Phytochemicals and Antioxidant Activities of Edible Herbs

Chodsana Sriket[1,*] and Phanat Kittiphattanabawon[2]

[1]Program in Food Science and Technology, Faculty of Agriculture, Ubon Ratchathani Rajabhat University, Ubon Ratchathani, Thailand
[2]Department of Agro-Industry Technology and Management, Faculty of Agro-Industry, King Mongkut's University of Technology North Bangkok, Prachinburi Campus, Prachinburi, Thailand

ABSTRACT

The contents of plant chemicals such as volatile compounds, phenolic compounds, fatty acids content and antioxidant activities from Thai edible herbs were determined. Eucalyptol was the most prominent volatile compound in *Alpinia galanga* (68%). The main phenolic acids (hydrocinnamic acids) in these herbs were ferulic acid, sinapic acid and syringic acid. Ferulic acid was the major hydrocinnamic acid derivative, ranging from 2 to 52 mg/g, followed by sinapic acid (16 to 24 mg/g) and syringic acid (1 to 8 mg/g). Polyunsaturated fatty acid (PUFA) was the most predominant fatty acid found in analyzed Thai herbs, followed by saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA). Four PUFAs including 18:3n-3, 18:2n-6, 18:3n-6 and 20:4n-6 was detected in the samples. *Alpinia galangal* had the highest omega-3 PUFA (18:3n-3) among all samples tested. *Boesenbergia pandurata* extract exhibited highest antioxidant activities. This research provided useful information for screening Thai edible herbs as potential sources of bioactive components for consumers and public health workers.

Keywords: Edible herbs, Phytochemicals, Volatile compounds, phenolic compounds, fatty acids, antioxidants

1. INTRODUCTION

Southeastern Asian countries especially, in Thailand, China and India have been used edible herbs as flavoring, seasoning, coloring agents and food preservatives as well as medicinal plants and folk medicines for a thousand years [1]. Edible herbs, medicinal herbs, vegetables and fruits have been known to process antioxidant properties. The antioxidant activities could be obtained from leave, roots, rhizome, flowers, fruits, seeds and bark [2].

Herbs have also been recognized as antimicrobial, anti-inflammatory, anti-mutagenic, anti-carcinogenic potential [3]. Edible herbs always contribute in Thai dishes, especially in dietary cultures where local edible herbs are used regularly [4]. Flavor is usually the result of many volatile and nonvolatile components presented in herbs, possessing diverse chemical and physicochemical properties [5]. 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 [6]. Phenolic compounds have strong antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions and chelate metals [7]. Increased consumption of phenolic compounds has been correlated with a reduced risk of cardiovascular disease and certain cancers [8-9]. Fatty acids play a major role as an energy source, affect cellular membrane structure and improve resistance to stress [10]. 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 [11-12]. 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 [13]. The demand for these commodities has grown in recent years as a consequence of continually increasing consumption of ready-to-eat foods, which include edible herbs as ingredients in Thai cuisine [14]. The selected 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, fatty acids and antioxidant properties of edible herbs consumed in Northeastern Thailand is scared. Therefore, the aim of this research was to investigate the volatile compounds, phenolic compounds, fatty acids and antioxidant properties of edible herbs from Northeastern Thailand. The receiving results could be

* Corresponding author, E-mail: psriket@hotmail.com

useful information for consumers and public health workers.

2. METERIAL AND METHODS

2.1 Chemicals

The compounds 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripiridyl-s-triazine (TPTZ), Folin–Ciocalteu's reagent, standards of gallic, ferulic, phydroxybenzoic, protocatechuic, p-coumaric, caffeic, syringic, sinapic, chlorogenic and vanillic acids were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO). The phosphoric acid, methanol and acetonitrile used in the HPLC analysis were purchased from Merck (Darmstadt, Germany). All other solvents were purchased from Fisher Scientific were of the highest available purity.

