esters, glycosides and bound complexes (Germano *et al*., 2006). In the analyzed samples, it was possible to identify 10 phenolic acids including gallic acid,

Table 3. Fatty acid composition (% of total fatty acids) of Thai local edible herbs

Herbs	C14:0	C15:0	C16:0	C18:0	SFA	C16:1	C18:1	MUFA	C18:2n-6	C18:3n-6	C18:3n-3	C20:4n-6	PUFA
Cassia siamea Britt.	0.93±0.08^{bcd}	ND	25.93±1.70^{b}	5.63±0.85^{b}	32.49±4.41^{b}	5.41±0.29^{a}	10.14±0.46^{b}	15.54±0.74^{a}	14.27±1.54^{c}	11.52±1.13^{d}	26.18±1.03^{b}	ND	51.97±3.41^{d}
Centella asiatica (Linn)Urban	ND	ND	15.06±0.78^{e}	4.24±0.22^{b}	19.31±1.13^{e}	ND	ND	ND	ND	67.87±6.31^{a}	12.82±1.04^{c}	ND	80.69±7.23^{a}
Citrus hystrix DC.	ND	ND	28.53±2.09^{a}	16.07±0.89^{a}	44.61±3.18^{a}	ND	16.11±0.95^{a}	16.11±0.95^{a}	ND	29.53±156^{c}	9.75±0.54^{d}	ND	39.28±3.10^{d}
Gynura divaricata DC.	1.49±0.06^{a}	0.23± 0.02^{b}	27.05±1.26^{a}	3.31±0.45^{c}	32.09±3.83^{b}	2.48±0.06^{c}	3.15±0.19^{e}	5.63±0.35^{d}	28.72±2.74^{b}	ND	33.62±3.13^{a}	ND	62.34±6.85^{c}
Limnophila aromatica Merrill.	1.02± 0.04^{c}	ND	11.39±1.06^{d}	2.08±0.11^{d}	14.48±1.03^{d}	1.32± 0.04^{c}	2.07±0.13^{f}	3.39±0.41^{e}	15.06±1.04^{c}	54.62±5.46^{b}	12.46±0.84^{c}	ND	82.13±6.05^{a}
Morinda citrifolia Linn.	1.17±0.06^{b}	ND	18.08±14^{b}	1.86±0.63^{d}	21.12±1.13^{c}	1.85±0.05^{d}	5.52±0.05^{c}	7.37±0.01^{c}	38.08±3.08^{a}	ND	33.13±1.06^{a}	ND	71.51±4.14^{b}
Morus alba Linn.	ND	ND	17.13±1.02^{b}	14.62±1.44^{a}	31.75±2.62^{b}	ND	10.56±0.83^{b}	10.56±0.83^{b}	30.26±2.84^{b}	ND	30.26±2.12^{a}	ND	57.69±5.26^{c}
Piper aurantuacum	ND	0.81±0.03^{a}	11.50±0.82^{d}	3.43±0.21^{c}	15.74±1.04^{d}	2.62±0.01^{b}	4.26±0.75^{d}	6.88±0.86^{d}	12.73±0.04^{d}	58.51±6.07^{b}	5.07±0.24^{e}	ND	77.40±4.05^{ab}

ND, not detectable
SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid
*Means ± SD (n = 3).
Different superscripts in the same column indicate the significant differences ($p < 0.05$).

