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Identification of Antioxidants in Lamiaceae Vegetables by HPLC-ABTS and HPLC-MS

Trakul Prommajak[1], Sang Moo Kim[2], Cheol-Ho Pan[3], Sang Min Kim[3], Suthat Surawang[1] and Nithiya Rattanapanone[1,4*]

[1]Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand
[2]Department of Marine Food Science and Technology, Gangneung-Wonju National University, Gangneung 210702, Republic of Korea
[3]Functional Food Center, Korea Institute of Science and Technology, Gangneung 210340, Republic of Korea
[4]Posthavest Technology Research Institute, Chiang Mai University, Chiang Mai 50200, Thailand

**Corresponding author. E-mail: agfsi001@gmail.com*

ABSTRACT

Antioxidants in five Lamiaceae vegetables, namely lemon balm, sweet basil, clove basil, holy basil, and lemon basil, were identified by HPLC-ABTS and HPLC-MS. The major antioxidants in the samples were hydroxycinnamic acids. Rosmarinic acid exhibited the highest antioxidant activity in each sample, except clove basil, in which chicoric acid contributed the highest antioxidant activity. Lemon balm had the highest concentration of rosmarinic acid. Chicoric acid (highest in clove basil) and dihydroxy-dimethoxyflavone (highest in basil) were also important antioxidants in many Ocimum spp. Other minor antioxidants were caffeic acid, caftaric acid, syringic acid, syringic acid, rutin, and acacetin-acetylglucoside.

Keywords: Lamiaceae, Antioxidant, HPLC-ABTS, Rosmarinic acid, Chicoric acid

INTRODUCTION

Free radicals in food cause lipid peroxidation, which adversely affects its nutritional and organoleptic properties (Yen and Duh, 1994). Free radicals in the human body are associated with many degenerative diseases, e.g. cardiovascular disease, diabetes, and cancer (Boeing et al., 2012). Consumption of fruits, vegetables, and antioxidants was associated with a lower risk of cancers and cardiovascular disease (Genkinger et al., 2004).

Lamiaceae is a family of annual or perennial aromatic herbs. It contains 236 genera that can be grown under a variety of soil and climatic conditions worldwide, with the exception of the cold regions of high latitude (Harley et al., 2004). Lavender, thyme, peppermint, patchouli, rosemary, and sage are used to extract volatile aromatic oil or prepare perfumes. Some plants are used for medicinal purposes. For example, the leaves of *Ocimum sanctum* are used to treat

the common cold. Flowers of *O. basillicum* are used to treat digestive and renal diseases. Leaves and tubers of some Lamiaceae plants are used as food (Reddy et al., 2004).

Lamiaceae plants also contain antioxidative phenolic compounds that effectively scavenge free radicals. The major antioxidants in the plants of this family are hydroxycinnamic acids and flavonoids. Rosmarinic acid has also been found, notably in many Lamiaceae plants, e.g. basil, sage, lemon balm, peppermint, thyme, lavender, rosemary, marjoram, hyssop, savory, and oregano (Zgórka and Głowniak, 2001; Dorman et al., 2003). The other hydroxycinnamic acids responsible for the antioxidant capacity of Lamiaceae plants include caffeic acid, chlorogenic acid, and ferulic acid (Chen and Ho, 1997). Chicoric acid, another hydroxycinnamic acid, was recently found in basil and had not yet been identified in other Lamiaceae plants (Lee and Scagel, 2009). Lamiaceae plants also contain flavonoids. Apigenin and luteolin derivatives have been identified in germander, sage, thyme, savory, marjoram, and oregano (Dorman et al., 2003; Valant-Vetschera et al., 2003).

Online HPLC-antioxidant assay is a technique developed for simultaneous determination of compounds and their antioxidant capacity. The compounds eluted from a column react with an oxidant probe, e.g. 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{\bullet+}$), and decrease the absorbance of the chromophore. This method avoids the decomposition of the compounds that occurs in a conventional process (He et al., 2010).

Lemon balm, basil, clove basil, holy basil, and lemon basil are common culinary herbs in Thailand. To the best of our knowledge, the profiles of the phenolic compounds with their antioxidant capacity of these plants cultivated in Thailand have not been reported. Therefore, the objective of this study was to identify and quantify the bioactive compounds that are responsible for antioxidant activity in Lamiaceae vegetables from Thailand using LC-ABTS and LC-MS assays.