2.2 Edible herbs

Edible herbs including galangal (*Alpinia galanga*), ginger (*Zingiber officinale* Roscoe.), turmeric (Curcuma longa Linn.) and kaempfer (*Boesenbergia pandurata*) were obtained from three representative markets in the Ubon Ratchathani province during September to November, 2012. At each market, 3 kg samples were sampled from three representative outlets. Single composite samples for each representative market, were prepared by combining about 500 g of homogenized single sample of the same rhizome variety from three representative outlets and then homogenizing again to obtain a uniform single composite sample. All samples were cleaned and freeze-dried using freeze-drier (Model DuratopTM lP/Dura DryTM lP, FTS® System, Inc., Stone Ridge, NY, USA) before analysis. Analyses were conducted in triplicate (n = 3).

2.3 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 [15]. 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 [16].

2.4 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 (Heidolph NIMAX 1010, Schwabach, Germany) set at 180 rpm. The mixture was centrifuged at 1400 x g for 20 min 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.* [17] as adapted from Velioglu *et al.*, [18]. 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 (Model RF-1500, Shimadzu Co., Kyoto, Japan). Results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g).

2.5 Identification and quantification of phenolic compounds

2.5.1 Extraction of herbal phenolics

The phenolic compounds in the herb samples were extracted using a modification of the procedure described by Bengoechea *et al.* [19] as adapted from Uzelac *et al.* [20]. Each sample (5 g) was mixed with 50 mL of methanol/HCl (100:1, v/v) which contained 2% tertbutylhydroquinone,in inert atmosphere (N2) during 12 h at 35°C in the dark. The extract was then centrifuged at 4000 rpm/min using a refrigerated centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Palo Alto, CA, USA), 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.

2.5.2 HPLC–DAD system for analysis of phenolic compounds

RP-HPLC system for analysis of phenolic compounds 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   250 mm i.d., 5   m). The composition of solvents and the gradient elution conditions used were described previously by Bengoechea *et al.* [19] Schieber *et al.* [21] and Butsat *et al.* [22] 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 60min, 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).

2.6 Determination of fatty acids content

2.6.1 Lipid extraction

Lipids were extracted according to the method of Bligh and Dyer [23]. 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.

2.6.2 Fatty acids content determination

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 H2 at 30 ± 1 mL/min, with a split ratio of 1:17. A fused silica capillary column (30 m × 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

of Yang et al. [24]. The emergent peaks were identified by comparing their retention time with internal standard fatty acid nanodecanoic acid (C19:0). Fatty acid contents were calculated as the following formulas [25].

Fatty acid content = (area under each peak/area of internal standard)×100×10/g sample

2.7 Antioxidant activities determination

2.7.1 DPPH free radical scavenging activity

The hydrogen atom or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-colored methanol solution of DPPH [26]. The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca et al. [27]. Ethanolic extract (0.1 ml) was added to 3 ml of a 0.001 M DPPH in methanol. The absorbance at 517 nm was determined after 30 min, and the percent inhibition of activity was calculated as $[(A_o-A_e)/A_o]/100$ (A_o = absorbance without extract; A_e = absorbance with extract).

2.7.2 Ferric reducing antioxidant power (FRAP)

The total reducing capacity was determined using the FRAP assay [28]. The FRAP reagent was initially prepared consisting of 300 mM acetate buffer, pH 3.6, 10 mM iron reagent (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3 6H_2O$ solution. The fresh working solution was warmed at 37ºC before using. Fruit extracts (100 µL) were allowed to react with 1.9 ml of the FRAP solution. After incubation for 4 min, the absorbance was read at 593 nm using a spectrophotometer. The results were calculated by standard curves prepared with known concentrations of $FeSO_4$, and were expressed as µmol $FeSO_4$/g.

