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DPPH Free-Radical Scavenging Activity, Total Phenolic Contents and Chemical Composition Analysis of Forty-Two Kinds of Essential Oils

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ABSTRACT

Forty-two commonly used essential oils were investigated for the antioxidant capabilities by DPPH free-radical scavenging activity, total phenolic contents and photochemiluminescence (PCL) assay. At the concentration of 5 mg/mL, cinnamon bark (91.4 ± 0.002%), origanum (86.66 ± 0.008%) and thyme wild (52.54 ± 0.016%) were shown to own the strongest DPPH free-radical scavenging activity. Their total phenolic contents were 658.40 ± 4.383, 1107.20 ± 0.768 and 275.50 ± 0.607 (μg GAE / 5 mg essential oil), respectively. To compare with the standard reference BHA (μg/mL), their EC_{50} were in the order: BHA (25.11 μg/mL) < cinnamon bark (90.63 μg/mL) <origanum (751.51 μg/mL). The photochemiluminescence assay was also employed to investigate the antioxidative capabilities of lipid-soluble substances (ACL). The results were as follow: cinnamon bark (133.9 ± 0.26 μmol trolox/g) > origanum (62.63 ± 1.73 μmol trolox/g) > theme wild (5.88 ± 0.16 μmol trolox/g). The chemical compositions of cinnamon bark, origanum and thyme wild were analyzed by GC-MS and followed by DPPH free-radical scavenging activity assay to confirm that eugenol, carvacrol and thymol were the major compositions contributing the antioxidative capabilities of the essential oils.

Key words: DPPH free-radical scavenging activity, total phenolic contents, photochemiluminescence, essential oil

INTRODUCTION

In recent years, there is an increasing interest in finding antioxidant phytochemicals, because they can inhibit the propagation of free-radical reactions and protect the human body from diseases[1]. Free-radicals and other reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, and hydrogen peroxide are an entire class of highly reactive molecules derived from the normal metabolism of oxygen or from exogenous factors and agents[2]. ROS's125 were reported to be a causative agent of various diseases such as arthritis, asthma, dementia, mongolism, carcinoma and Parkinson's disease[3].

Phenolic compounds are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers[4]. It is reported that phenolic compounds in plants possess strong antioxidant activity and may help to protect cells against the oxidative damage caused by free-radicals[5]. Consumption of fruits and vegetables with high content of antioxidative phytochemicals such as phenolic compounds may reduce the risk of cancer, cardiovascular disease and many other diseases[6,7]. About 100 pure components of essential oils have been tested for their antioxidant effectiveness by Ruberto and Baratta[8]. From a general point of view phenols (eugenol, carvacrol and thymol) were confirmed to possess the highest antioxidant activity. Yanishlieva *et al*. showed thymol and carvacrol participated in one side reaction during inhibited TGL (triacylglycerols of lard) oxidation, and thymol took part in two side reactions during TGSO (triacylglycerols of sunflower oil) oxidation. In general, during autoxidation of lipids at ambient temperature, thymol is a more effective and more active antioxidant than carvacrol[9].

Essential oils are known to possess potential as natural agents for food preservation[10,11]. In addition, essential oils and components are gaining increasing interest because of relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-functional use[12]. Bioactive compounds commonly found in fruits, vegetables, herbs, and other plants have been shown to have possible health benefits with antioxidative, anticarcinogenic, atherosclerosis, antimutagenic,

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and angiogenesis inhibitory activities[13-15]. Mathew and Abraham evaluated the antioxidant activities of the methanolic extract of *Cinnamomum verum* barks (CBE), which was found to be potent in free radical scavenging activity especially against DPPH radicals and ABTS radical cations. The peroxidation inhibiting activity of CBE, recorded using a linoleic acid emulsion system, showed very good antioxidant activity[16]. Singh *et al.* reported (*E*)-cinnamaldehyde (97.7%), δ -cadinene (0.9%) and a -copaene (0.8%) were the major components of cinnamon bark volatile oil[17]. Raina *et al.* showed eugenol (76.6%), linalool (8.5%) and pipertone (3.3%) as major components from cinnamon leaves[18].

Many authors, in fact, have reported antimicrobial, antifungal, antioxidant and radical-scavenging properties by spices and essential oils[19]. Most reports of essential oils were determined by original extraction. These essential oils of original extraction were different from commercially used essential oils. In our laboratory, forty-five essential oils have been published by antioxidant assay of DPPH free-radical scavenging activity and total phenolic content (TPC)[20]. The present study was aimed to analyze the relative content of phenolics in another forty-two commonly commercially available essential oils to evaluate their antioxidant capacity and to identify the components.