MATERIAL AND METHODS

Materials

Lemon balm (*Melissa officinalis* L.), sweet basil (*Ocimum basilicum* L.), clove basil (*Ocimum gratissimum* L.), holy basil (*Ocimum sanctum* L.), and lemon basil (*Ocimum* × *citriodorum*) were purchased from a local market in Chiang Mai Province, Thailand during August-September 2012. Methanol and water for HPLC analysis were purchased from Fisher (Seoul, Korea). 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and potassium persulfate were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of vegetable extracts

The edible parts of vegetables, without bruises or visual defects, were selected and washed in water for about 1 min. The vegetables (10 g) were extracted in 60% ethanol (50 ml) using a blender (Model MR 4050 CA, Braun, Spain) for 30 sec. Then, the extracts were filtered through a double layer of muslin cloth and centrifuged at 2,500xg for 20 min. The supernatant was kept at -80°C until

analysis. Extraction was performed in duplicates.

Identification and quantification of antioxidants

Separation of the compounds in the crude vegetable extracts was performed by Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA, USA) using the method described by He et al. (2010) with slight modification. Sample (10 µl) was eluted by 100% methanol (solvent A) and methanol/water/formic acid (20/80/0.25, v/v/v) (solvent B) through a Kromasil 100-5C18 (250×46 mm) column with a flow rate of 0.7 ml/min and column temperature of 30°C. Gradient elution began with the initial condition of 100% B, then changed to 90% B at 6.25 min, 80% B at 12.5 min, 55% B at 50 min, and 20% B at 68.75 min, before changing back to 100% B at 75 min. The compounds were detected by diode array detector (DAD) at 210, 254, 280, 330, and 520 nm.

The compounds that escaped from DAD were subjected to online-ABTS analysis. ABTS reagent (2 mM ABTS and 3.5 mM potassium persulphate) was prepared and kept in a dark condition at room temperature for 16 h to stabilize the radical before use. The reagent was pumped at a flow rate of 0.5 ml/min to react with the eluate, before passing through a multiple wavelength detector (MWD) for detection of ABTS at 734 nm. The presence of antioxidants in a sample resulted in negative peaks in the chromatogram.

Antioxidant concentrations were quantified from calibration curves. Flavonoids were quantified as the molar equivalent of quercetin. Other phenolic compounds were quantified as the molar equivalent of caffeic acid. Antioxidant capacities of the compounds were calculated from the area of the negative peak and expressed as Trolox equivalents.

For HPLC-MS analyses, compound separation and UV-Vis detection were performed using the same conditions as HPLC-ABTS. Atmospheric pressure ionization-electrospray (API-ES) ionization was performed by an Agilent 6120 quadrupole mass spectrometer in both positive and negative modes. Capillary voltages were 4,000 V for positive and 3,500 V for negative ionization. The drying gas flow rate was 9 L/min, the drying gas temperature was 350°C, and the nebulizer pressure was 35 psig. Mass spectra were recorded in the range of m/z 100-1,500. The compounds were identified by comparing molecular weights, fragments, and UV absorption with that reported in the literature.

Statistical analysis was performed by R version 2.15.1.

RESULTS

Identification of antioxidants

Various compounds accounted for the antioxidant activity of the ethanol extracts of the five Lamiaceae vegetables. HPLC-ABTS chromatograms revealed that the vegetables had similar antioxidant profiles (Figure 1). Mass spectra of identified compounds are shown in Figure 2. Compound 1 was identified as syringic acid (Table 1). Compounds 2, 3, 4, and 7 were hydroxycinnamic acids, identified as caftaric acid, caffeic acid, chicoric acid, and rosmarinic acid, respectively. Compounds 5, 6, and 9 were flavonoids, identified as acacetin-acetyl glucoside,

rutin (quercetin rutinoside), and dihydroxy-dimethoxyflavone, respectively. For general phenolic compounds, UV absorption at 280 nm was detected. But for hydroxycinnamic acids and flavonoids, absorption maxima around 330 nm were