2.8 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) [29]. Statistical analyses were performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1 Volatile compounds

The extraction of the volatile compounds from Thai edible herbs was carried out following the headspace sampling and analysis by means of coupled GC–MS method. Fifteen volatile compounds were identified in the rhizome of all samples. There were significant differences among different varieties tested. Eucalyptol was the most prominent volatile compound in Alpinia galanga (68%) (Table1). Isoserine was found to be a volatile in Curcuma longa Linn. (34%) and Alpinia galangal (10%) (Table 1).

However, paeonol, (E)-carveol, (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) [30]. The different types of volatile compounds in different herb species might be resulted in the various tastes and flavor of those herbs [31]. Volatile compounds are responsible for plant flavor [32]. 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.

TABLE 1 VOLATILE COMPOUNDS (% AREA) OF EDIBLE HERBS

Edible herbs	Volatile compounds
Alpinia galangal	Eucalyptol (68.80%), Isoserine (10.33%), Caryophyllene (8.02%), 4-Carvomenthenol (6.88%), Alpha-humulene (5.97%)
Boesenbergia pandurata (Roxb.)Schltro	Alpha-tumerone (41.85%), Alpha-phellandrene (26.98%), Zingiberene (16.06%), Camphene (15.11%)
Curcuma longa Linn.	Isoserine (34.38%), 1-heptadecene (22.91%), Carotol (12.65%), Alpha-phellandrene (10.85%)
Zingiber officinale Roscoe.	Zingiberene (26.84%), Eucalyptol (26.07%), Camphene (18.19%), Beta-sesqulphellandrene (12.09%), Citral (8.55%), Hexaldehyde (8.27%)

TABLE 2 PHENOLIC ACIDS OF EDIBLE HERBS

Edible herbs	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	
Alpinia alangal (Linn.) Swartz.	ND	1.72±0.29*	ND	ND	ND	1.52±0.39	1.47±0.44	1.48±0.05	2.46±0.22	16.34±1.45	24.99±1.26
Boesenbergia pandurata (Roxb.) Schltro.	ND	3.96±0.56	ND	ND	3.35±0.34	1.70±0.27	8.34±0.51	6.36±0.34	52.28±3.46	24.38±2.66	100.37±5.73
Curcuma Longa Linn.	ND	1.65±0.05	ND	ND	ND	1.64±0.39	1.67±0.22	1.74±0.28	2.29±0.14	17.41±1.52	26.40±1.18
Zingiber officinale Roscoe.	ND	ND	1.79±0.19	ND	ND	ND	17.58±1.37	ND	ND	21.86±2.66	41.23±2.83

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).

3.2 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 [34]. 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 edible herbs varied from 3 mg GAE/g DW in *Zingiber officinale* Roscoe.Wall. to 12 mg GAE/g DW in *Boesenbergia pandurata* (Roxb.) Schltro (Fig. 1). The highest value of TPC was found in *Boesenbergia pandurata* (Roxb.) Schltro. (12 mg GAE/g DW), followed by *Alpinia galangal* (8 mg GAE/g DW) compared to other samples. However, high phenolic content was found in villous amomum, *Fructus amomi* (83.47 mg GAE/g) [35]. The different phenolic content in various herb species might be different from cultivars. Phenolic compounds are the main bioactive compounds in fruits and vegetables [36]. Maqsood *et al.* [37] reported that phenolic compounds have been received considerable attention, due to their potential antioxidant activities, which potentially have beneficial implications in human health.
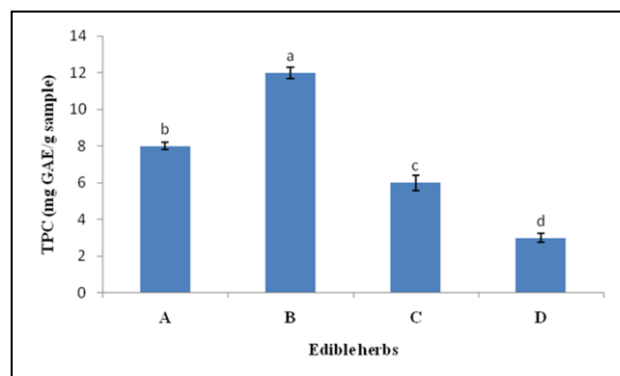