Table 4. Fatty acid concentration (mg/100 g) of Thai local edible herbs

Herbs	C14:0	C15:0	C16:0	C18:0	SFA	C16:1	C18:1	MUFA	C18:2n-6	C18:3n-6	C18:3n-3	C20:4n-6	PUFA
Cassia siamea Britt.	27.43±7.30^{a,a}	ND	836.78±20.18^{a}	183.70±9.18^{b}	1047.90±105.48^{a}	166.65±18.45^{a}	333.66±27.30^{a}	500.32±49.22^{a}	473.58±36.20^{c}	369.24±18.36^{d}	886.62±45.37^{a}	ND	1729.43±14.15^{b}
Centella asiatica (Linn)Urban	ND	ND	441.61±32.53^{e}	124.47±9.24^{c}	566.07±60.83^{e}	ND	ND	ND	ND	1990.05±104.13^{a}	376.00±9.23^{c}	ND	2366.05±118.93^{a}
Citrus hystrix DC.	ND	ND	539.75±10.28^{b}	304.37±17.19^{a}	844.12±47.10^{b}	ND	304.72±19.28^{b}	304.72±30.28^{b}	ND	558.73±52.17^{c}	184.55±9.43^{e}	ND	743.27±57.56^{f}
Gynura divaricata DC.	28.44±5.34^{a}	4.13±0.61^{a}	503.63±47.21^{b}	61.97±9.17^{d}	598.17±29.31^{c}	46.58±9.10^{b}	59.46±9.34^{c}	106.04±8.49^{c}	539.56±68.42^{b}	ND	616.47±83.49^{b}	ND	1156.03±90.80^{d}
Limnophila aromatica Merrill.	10.35±1.87^{b}	ND	116.14±7.10^{f}	21.10±9.06^{f}	147.59±8.12^{g}	13.49±6.02^{c}	21.17±7.06^{g}	34.65±14.07^{e}	153.67±18.04^{e}	556.86±39.31^{c}	127.15±8.07^{f}	ND	837.68±29.27^{e}
Morinda citrifolia Linn.	11.57±0.64^{b}	ND	176.96±9.43^{c}	18.18± 0.97^{f}	206.71±14.30^{g}	18.13±0.69^{a}	53.95±12.05^{c}	72.08±13.48^{d}	375.87±40.24^{d}	ND	323.21±21.02^{d}	ND	699.08±47.95^{f}
Morus alba Linn.	ND	ND	157.28±18.26^{d}	181.13±10.52^{b}	338.41±18.62^{d}	ND	315.64±20.18^{a}	315.64±30.14^{b}	751.32±54.83^{a}	ND	673.34±48.32^{b}	ND	1424.66±113.93^{c}
Piper aurantuacum	ND	9.41±1.57^{b}	135.09±9.05^{e}	40.24±7.05^{e}	184.74±12.11^{f}	30.82±10.09^{c}	50.14±18.17^{c}	80.96±12.08^{d}	150.37±18.09^{e}	685.48±47.43^{b}	59.19±7.06^{g}	ND	907.84±35.56^{d}

ND, not detectable
SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid
*Means ± SD (n = 3).
Different superscripts in the same column indicate the significant differences ($p < 0.05$).

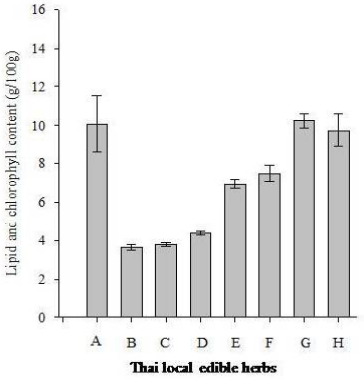

Figure 2. Lipid and chlorophyll content of Thai local edible herbs. *Citrus hystrix* DC., A; *Morinda citrifolia* Linn., B; *Limnophila aromatica* Merrill., C; *Piper aurantuacum*, D; *Gynura divaricata* DC., E; *Morus alba* Linn., F; *Cassia siamea* Britt., G; *Centella asiatica* (Linn) Urban, H.