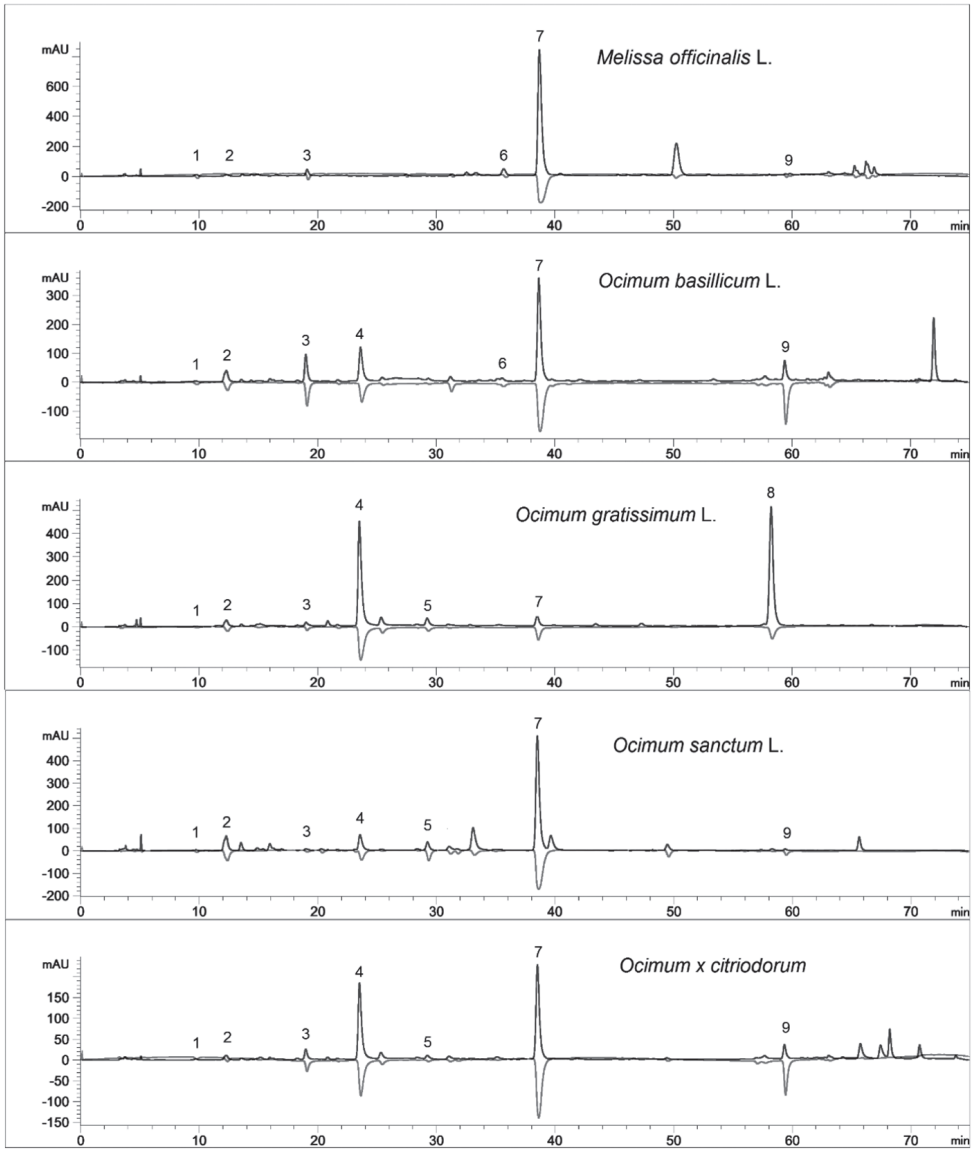

Figure 1. HPLC-ABTS chromatograms of the ethanolic extract of Lamiaceae vegetables. Positive peaks: DAD signal at 280 nm for detection of phenolic compounds. Negative peaks: MWD signal at 734 nm for detection of ABTS radical scavenging activity.