Figure 1 Total phenolic content (TPC) of edible herbs. Alpinia galangal (A), Boesenbergia pandurata (B), Curcuma longa Linn. (C), Zingiber officinale Roscoe. (D) Means ± SD (n=3). Different superscripts indicate the significant differences (p < 0.05)

3.3 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 [38]. In the analyzed samples, it was possible to identify 10 phenolic acids including gallic acid, 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 *Boesenbergia pandurata* (Roxb.) Schltro. (100 mg/g DW), followed by *Zingiber officinale* Roscoe. (41 mg/g DW), *Curcuma Longa* Linn. (26 mg/g DW) and *Alpinia alangal* (Linn.) (24 mg/g DW). The main phenolic acids (hydrocinnamic acids) in these samples were ferulic acid, sinapic acid and syringic acid. Ferulic acid was the major hydrocinnamic acid derivative, ranging from 2 to 52 mg/g DW, followed by sinapic acid (16 to 24 mg/g DW) and syringic acid (1 to 8 mg/g DW). The highest content of ferulic acid, sinapic acid and syringic acid was found in Boesenbergia pandurata (Roxb.) Schltro. (Table 2). High levels of ferulic acid are found in herbs, vegetables, fruits, cereals, and coffee [39]. Ferulic acid is an abundant dietary antioxidant which may offer beneficial effects against cancer, cardiovascular disease and diabetes [39]. The hydroxybenzoic acid, gallic acid, vanilic acid and p-hydroxy benzoic acid occurred in low quantities (Table 2). Natural antioxidants are important ingredients that facilitate the control of the oxidative deterioration of foods [40]. Herbs extracts containing high amounts of total and individual phenolics, were found to exhibit antioxidant activities [41]. Phenolic compounds found in plants have been reported to have a strong antioxidant activity [41-43]. 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 [42].

TABLE 3 FATTY ACID CONTENTS (MG/100 G) OF EDIBLE HERBS

Edible 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
Alpinia alangal (Linn.) Swartz.	22.13 ±4.10*	ND	154.58 ±8.41	24.63 ±8.27	201.34 ±0.19	ND	80.06 ±0.08	80.06 ±0.08	200.51 ±0.49	573.62 ±0.65	58.85 ±0.05	ND	832.98 ±1.09
Boesenbergia pandurata (Roxb.) Schltro.	ND	ND	1218.77 ±108.79	394.02 ±19.32	1612.79 ±90.72	ND	ND	ND	600.06 ±87.83	1382.10 ±114.86	145.48 ±7.18	150.96 ±8.04	2278.61 ±126.64
Curcuma Longa Linn.	ND	ND	187.46 ±9.21	77.54 ±9.75	265.00 ±0.51	87.54 ±0.31	ND	87.54 ±0.31	785.37 ±0.53	1972.39 ±1.03	34.54 ±0.12	ND	2792.30 ±1.02
Zingiber officinale Roscoe.	ND	4.95 ±1.86	96.42 ±8.03	ND	101.36 ±0.06	ND	57.11 ±0.05	57.11 ±0.05	167.82 ±0.06	359.39 ±1.51	19.76 ±0.06	ND	546.96 ±1.46

ND= not detectable

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

*Means ± SD (n=3)