MATERIALS AND METHODS

I. *Materials*

Ethanol (EtOH) was of HPLC grade purchased from Echo Chemical Co. (Taiwan). The chemicals 1, 1-diphenyl-2-picrylydrazyl (DPPH), butyl hydroxy anisole (BHA) and β-caryophyllene were from TCI Shanghai, Japan. Folin-Ciocalteau's phenol reagent and eugenol were from Merck, Germany. Thymol and *ρ*-cymene were from Acros Organics, Belgium. Gallic acid, carvacrol and *trans*-cinnamaldehyde were from Lancaster (England), SAFC (USA) and Alfa Aesar (China), respectively. Forty-two essential oils were purchased from Australian Botanical Products (TGA warrant by Australian government, USDA and ACO certification). Information of the forty-two essential oils was listed in Table 1.

II. *Methods*

(I) *DPPH Free- Radical Scavenging Assay*

The DPPH free-radical scavenging activity was determined by the methods described by Liu *et al.*[21] and Yang *et al.*[22] with modifications. Two hundred and fifty microliters of essential oil in EtOH solution (5 mg/mL) was added to 250 μL of 5.07×10^{-4} M DPPH EtOH solution. The reaction mixture was incubated in the dark at room temperature; the absorbance was measured at 517

Table 1. Information on forty-two essential oils

NO.	Name	Scientific name	Origin	Department	Extraction method	Extraction part
1	Highly Lavendr	*Lavandula angustifolia*	France	Lamiaceae	Distillation	fresh flowering tops
2	Camphor	*Cinnamomum camphora*	China	Lauraceae	Distillation	leaves
3	Angelica root	*Angelica archangelica*	Europe	Apiaceae	Distillation	roots
4	Patchouli	*Pogostemon cablin*	Indonesia	Lamiaceae	Distillation	leaves
5	Lavendin	*Lavandula X.intermedia*	Europe	Lamiaceae	Distillation	fresh flowering tops
6	Palmarosa	*Cymbopogon martini var.motia*	India/South America	Poaceae	Distillation	leaves
7	Marioram	*Origanum maforana*	Egypt	Lamiaceae	Distillation	leaves
8	Origanum	*Origanum vulgare*	Europe	Lamiaceae	Distillation	leaves
9	Citronella Ceylon	*Cymbopogon nardus*	Sri Lanka	Poaceae	Distillation	leaves
10	Cubeb	*Piper cubeba L.*	Indonesia	piperaceae	Distillation	seeds
11	Galbanm	*Ferula galvaniflua*	Europe/Middle East	Apiaceae	Distillation	gum
12	Fir nedle Siberian	*Abies sibirica*	Ukraine	Pinaceae	Distillation	needles
13	Litsea cubeba	*Litsea cubeba*	China	Lauraceae	Distillation	berries & leaves

Table 1. Continued

NO.	Name	Scientific name	Origin	Department	Extraction method	Extraction part
14	Melissa genuine	*Melissa officinalis*	Europe	Lamiaceae	Distillation	leaves
15	Grapefruit	*Citrus paradisi*	USA	Rutaceae	Cold compression	fruit rind
16	Kanuka	*Kunzea ericiodes*	New Zealand	Myrtaceae	Distillation	Leaves
17	Elemi	*Canarium luzonicum*	Europe	Burseraceae	Distillation	Gum
18	Peru balsam	*Myroxylon pereirae*	Peru	Fabaceae	Distillation	Balsam
19	Roman chamomile	*Anthemis nobilis*	France	Asteraceae	Distillation	Flowers
20	Cabreuva	*Myrocarpus fastigiatus*	South America	Fabaceae	Distillation	Wood
21	Cananga Java	*Cananga odorata*	Indonesia	Annonaceae	Distillation	Flowers
22	Lime	*Citrus aurantifolia*	South America	Rutaceae	Cold compression	fruit rind
23	Lavender spike	*Lavandula latifolia*	Europe	Lamiaceae	Distillation	fresh flowering tops
24	Lemongrass	*Cymbopogon flexuosus*	India	Poaceae	Distillation	Leaves
25	Orange Bitter	*Citrus aurantium biagarade*	Sicily	Rutaceae	Cold compression	fruit rind
26	Spikenard	*Nardostacys jatamansi*	Nepal	Valerianaceae	Distillation	roots
27	Tarragon	*Artemisia dracunculus*	Europe/Middle East	Asteraceae	Distillation	leaves
28	Thyme linalool	*Thymus vulgaris*	Europe	Lamiaceae	Distillation	leaves
29	Thyme wild	*Thymus serpyllum*	Europe	Lamiaceae	Distillation	leaves
30	Dill weed	*Anethum graveolens*	Europe	Apiaceae	Distillation	leaves & flowering tops
31	Eucalyptus dives “C”	*Eucalyptus dives var.C*	Australia	Myrtaceae	Distillation	leaves & terminal branchlets
32	Eucalyptus peppermint	*Eucalyptus dives”Type”*	Australia	Myrtaceae	Distillation	leaves & terminal branchlets
33	Eucalyptus blue gum	*Eucalyptus globules*	Paraquay	Myrtaceae	Distillation	leaves & terminal branchlets
34	Spearmint	*Minthe spicata*	China	Lamiaceae	Distillation	leaves
35	Rosemary verbenone	*Rosmarinus officinalis*	Europe	Lamiaceae	Distillation	leaves
36	Niaouli pacific islands	*Melaleuca quinqunervia*	Madagascar	Myrtaceae	Distillation	leaves
37	Hyssop	*Hyssopus officinalis*	Europe	Lamiaceae	Distillation	leaves
38	Cinnamon bark	*Cinnamomum zeylanicum*	Sri Lanka	Lauraceae	Distillation	bark
39	Caraway	*Elettaria cardamomum*	Europe	Apiaceae	Distillation	seeds
40	Carrot seed	*Daucrs carota*	India	Apiaceae	Distillation	seeds
41	Parsley herb	*Petroselinium crispum*	Australia	Apiaceae	Distillation	seeds
42	Celery seed	*Apium graveolens*	India	Apiaceae	Distillation	seeds