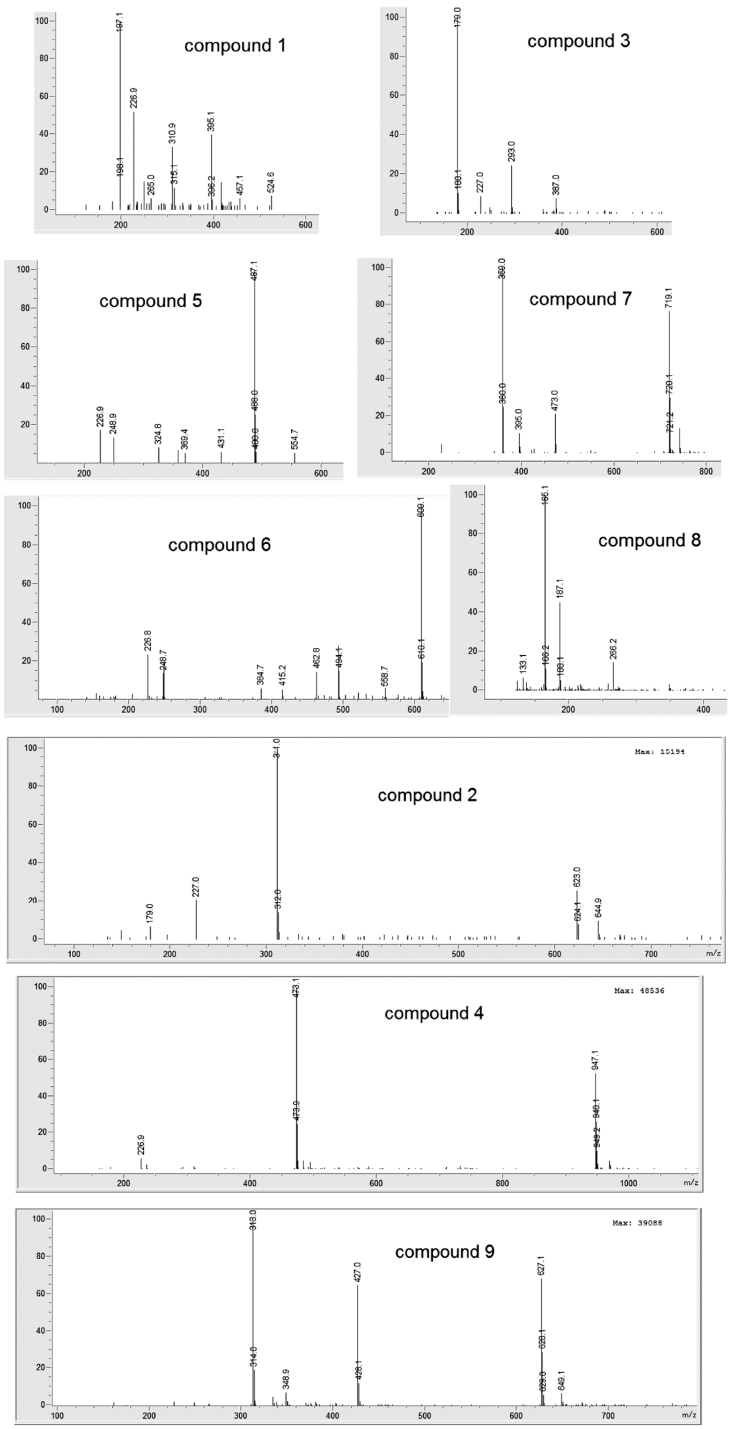

Figure 2. Mass spectra of antioxidative compounds identified from Lamiaceae vegetables. All mass spectra were from negative-ion mode, except compound 8.

Table 1. Identification of antioxidants in Lamiaceae vegetables by HPLC-MS.

No.	λ_{max} (nm)			MS negative ions (*m/z*)			MS positive ions (*m/z*)				MW	Proposed name	References
				$[M-H]^-$	$[2M-H]^-$	Other	$[M+H]^+$	$[M+Na]^+$	$[2M+Na]^+$	Other			
1	221	280		197	395	–	199	221	–	153 [M–COOH] 166 [M–OCH_3]	198	Syringic acid	Sun et al. (2007)
2	297sh	330		311	623	149 [tartaric acid–H]$^-$	–	335	647	163 [caffeoyl]	312	Caftaric acid	Lee and Scagel (2009)
3	245	324		179	–	–	181	–	–	135 [M–COOH]	180	Caffeic acid	(Hossain et al. (2010)
4	244	330		473	947	–	–	497	971	163 [caffeoyl] 295 [M–caffeic acid+H]$^+$	474	Chicoric acid	Lee and Scagel (2009)
5	222	244	330	487	–	–	–	511	–	163 [glucose+H]$^+$ 221 [acetyl glucoside] 285 [acacetin+H]$^+$	488	Acacetin-acetyl glucoside	Lai et al. (2007)
6	284	230	330sh	609	–	–	611	633	1243	303 [quercetin+H]$^+$ 449 [M–hexose]	610	Rutin	Eyles et al. (2007)
7	220	330		359	719	–	–	383	743	163 [caffeoyl]	360	Rosmarinic acid	Lee and Scagel (2009)
8	202	232	280		–	–	165	187	–	–	164	Coumaric acid	Sánchez-Rabaneda et al. (2003)
9	252	332		313	627	–	315	337	–	–	314	Dihydroxy-dimethoxyflavone	Vieira et al. (2003)

also detected (Tomas-Barberan and Ferreres, 2012).

Quantification of antioxidative compounds

The phenolic acid concentrations in five Lamiaceae vegetables are shown in Table 2. Of the phenolic compounds, rosmarinic acid was the most abundant in lemon balm, sweet basil, and holy basil, while chicoric acid was the most abundant in clove basil and lemon basil.

Table 2. The phenolic acid concentrations in Lamiaceae vegetables.