3.4 Fatty acid content

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 3).The content of PUFAs in samples ranged from 547 mg/100 g in *Zingiber officinale* Roscoe. to 2792 mg/100 g in *Curcuma longa* Linn. (Table 3). 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 [44]. Linoleic acid (C18:2n-6) content of LA ranged from 167 mg/100 g in *Zingiber officinale* Roscoe. to 785 mg/100 g in *Curcuma longa* Linn. (Table 3). *Curcuma longa* Linn. contained the highest GLA (18:3n-6) (1972 mg/100 g). The content of LA (C18:2n-6) ranged from 167 mg/100 g in *Zingiber officinale* Roscoe. In addition, arachidonic acid (AA, 20:4n-6) was found only in *Boesenbergia pandurata* (Roxb.) (151 mg/100 g). Essential fatty acids are related to human health [45-46]. Plants oil provide rich sources of triglycerides containing n-6 PUFAs mainly LA [47]. 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) [11]. Vegetables contribute polyunsaturated fatty acids (PUFAs) of the n-3 variety, mainly ALA [12]. 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 [48]. 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 [46, 48-49]. 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. 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) [50]. AA is the precursor of prostaglandin E2 (PGE2) [51-52]. Dietary n-6 fatty acids also have been shown to posse's 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 [53-54]. The content of total saturated fatty acids (SFAs) in samples ranged from 101 mg/100 g in *Zingiber officinale* Roscoe. to 1,613 mg/100 g in *Boesenbergia pandurata* (Roxb.) (Table 3). The main SFA in samples were palmitic acid (16:0) and stearic acid (18:0). Kelley *et al.* [55] 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.* [56] 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 content of total MUFA ranged from 57 mg/100 g in *Zingiber officinale* Roscoe. to 87 mg/100 g in Curcuma longa Linn. (Table 3). 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 [54].

3.5 Antioxidant activities of edible herbs

3.5.1 DPPH radical scavenging activity

The DPPH radical is widely used to evaluate the radical scavenging activity of antioxidant compounds. The ability to act as donor of hydrogen atoms in the transformation of the DPPH radical to its reduced form was investigated for different edible herb extracts. The antioxidant activities of Thai edible herbs are shown in Table 4. The results showed that the DPPH radical scavenging activity of edible herbs ranged from 78% to 86% Table 4. It was observed that Curcuma longa Linn. having high inhibition activity was a very potent radical scavenger. High DPPH radical scavenging activity of some edible herbs extract (kesum (82.6 %), ginger (79.0%) and turmeric (64.6%)) were also reported [57]. The varied radical scavenging activity of the herbs extracts might be depended on the amount of total phenolic content in each sample (Fig. 1). Several flavonoids and polyphenols have been isolated from plant extracts with potent DPPH radical scavenging activities [44]. Marwah *et al.* [58] reported that DPPH radical scavenging activity of plants depends upon species.

3.5.2 Ferric reducing antioxidant power (FRAP)

The FRAP assay is a method of measuring the ability of antioxidants to reduce Fe^{3+} to be Fe^{2+} [28]. The formation of blue color from Fe^{2+}–TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm. The FRAP values of the extracts of edible herbs are shown in Table 4. The FRAP value of different edible herbs extracts indicated that the *Boesenbergia pandurata* extract had the greatest reducing power, followed by *Alpinia galangal*, *Curcuma longa* and *Zingiber officinale* (Table 4). High antioxidant activity determined by using FRAP method was also found in Chinese edible spices [32]. The *Boesenbergia pandurata* extract gave high FRAP values; these might be due to their higher ferulic and total phenolic acids contents (Table 2). Phenolic acids was positively associated with the FRAP [37]. Polyphenolic compounds found in edible plants have been reported to have multiple biological effects, including antioxidant activity [38].

TABLE 4 ANTIOXIDANT ACTIVITY OF EDIBLE HERBS

Edible herbs	Antioxidant activity	
	DPPH (% inhibition)	FRAP (μmol FeSO$_4$/100 g sample)
Alpinia galangal	85.29±0.18*[b]	563.09±2.46[b]
Boesenbergia pandurata (Roxb.) Schltro	86.42±0.32[a]	606.39±2.25[a]
Curcuma longa Linn.	82.57±0.94[c]	472.06±1.46[c]
Zingiber officinale Roscoe.	78.26±0.74[d]	421.24±1.78[d]

*Means ± SD (n=3).

Different superscripts in the same column indicate the significant differences (p < 0.05).