protocatechuic acid, *p*-hydroxy benzoic acid, chorogenic acid, vanilic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid and sinapic acid. The distribution of phenolic acids in all samples is presented in Table 2. The highest concentrations of total phenolic acids were found in *Cassia siamea* Britt. (502 mg/g DW), followed by *Piper sarmentosum* Roxb. (435 mg/g DW), *Morinda citrifolia* Linn. (258 mg/g DW) and *Centela asiatica* Linn. (123 mg/g DW). The lowest amounts of phenolic acids were found in *Gynura divaricata* Subsp. (Table 2). The main phenolic acids (hydrocinnamic acids) in these samples were ferulic acid, sinapic acid and protocatechuic acid. Ferulic acid was the major hydrocinnamic acid derivative, ranging from 2 to 359 mg/g, followed by sinapic acid (16 to 225 mg/g) and syringic acid (1 to 41 mg/g). The highest content of ferulic acid was found in *Piper sarmentosum* Roxb. (Table 2). High levels of ferulic acid are found in herbs, vegetables, fruits, cereals, and coffee (Zhao and Moghadasian, 2008). Ferulic acid is an abundant dietary antioxidant which may offer beneficial effects against cancer, cardiovascular disease and diabetes (Zhao and Moghadasian, 2008). *Morinda citrifolia* Linn. had the most dominant contents of sinapic acid (Table 2). The hydroxybenzoic acid, gallic acid, vanilic acid and *p*-hydroxy benzoic acid occurred in low quantities, but not in all samples investigated (Table 2). The highest content of protocatechuic acid and chlorogenic acid were found in *Cassia siamea* Britt. Natural antioxidants are important ingredients that facilitate the control of the oxidative deterioration of foods (Chanwitheesuk *et al.*, 2005). Herbs extracts containing high amounts of total and individual phenolics, were found to exhibite antioxidant activities (Wojdyło *et al.*, 2007). Phenolic compounds found in plants have been reported to have a strong antioxidant activity (Elzaawely *et al*., 2005; Mansouri *et al*., 2005; Wojdyło *et al*., 2007). The antioxidant potential of phenolic compounds is dependent on the number and arrangement of the hydroxyl groups as well as the presence of electron donating substituent in the ring structure (Elzaawely *et al.*, 2005).

Total lipid and chlorophyll contents

The total lipid and chlorophyll contents of Thai local edible herbs are shown in Figure 2. In samples tested, total lipid and chlorophyll contents ranged from 3% in *Morinda citrifolia* Linn. to 10% in *Cassia siamea* Britt. Organic chemicals uptake such as phenanthrene and pyrened from soil and water exhibit significantly positive correlation with lipid contents (Gao *et al*., 2005). Tlili *et al.* (2011) reported that lipid content in cactus seeds was 5.5%. Difference

in lipid contents, fatty acids and chlorophyll contents might be caused by water supplement. These results suggested that organic chemicals absorb from soil and water may correlate with lipid content.

Fatty acid composition and concentration

Polyunsaturated fatty acid (PUFA) was the most predominant fatty acid found in analyzed samples, followed by saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) (Table 4).The concentration of PUFAs in samples ranged from 699 mg/100 g in *Morinda citrifolia* Linn. to 2366 mg/100 g in *Centella asiatica* (Linn) Urban. Consumption of PUFAs, especially n-3 PUFA is increasing because of beneficial effects on reducing the risk of diabetes by reduction of glucose intolerance and lowering blood pressure (Nieuwenhuys and Hornstra, 1998). Linoleic acid concentration of ALA ranged from 59 mg/100 g in *Piper aurantuacum* to 886 mg/100 g in *Cassia siamea* Britt. (Table 4). *Centella Asiatic* (Linn) Urban contained the highest GLA (1990 mg/100 g). The concentration of LA ranged from 167 mg/100 g in *Zingiber officinale* Roscoe. (LA,18:2n-6), alpha-linolenic acid (ALA,18:3n-3), gamma-linoleic acid (GLA,18:3n-6) and arachidonic acid (AA, 20:4n-6) detected in the samples. In addition, AA was found in only *Kaempferia parviflora* Wall. (150 mg/100 g). Essential fatty acids are related to human health (Horrobin, 1993; Innis, 1996). Plants oil provide rich sources of triglycerides containing n-6 PUFAs mainly LA (Lee and Lip, 2003). Additionally, green vegetables (watercress, mint, parsley, spinach, Chinese cabbage, brussels sprouts, bok choy, cobs lettuce, broccoli, chinese broccoli, baby bok choy) are known to contain a relatively high proportion of omega-3 PUFAs, primarily in the form of α-linolenic acid (ALA) (Pereiar *et al.*, 2001). Leafy vegetables contribute polyunsaturated fatty acids (PUFAs) of the n-3 variety, mainly ALA (Youdim *et al*., 2000). ALA was found in plants, animals, zooplankton, phytoplankton and marine species. The most predominant n-3 polyunsaturated fatty acid (PUFA) in terrestrial plants is ALA (Sinclair *et al*., 2002; Li *et al*., 2003). LA and ALA are essential fatty acids for human because they cannot be synthesized by human. The two most important metabolically active polyunsaturated fatty acids are the parent fatty acids LA and ALA (Innis, 1996; Sinclair *et al.*, 2002; Seo *et al.*, 2005). LA is only fatty acid to appreciably lower plasma/serum total and low-density lipoprotein (LDL) cholesterol levels when substituted for carbohydrate in the diet (Li *et al.*, 2002). Gamma linoleic acid (18:3n-6) has also been shown to support the treatment of rheumatoid arthritis, diabetic neuropathy, atopic eczema, cancer, asthma, osteoporosis and possibly coronary heart disease (CHD) (Andreassi *et al.*, 1997). AA is the precursor of prostaglandin E2 (PGE2) (Stanley-Samuelson *et al*., 1988; Mamalakis *et al*., 1998). Dietary n-6 fatty acids also have been shown to posses effective tumoricidal properties, when taken according to their recommended daily intake, against prostate and breast cancers as well as malignant gliomas pancreas tumors and lymphpocytic leukaemia (Agombar *et al.*, 2004; Bidoli *et al*., 2005). The concentration of total saturated fatty acids (SFAs) in samples ranged from 147 mg/100 g in *Limnophila aromatica* Merr.to 1047 mg/100 g in *Cassia siamea* Britt. (Table 4). The main SFA in samples were palmitic acid (16:0) and stearic acid (18:0). Kelly *et al.* (2001) reported that diet enriched in palmitic acid (16:0) resulted in an increased *ex vivo* collagen and adenosine diphosphate (ADP) induced whole blood platelet aggregation. Li *et al.* (2002) also reported that diet rich in SFA may influence Thromboxane A2 (TXA2) formation via effect on tissue proportion of AA. Palmitoleic acid (16:1) and oleic acid (18:1) were detected in the samples. The concentration of total MUFA ranged from 34 mg/100 g in *Limnophila aromatica* Merr. to 500 mg/100 g in *Cassia siamea* Britt. (Table 4). Consumption of MUFA has the beneficial effect on reducing LDL cholesterol and fasting glucose, increasing immune response, which means having effects on reducing the risk of diabetes (Bidoli *et al.*, 2005).