Compounds	Concentration[a] (mg/g DW)				
	Lemon balm	Sweet basil	Clove basil	Holy basil	Lemon basil
Syringic acid	0.30±0.01	0.07±0.01	0.07±0.00	0.12±0.03	0.08±0.00
Caftaric acid	0.52±0.12	2.60±0.35	1.26±0.02	4.13±0.12	0.71±0.02
Caffeic acid	1.03±0.03	2.44±0.53	0.35±0.07	0.22±0.03	0.63±0.09
Chicoric acid	ND[b]	5.67±0.25	17.12±0.41	3.32±0.43	8.46±0.23
Acacetin-acetyl glucoside	ND	0.58±0.01	2.30±0.02	3.19±0.04	0.75±0.03
Rutin	5.32±0.64	1.31±0.04	ND	ND	ND
Rosmarinic acid	32.31±2.33	13.32±1.12	1.21±0.08	17.19±1.19	7.65±0.07
Coumaric acid	ND	ND	14.08±0.16	ND	ND
Dihydroxy-dimethoxyflavone	0.14±0.00	3.35±0.44	ND	0.83±0.81	1.53±0.04

Note: [a]Means of duplicate analyses. Flavonoids were quantified as molar equivalent of quercetin. Other phenolic compounds were quantified as molar equivalent of caffeic acid. [b]ND, not detected.

Antioxidant capacity of phytochemicals

ABTS radical cation ($ABTS^{\bullet+}$) is a blue/green chromophore produced by the reaction between ABTS and potassium persulfate. It has an absorption maximum at 734 nm. In the presence of hydrogen-donating antioxidants, e.g. polyphenols and flavonoids, the radical cation becomes colorless. In an online HPLC-ABTS, it produced a negative peak in the presence of antioxidants (Koleva et al., 2001).

Rosmarinic acid possessed the highest antioxidant capacity among the compounds in the tested samples, except clove basil, in which chicoric acid contributed the highest antioxidant capacity (Table 3). Chicoric acid and dihydroxy-dimethoxyflavone were also responsible for a moderately large portion of antioxidant capacity in sweet basil and lemon basil. Caffeic acid, identified in all samples, was highest in sweet basil.

Table 3. Antioxidant capacity of phenolic compounds in Lamiaceae vegetables.

Compounds	Antioxidant capacity[a] (mmol TE/g DW)				
	Lemon balm	Sweet basil	Clove basil	Holy basil	Lemon basil
Syringic acid	0.37±0.04	0.12±0.03	0.06±0.01	0.13±0.02	0.10±0.01
Caftaric acid	0.19±0.03	0.83±0.08	0.39±0.01	1.21±0.06	0.19±0.00
Caffeic acid	0.78±0.06	1.77±0.16	0.28±0.03	0.14±0.03	0.50±0.02
Chicoric acid	ND[b]	2.10±0.04	4.18±0.11	1.18±0.13	2.65±0.01
Acacetin-acetyl glucoside	ND	0.08±0.00	0.30±0.00	1.22±0.20	0.06±0.02
Rutin	0.44±0.05	0.20±0.00	ND	ND	ND
Rosmarinic acid	9.29±0.32	7.11±0.05	1.16±0.07	7.46±0.06	4.85±0.03
Coumaric acid	ND	ND	1.19±0.08	ND	ND
Dihydroxy-dimethoxyflavone	0.18±0.01	3.54±0.19	ND	0.24±0.10	1.86±0.06

Note: [a]Means of duplicate analyses. [b]ND, not detected.

Trolox equivalent antioxidant capacity (TEAC) of antioxidants

The TEAC value is the specific antioxidant capacity of a given substance compared to the standard Trolox (Huang et al., 2005). It is calculated as the ratio between the antioxidant capacity (molar equivalent of Trolox from area of negative peak) and molarity of a compound. The antioxidant activity of the hydroxycinnamic acids decreased in the following order: rosmarinic acid > chicoric acid > caffeic acid > caftaric acid (Table 4).

Table 4. Trolox equivalent antioxidant capacity (TEAC) of phenolic compounds in Lamiaceae vegetables.

Compounds	TEAC[a]					
	Lemon balm	Sweet basil	Clove basil	Holy basil	Lemon basil	Mean±SD
Syringic acid	2.46	3.47	1.89	2.19	2.51	2.50±0.59
Caftaric acid	1.10	1.00	0.97	0.91	0.81	0.96±0.11
Caffeic acid	1.35	1.31	1.48	1.13	1.43	1.34±0.13
Chicoric acid	NDb	0.75	1.16	1.69	1.48	1.27±0.41
Acacetin-acetyl glucoside	ND	0.71	0.63	1.87	0.41	0.91±0.66
Rutin	0.50	0.91	ND	ND	ND	0.71±0.29
Rosmarinic acid	1.04	1.93	3.45	1.56	2.28	2.05±0.91
Coumaric acid	ND	ND	0.14	ND	ND	0.14
Dihydroxy-dimethoxyflavone	4.17	3.33	ND	0.92	3.81	3.06±1.47

Note: [a]Molar of Trolox with equivalent antioxidant capacity of a 1 molar substance. [b]ND, not detected.