4. CONCLUSION

The study shows that there are differences in phytochemicals and antioxidant properties of edible herbs consumed in Thailand. Eucalyptol was the most prominent volatile compound in *Alpinia galangal*. The main phenolics (hydrocinnamic acids) in those herbs were ferulic acid, sinapic acid and syringic 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. *Boesenbergia pandurata* extract exhibited highest antioxidant activities. There were significant differences among different varieties tested. This research generated useful information for consumers and researchers to utilize herbs as sources of bioactive compounds.

ACKNOWLEDGEMENTS

RERERENCES

[1] Shan, B., Cai, Y. Z., Sun, M. and Corke, H. Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents, Journal of Agricultural and Food Chemistry, 53, 2005, 7749-7759.

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

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

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

[5] Lee, S.-J., Umano, K., Shibamoto, T. and Lee, K.-G. Identification of volatile components in basil (Ocimum basilicum L.) and thyme leaves (Thymus vulgaris L.) and their antioxidant properties, Food Chemistry 91(1), 2005, 131-137.

[6] Urbach, G. The flavour of milk and dairy products: II. Cheese: Contribution of volatile compounds, International Journal of Dairy Technology, 50, 1997, 79–89.

[7] Shahidi, F. and Naczk, M. Phenolics in food and nutraceuticals. Boca Raton, FL: CRC Press. 2004.

[8] Shahidi, F. 2000. Antioxidants in food and food antioxidants, Nahrung, 44, 158-63.

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

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

[11] Pereira, C., Li, D., and Sinclair, A.J. The α-linolenic acid content of green vegetables commonly available in Australia, International Journal for Vitamin and Nutrition Research, 71, 2001, 223–228.

[12] Youdim, K.A., Martin, A. and Joseph, J.A. Essential fatty acids and the brain: possible health implications, International Journal of Developmental Neuroscience, 18, (4-5), 2000, 383-99.

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

[14] Douglas, M.; Heyes, J. and Smallfield, B. 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) 2005.

[15] Barcarolo, R. and Casson, P. 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, 1997, 24–28.

[16] Diaz-Maroto, M.C., Perez-Coello, M.S., Cabezudo, M.D. Effect of different drying methods on the volatile components of parsley (Petroselinum crispum L.), European Food Research and Technology, 215, 2002, 227–230.

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

[18] Velioglu, Y. S., Mazza, G., Gao, L. and Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products, Journal of Agricultural and Food Chemistry, 46(10), 1998, 4113–4117.

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

[20] Uzelac, D. V., Pospišil, J., Levaj, B. and Delonga, K. 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, 2005, 373–383.

[21] Schieber, A., Keller, P. and Carle, R. Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography, Journal of Chromatography A, 910, 2001, 265–273.

[22] Butsat, N., Weerapreeyakul, N. and Siriamornpun, S. 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, 2009, 4566-4571.

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

[24] Yang, L. F., Siriamornpun, S. and Li, D. Polyunsaturated fatty acid content of edible insects in Thailand, Journal of Food Lipids, 13, 2006, 277–285.

[25] Raksakantong, P., Meeso, N., Kubola, J. and Siriamornpun, S. Fatty acids and proximate composition of eight Thai edible terricolous insects, Food Research International, 43, 2010, 350–355.

[26] Gulluce, M., Sahin, F., Sokmen, M., Ozer, H., Daferera, D., Sokmen, A., et al. Antimicrobial and antioxidant properties of the essential oils and methanol extract from Mentha longifolia L. ssp. Longifolia, Food Chemistry, 1, 2007, 1449–1456.

[27] Braca, A., Tommasi, N. D., Bari, L. D., Pizza, C., Politi, M. and Morelli, I. Antioxidant principles from Bauhinia terapotensis, Journal of Natural Products, 64, 2001, 892–895.

[28] Benzie, I. F. and Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay, Analytical Biochemistry, 239, 1996, 70–76.