Conclusion

Edible herbs used in this study are ingredients in Thai traditional foods which play important roles in human nutrition. *Piper aurantuacum* had the highest content of 7-hydroxy-5,6,7,8-tetrahydroind (67%). TPC was highest in *Limnophila aromatic* compared with other samples. The main phenolic acids (hydrocinnamic acids) in these herbs were ferulic acid, sinapic acid and protocatechuic acid. Linoleic acid (LA, 18:2n-6), alpha-linolenic acid (ALA, 18:3n-3), gamma-linoleic acid (GLA, 18:3n-6), and arachidonic acid (AA, 20:4n-6) were detected in the samples. This research generated useful information for consumers and researchers to utilize herbs as sources of bioactive compounds.

References

Abu-Bakar, M.F., Mohamed, M., Rahmat, A. and Fry, J. 2009. Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*). Food Chemistry 113: 479–483.

Agombar, A, Cooper, A.J. And Johnson, C.D. 2004. An aqueous formulation of gamma-linolenic acid with anti-proliferative action on human pancreatic cancer cell lines. Anticancer Drugs 15(2): 157-60.

Andreassi, M., Forleo, P., Di Lorio, A., Masci, S., Abate, G. and Amerio, P. 1997. Efficacy of γ-linolenic acid in the treatment of patients with atopic dermatitis. Journal of International Medical Research 25: 266–274.

Balange, A. and Benjakul, S. 2009. Use of kiam wood extract as gel enhancer for mackerel (*Rastrelliger kanagurta*) surimi. International Journal of Food Science and Technology 44: 1661–1669.

Barcarolo, R. and Casson, P. 1997. Modified capillary GC/MS system enabling dynamic head space sampling with on-line cryofocusing and cold on-column injection of liquid samples. Journal of High Resolution Chromatography 20: 24–28.

Barreira, J.C.M., Ferreira, I.C.F.R., Oliveira, B.M.P.P. and Pereira, J.A. 2008. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. Food Chemistry 107: 1106–1113.

Bengoechea, M.L., Sancho, A.I., Bartolome, B., Estrella, I., GomesCordoves, C. and Hernandez, M.T. 1997. Phenolic composition of industrially manufactured purees and concentrates from peach and apple fruits. Food Chemistry, 45: 4071–4075.