DISCUSSION

Phenolic compounds are secondary metabolites in plants that play a role in plant adaptation to the environment, for example, protection against ultraviolet radiation, herbivores, pathogens, and abiotic stress (Bourgaud et al., 2001). The identified phenolic compounds were synthesized through the phenylpropanoid

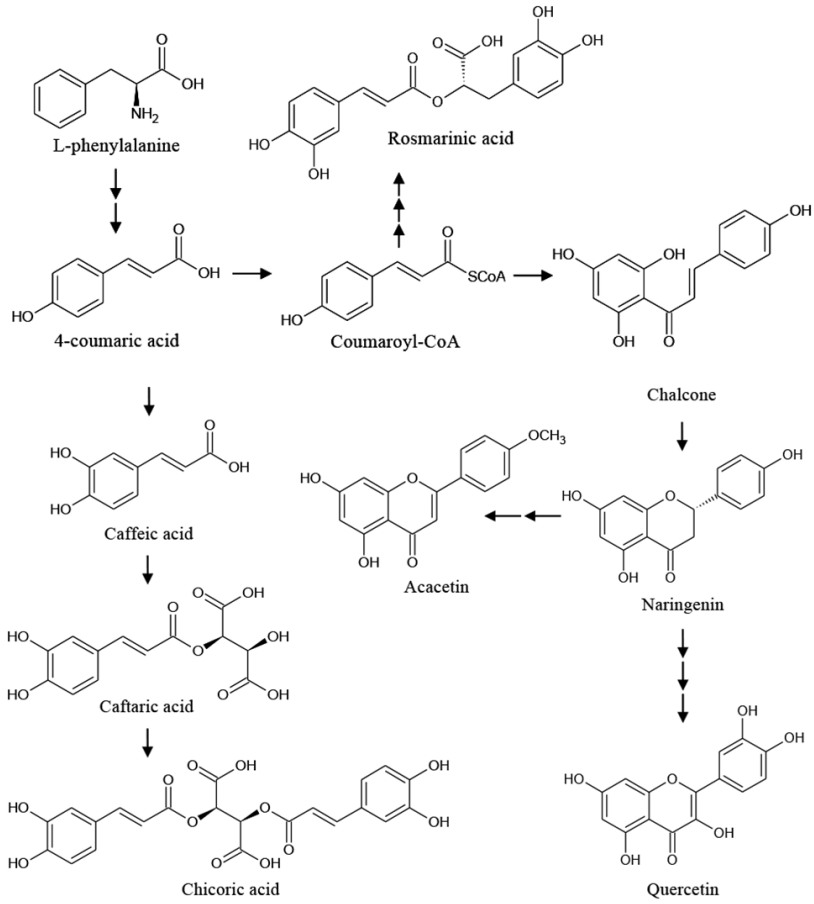

Figure 3. Phenylpropanoid pathway for synthesis of some phenolic compounds in Lamiaceae plants. Each arrow represents one enzymatic reaction. Adapted from: Petersen et al. (2009); Saltveit (2009); and Bel-Rhlid et al. (2012).

pathway as shown in Figure 3.

Although the culinary herbs used in this study contained similar antioxidant compounds, their concentrations varied; this affected the total antioxidant capacity of the extracts. The variation in phenolic compounds in plants is affected by species, genotypes, climate, location, and growing conditions (Asami et al., 2003; Anttonen and Karjalainen, 2005; Scalzo et al., 2005).

The caffeic acid concentrations in the Lamiaceae plants in this study (from 0.22 mg/g DW in holy basil to 2.44 mg/g DW in sweet basil) were similar to the range found in 10 Lamiaceae plants (from 0.1 mg/g DW in lavender to 2.6 mg/g DW in sweet basil) by Zgórka and Głowniak (2001).

The concentration of caftaric acid in sweet basil (2.82 mg/g DW) found here was higher than those reported in 15 basil cultivars (from 0.09 to 0.49 mg/g DW) by Kwee and Niemeyer (2011). Caftaric acid has also been found in other plant families – 0.51-1.68 mg/g DW in manatee grass (*Syringodium filiforme*) (Nuissier et al., 2010) and 1.9-6.5 mg/g DW in purple coneflower (*Echinacea purpurea*), depending on growth temperature and light adiation (Wu et al., 2007).