[29] Steel, R. G. D. and Torrie, J. H. Principles and procedures of statistics: A biometrical approach. New York, McGraw-Hill, 1980.

[30] Sriket, C. and Sriket, P. Phytochemicals of Thai local edible herbs, International Food Research Journal, Accepted, 2014.

[31] Quintavalla, S. and Vicini, L. Antimicrobial food packaging in meat industry, Meat Science, 62, 2002, 373-380.

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

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

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

[35] Lee, S. K., Mbwambo, Z. H., Chung, H., Luyengi, L., Gamez, E. J. C., Mehta, R. G., *et al.* Evaluation of the antioxidant potential of natural products, Combinatorial Chemistry and High Throughput Screening, 1, 1998, 35–46.

[36] Shim, S.-L., Hwang, I.-M., Ryu, K.-Y., Jung, M.-S., Seo, H.-y., Kim, H.-Y., *et al.* Effect of γ-irradiation on the volatile compounds of medicinal herb, Paeoniae Radix, Radiation Physics and Chemistry 78, 2009, 665-669.

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

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

[39] Zhao, Z. and Moghadasian, M.H. Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review, Food Chemistry, 109, 2008, 691–702.

[40] Chanwitheesuk, A., Teerawutgulrag, A. and Rakariyatham, N. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand, Food Chemistry 92(3) 2005, 491-497.

[41] Wojdyło, A., ski, O. and Czemerys, R. Antioxidant activity and phenolic compounds in 32 selected herb, Food Chemistry, 105, 2007, 940-949.

[42] Elzaawely, A. A., Xuan, T.D. and Tawata, S. Antioxidant and antibacterial Activities of Rumex japonicus Houtt. aerial parts, Biological & Pharmaceutical Bulletin, 28,2005, 2225–2230.

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

[44] Nieuwenhuys, C. M. A. and Hornstra, G. The effects of purified eicosapentaenoic and docosahexaenoic acids on arterial thrombosis tendency and platelet function in rats, Biochimica Biophysica Acta, 1390, 1998, 313–322.

[45] Horrobin, D.F. Omega-6 and Omega-3 essential fatty acids in Atheroselerosis, Siminars in Thrombosis and Hemostasis, 19, 1993, 129-137.

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

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

[48] Sinclair, H.M. Essential fatty acids: a historical perspective, Biochemical Society Transactions, 18, 1990, 756-761.

[49] Seo,T., Blaner, W.S. and Deckelbaum, R.J. Omega-3 fatty acids: molecular approaches to optimal biological outcomes, Curr Op Lipidiol, 16, 2005, 8-11.

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

[51] Stanley-Samuelson, D. W., Jurenka, R. A., Cripps, C., Blomquist, J. G., De Renobales, M., Loher, W., *et al.* Fatty acids in insects: Composition, metabolism, and biological significance, Journal of Insect Biochemistry and Physiology, 9, 1988, 1–33.

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

[53] Agombar A, Cooper A.J. and Johnson CD. An aqueous formulation of gamma-linolenic acid with anti-proliferative action on human pancreatic cancer cell lines, Anticancer Drugs, 15(2), 2004, 157-60.

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

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

[56] Li, D., Habito, R., Angelos, G. Sinclair, A.J. and Ball, M.J. Rich in Saturated fat decreases the ratio of Thromboxane, prostacycn in healthy men, Biomedical and Environmental Science, 16, 2003, 333-339.

[57] Maizura, M., Aminah, A. and Wan Aida, W. Total phenolic content and antioxidant activity of kesum (Polygonum minus), ginger (Zingiber officinale) and turmeric (Curcuma longa) extract, International Food Research Journal, 18, 2011, 529-534.

[58] Marwah, R. G., Fatope, M. O., Mahrooqi, R. A., Varma, G. B., Abadi, H. A. and Al-Burtamani, S. K. S. Antioxidant capacity of some edible and wound healing plants in Oman, Food Chemistry 101(2) 2007, 465-470