Bidoli, E., Talamini, R., Bosetti, C., Negri, E., Maruzzi, D., Montella, M. *et al.* 2005. Macronutrients, fatty acids, cholesterol and prostate cancer risk. Annals of Oncology 16: 152–157.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911–917.

Butsat, N., Weerapreeyakul, N. and Siriamornpun, S. 2009. Changes in phenolic acids and antioxidant activity in Thai rice husk at five growth stages during grain development. Journal of Agricultural and Food Chemistry 57: 4566-4571.

Carlsen, H.M., Halvorsen, L.B., Holte, K., Bøhn, K.S., Dragland, S., Sampson, L. *et al.* 2010. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutrition journal 9(3): 1-11.

Ceylan, E. and Fung, D.Y.C. 2004. Antimicrobial activity of spices. Journal of Rapid Methods and Automation in Microbiology 12: 1–55.

Douglas, M.; Heyes, J. and Smallfield, B. 2005. Herbs, spices and essential oils: post-harvest operations in developing countries. New Zeland: New Zealand Institute for Crop and Field Research, Food and Agriculture Organization of the United Nations 20(10).

Elzaawely, A.A., Xuan, T.D. and Tawata, S. 2005. Antioxidant and antibacterial Activities of *Rumex japonicus* Houtt. aerial parts. Biol Pharmaceut Bull 28: 2225–2230.

Erasto, P., Grierson, D.S. and Afolayan, A.J. 2007. Evaluation of antioxidant activity and the fatty acid profile of the leaves of *Vernonia amygdalina* growing in South Africa. Food Chemistry 104: 636–642.

Gao, Y.Z., Zhu, L.Z. and Ling, W.T. 2005. Application of the partition limited model for plant uptake of organic chemicals from soil and water. Science of the Total Environment 336: 171–182.

Germano, M.P., D'Angelo, V., Biasini, T., Sanogo, R., De Pasquale, R. and Catania, S. 2006. Evaluation of the antioxidant properties and bioavailability of free and bound phenolic acids from *Trichilia emetic* Vahl. Journal of Ethnopharmacology 105: 368–373.

Horrobin, D.F. 1993. Omega-6 and Omega-3 essential fatty acids in Atheroselerosis. Siminars in thrombosis and hemostasis 19: 129-137.

Innis, S.M. 1996. Essential dietary lipids in present knowledge in nutrition. Washington, D.C.: U.SI press. 58-142.

Kamler, E., Wolnicki, J., Kamiński, R. and Sikorska, J. 2008. Fatty acid composition, growth and morphological deformities in juvenile cyprinid, *Scardinius erythrophthalmus* fed formulated diet supplemented with natural food. Aquaculture 278: 69–76.

Kelly, K.M., Cameron, A.C., Biernbaumb, A.J. and Poff, K.L. 2003. Effect of storage temperature on the quality of edible flowers. Postharvest Biology and Technology 27: 341-344.

King, A. and Young, G. 1999. Characteristics and occurrence of phenolic phytochemicals. Journal of the American Dietetic Association 99: 213-218.

Konczak, I., Zabaras, D., Dunstan, M. and Aguas, P. 2010. Antioxidant capacity and phenolic compounds in commercially grown native Australian herbs and spices. Food Chemistry 122: 260–266.

Lee, K.W. and LIP, G.Y.H. 2003. The role of omega-3 fatty acids in the secondary prevention of cardiovascular disease. An International Journal of Medicine 96: 465-480.

Li, D., Mansor, M., Zhuo, S.R., Woon, T., Anthony, M.A. and Sinclair, A.J. 2002. Omega-3 polyunsaturated fatty acid contents of canned meats available in Australia. Food Australia 54: 311–315.

Lu, M., Yuan, B., Zeng, M. and Chen, J. 2011. Antioxidant capacity and major phenolic compounds of spices commonly consumed in China. Food Research International 44: 530-536.

Mansouri, A., Embarek, G., Kokkalou, E. and Kefalas, P. 2005. Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). Food Chemistry 89: 411–420.

Mamalakis, G., Kafatos, A., Tornaritis M. and Alevizos, B. 1998. Anxiety and adipose essential fatty acid precursors for prostaglandin E1 and E2. Journal of the American College of Nutrition 17(3): 239–243.