The concentration of chicoric acid in sweet basil (6.17 mg/100 g DW) found here was higher than those reported in 15 sweet basil cultivars (0.03-2.78 mg/g DW) by Kwee and Niemeyer (2011) and those reported in commercial dry basil (0.06-0.16 mg/g DW) by Lee and Scagel (2010). For other plant families, the concentration of chicoric acid has been reported as 0.94-5.26 mg/g DW for manatee grass (Nuissier et al., 2010) and 0.43-19.27 mg/g DW for *Echinacea* spp. (Pellati et al., 2004).

Chicoric acid has been previously identified as a major phenolic compounds in *Echinacea* spp. (Perry et al., 2001). In addition to its antioxidant activity, this compound also has been shown to have antiviral properties by targeting human immuno-deficiency virus type 1 (HIV-1) integrase (Robinson Jr, 1998). Echinacea, indigenous to North America, has been commercialized worldwide for treating the common cold (Barrett, 2003). This study found chicoric acid, a main bioactive compound in Echinacea, in clove basil, which is widely cultivated in Southeast Asia.

Rosmarinic acid was the most abundant of the hydroxycinnamic acids found in basil (13.25 mg/g DW); others have also reported this (Lee and Scagel, 2010; Bušić et al., 2013). The rosmarinic acid concentration in basil (13.25 mg/g DW) was similar to that of sweet basil from New Zealand (10.86 mg/g DW) (Shan et al., 2005). However, this is higher than Kwee and Niemeyer (2011) found in 15 different basil cultivars (0.10 to 6.09 mg/g DW). Basil variety and nitrogen fertilization affected the concentration of the phenolic compounds. Rosmarinic acid concentrations ranged from 5.41 mg/g DW in basil cv. Sweet Thai irrigated with 5 mM nitrogen to 47.89 mg/g DW in basil cv. Dark Opal grown in the summer and irrigated with 0.1 mM nitrogen (Nguyen and Niemeyer, 2008). Rosmarinic acid content in sage (*Salvia* spp.), another Lamiaceae plant, has been shown to vary from 13.3 to 47.3 mg/g DW (Bandoniene et al., 2005). In addition to its antioxidant activity, rosmarinic acid also has been shown to have anti-inflammatory activity by inhibiting diesel exhaust particles-induced lung injury (Sanbongi et al.,

2003) and antiviral activity against herpes simplex virus type 1 and 2 (Mazzanti et al., 2008; Astani et al., 2012).

Syringic acid has been identified in other Lamiaceae plants, e.g., basil, oregano, rosemary, sage, spearmint, and thyme, in concentrations ranging from 0.05 to 0.12 mg/g DW (Kivilompolo and Hyötyläinen, 2007).

Aglycone of acacetin has been previously identified in many Ocimum spp., e.g., *O. basillicum, O.* × *citriodorum, O. americanum,* and *O. minimum* (Grayer et al., 2004).

Rutin or quercetin-3-O-rutinoside has been previously identified in Lamiaceae plants, e.g., rosemary, oregano, basil, and thyme, with concentrations ranging from 0.3 to 34.0 mg/g FW (Lee and Scagel, 2009; Hossain et al., 2010; Sofic et al., 2010). Rutin has also been identified in clove basil (Grayer et al., 2000), although it was not detected in this study.

The isomers of dihydroxy-dimethoxyflavone, including cirsimaritin (5,4'diOH-6,7-diOMe flavone) and ladanein (5,6-diOH-7,4'-diOMe flavone), have been identified in hoary basil (*O. americanum*), sweet basil, and lemon basil (Grayer et al., 2004). The isomers have also been found in other Lamiaceae plants. For example, cirsimaritin and ladanein were identified in catmint (*Nepata* spp.) (Jamzad et al., 2003). Cirsimaritin and 5,4'-diOH-7,3-diOMe flavone have been identified in *Teucrium* spp. Cirsimaritin, ladanein, and velutin (5,4'-diOH-7,3'-diOMe flavone) have been identified in *Salvia* spp. (Valant-Vetschera et al., 2003).

The number and position of hydroxyl groups has been shown to influence the antioxidant activity of phenolic compounds (Arts et al., 2003). Rosmarinic acid and chicoric acid have four hydroxyl groups attached to two aromatic rings, while caffeic acid and caftaric acid have two hydroxyl groups attached to an aromatic ring. While the number and position of the hydroxyl groups attached to the aromatic rings is similar in rosmarinic acid and chicoric acid, rosmarinic acid has a higher TEAC value. The difference might due to the presence of a tartaroyl group in chicoric acid. A similar trend has also been found between caffeic acid and caftaric acid, with the latter containing a tartaroyl group.