Maqsood, S., Benjakul, S. and Shahidi, F. 2013. Emerging Role of Phenolic Compounds as Natural Food Additives in Fish and Fish Products. Critical Reviews in Food Science and Nutrition 53(2): 162-179.

Nieuwenhuys, C.M.A. and Hornstra, G. 1998. The effects of purified eicosapentaenoic and docosahexaenoic acids on arterial thrombosis tendency and platelet function in rats. Biochimica Biophysica Acta 1390:

313–322.
Pereira, C., Li, D. and Sinclair, A.J. 2001. The α-linolenic acid content of green vegetables commonly available in Australia. International Journal for Vitamin and Nutrition Research 71: 223–228.
Quintavalla, S. and Vicini, L. 2002. Antimicrobial food packaging in meat industry. Meat Science 62: 373−380.
Raksakantong, P., Meeso, N., Kubola, J. and Siriamornpun, S. 2010. Fatty acids and proximate composition of eight Thai edible terricolous insects. Food Research International 43: 350–355.
Schieber, A., Keller, P. and Carle, R. 2001. Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography. Journal of Chromatography A 910: 265–273.
Seo, T., Blaner, W.S. and Deckelbaum, R.J. 2005. Omega-3 fatty acids: molecular approaches to optimal biological outcomes. Curr Op Lipidiol 16: 8-11.
Shahidi, F. 2000. Antioxidants in food and food antioxidants. Nahrung, 44, 158-63. Shahidi, F. and Naczk, M. (2004). Phenolics in food and nutraceuticals. Boca Raton, FL: CRC Press.
Shan, B., Cai, Y. Z., Sun, M. and Corke, H. 2005. Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents. Journal of Agricultural and Food Chemistry 53: 7749-7759.
Shim, S.-L., Hwang, I.-M., Ryu, K.-Y., Jung, M.-S., Seo, H.-y., Kim, H.-Y., et al. 2009. Effect of γ-irradiation on the volatile compounds of medicinal herb, Paeoniae Radix. Radiation Physics and Chemistry 78: 665-669.
Sinclair, A.J., Attar-Bashi, N.M. and Li, D. 2002. What is the role of α-linolenic acid for mammal?. Lipids 37: 1113-1123.
Sinclair, H.M. 1990. Essential fatty acids: a historical perspective. Biochemical society transactions 18: 756-761.
Stanley-Samuelson, D.W., Jurenka, R.A., Cripps, C., Blomquist, J.G., De Renobales, M., Loher, W., *et al.* 1988. Fatty acids in insects: Composition, metabolism, and biological significance. Journal of Insect Biochemistry and Physiology 9: 1–33.
Steel, R.G.D. and Torrie, J.H. 1980. Principles and procedures of statistics: A biometrical approach. McGraw-Hill New York.
Tlili, N., Bargougui, A., Elfalleh, W., Triki, S. and Nasri, N. 2011. Phenolic compounds, protein, lipid content and fatty acids compositions of cactus seeds. Journal of Medicinal Plant Research 5: 4519-4524.
Urbach, G. 1997. The flavour of milk and dairy products: II. Cheese: Contribution of volatile compounds. International Journal of Dairy Technology 50: 79–89.
Uzelac, D.V., Pospišil, J., Levaj, B. and Delonga, K. 2005. The study of phenolic profiles of raw apricots and apples and their purees by HPLC for the evaluation of apricot nectars and jams authenticity. Food Chemistry 91: 373–383.
Velioglu, Y.S., Mazza, G., Gao, L. and Oomah, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry 46(10): 4113–4117.
Wojdyło, A., ski, O. and Czemerys, R. 2007. Antioxidant activity and phenolic compounds in 32 selected herb. Food Chemistry 105: 940-949.
Yang, L.F., Siriamornpun, S. and Li, D. 2006. Polyunsaturated fatty acid content of edible insects in Thailand. Journal of Food Lipids 13: 277–285.
Youdim, K.A., Martin, A. and Joseph, J.A. 2000. Essential fatty acids and the brain: possible health implications. International Journal of Developmental Neuroscience 18(4-5): 383-99.
Zhao, Z. and Moghadasian, M.H. 2008. Chemistry, natural sources, dietary intake apharmacokinetic properties of ferulic acid: A review. Food Chemistry 109: 691–702.