Flavonoid aglycone (dihydroxy-dimethoxyflavone) had a higher TEAC value than flavonoid glycosides (rutin and acacetin-acetyl glucoside). Glycosylation negatively affected the antioxidant activity of flavonoids, due to a steric effect (Rice-Evans et al., 1996).

The TEAC values of the phenolic compounds found here (Table 4) were similar to those reported previously in the literature – 1.31 and 1.26 for caffeic acid (Cai et al., 2006; Rice-Evans et al., 1996) and 2.13 for rosmarinic acid (Nenadis et al., 2004).

The TEAC value represents the specific antioxidant capacity of a compound. The same compounds, even though analyzed from different samples, should have similar TEAC value. However, some of the same compounds extracted from different Lamiaceae plants had different TEAC values. A possible explanation for this variation is the low reaction kinetics of an ABTS assay, which takes a long time to reach the end point (Karadag et al., 2009). A normal reaction time

for an ABTS assay has been reported as 6 min (Re et al., 1999). In contrast, the reaction in an online HPLC-ABTS assay takes only a few minutes before reaching a detector. Different compounds require different reaction times with the ABTS radical to reach the end point. Walker and Everette (2009) have reported end points for Trolox and ascorbic acid of within 2 s; gallic acid of more than 15 s; rutin, quercetin, and curcumin of about 1 min; and chlorogenic acid, caffeic acid, and ferulic acid of more than 1 min. Higher antioxidant concentrations prolonged the reaction rates. Gallic acid at the concentration of 50 μM required upwards of 5 min to reach steady state, while 250 μM gallic acid required 15 min (Pérez-Jiménez and Saura-Calixto, 2008). Although the reaction time between the ABTS radical and different substances may differ, each substance had the same reaction time from mixing point to detector (as determined by flow rate of HPLC). Therefore, the same substance from different samples had the same reaction time, and should respond according to their concentration.

In this study, the TEAC value of rosmarinic acid (derivative of caffeic acid) varied from 1.04 to 3.45. A plot between concentration and TEAC value of rosmarinic acid showed a negative relationship (Figure 4). The higher the concentration of rosmarinic acid in the samples, the lower the TEAC values. These results indicated that ABTS might not be appropriate for quantifying the antioxidant capacity of antioxidants with slow reaction kinetics by an online HPLC-ABTS, unless the antioxidant concentrations were low enough to reach steady state before reaching a detector.

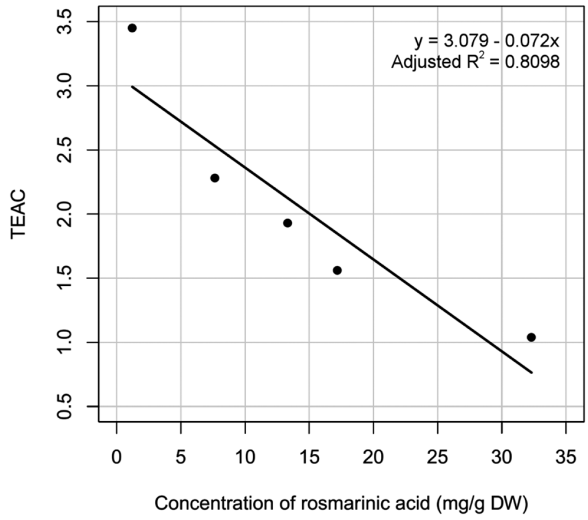

Figure 4. Relation between rosmarinic acid concentration and TEAC value obtained by HPLC-ABTS.

CONCLUSION

The Lamiaceae vegetables studied here contained similar compounds that were responsible for their antioxidant activity. However, these compounds were present at different concentrations. The major antioxidants were hydroxycinnamic acids and flavonoids. Rosmarinic acid was responsible for the highest antioxidant activity in all samples, except clove basil. The rosmarinic acid content was highest in lemon balm. Other major antioxidants were chicoric acid and dihydroxy-dimethoxyflavone. Chicoric acid content was highest in clove basil. Dihydroxy-dimethoxyflavone content was highest in sweet basil. The minor antioxidants were caffeic acid, caftaric acid, syringic acid, rutin, and acacetin-acetylglucoside. In addition to antioxidant activity, Lamiaceae plants also possessed other medicinal properties. *Ocimum* spp., especially clove basil, contained a significant amount of antiviral chicoric acid, making it an alternative source to Echinacea.

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