nm after one hour. EtOH was used as the control and BHA was used as a standard reference.

DPPH radical scavenging activity was calculated by using the following equation: Scavenging activity (%) = (1- absorbance of essential oil/absorbance of control) × 100%

(II) *Determination of Total Phenolic Contents*

Total phenolic contents (TPC) were determined according to Folin-Ciocalteu's methods[23]. The essential oils were diluted to a suitable concentration for analysis. A half militers of essential oil, 1 mL of 1N Folin-Ciocalteu's reagent and 1 mL of 20% Na_2CO_3 (w/v) were mixed. After 2 hours of incubating at ambient temperature, the mixture was centrifuged for 10 min (8000 rpm). 'The supernatant was measured at 765 nm.

Different concentrations of gallic acid (10-90 μg/mL) was determined to be a calibration curve (y = 0.0315x - 0.0296; $\gamma^2 = 0.9997$; y is absorbance, x is concentration of gallic acid). The results were shown as μg gallic acid equivalents (GAE)/5mg essential oil.

(III) *Photochemiluminescence (PCL) Assay*

The luminol-photochemiluminescence was used to measure antioxidative capabilities of lipid-soluble substances (ACL) with a standard protocol in photochem system (Analytik Jena, Taiwan). All kits were supplied by the company. The photosensitizer luminal generates superoxide radicals and a chemiluminogenic probe for free-radicals.

$S + h\nu + O_2 \rightarrow [S^{\bullet+} \times O_2^{\bullet-}] \rightarrow S + O_2$ [24]

To establish the standard calibration curve, 2270 μL reagent 1 (methanol), 250 μL reagent 2 (reaction buffer), 25 μL reagent 3 (luminol) and different volume of reagent 4 (trolox) were mixed. Essential oils were replaced by reagent 4 to measure the antioxidant activities. The antioxidant potential was determined by means of the area under the curve at different concentrations and expressed as μmol of trolox/g[25].

(IV) *Gas Chromatography-mass Spectrometry (GC-MS)*

The components of essential oils were analyzed by using a Thermo GC-MS system (Trace GC 2000, Trace DSQ-Mass Spectrometer, MSD 201351, Thermo, USA). The capillary column was a TR-5MS (5% phenyl polysilphenylene-siloxane, Thermo, USA) with a length of 30 m, an inside diameter of 0.25 mm and a film thickness of 0.25 μm[15]. The carrier gas was helium. The analysis condition of GC oven was followed the procedure: initial temperature 40°C for 1 min, programmed rate at 8°C /min up to final temperature and held isothermally for 10 min. The temperature of injector and detector were set at 200 and 250°C, respectively. An aliquot of 1 μL essential oil dissolved in EtOH and adjusted to 0.5 mg/mL was injected for analysis. Identification of the components were based on comparisons of retention times and mass spectral fragmentation pattern with NIST98 database.

RESULTS AND DISCUSSION

I. *DPPH Free-Radical Scavenging Activity*

According to the results obtained, cinnamon bark was found the strongest DPPH free-radical scavenging activity (91.4 ± 0.002%). This activity was followed by origanum (86.66 ± 0.008%) and thyme wild (52.54 ± 0.016%). Niaouli pacific islands (4.29 ± 0.007%) and grapefruit (6.3 ± 0.010%) were the lowest two essential oils of the DPPH free-radical scavenging activity. All data were listed in Table 2. To compare with the standard reference BHA, Figure 1 and Figure 2 were shown

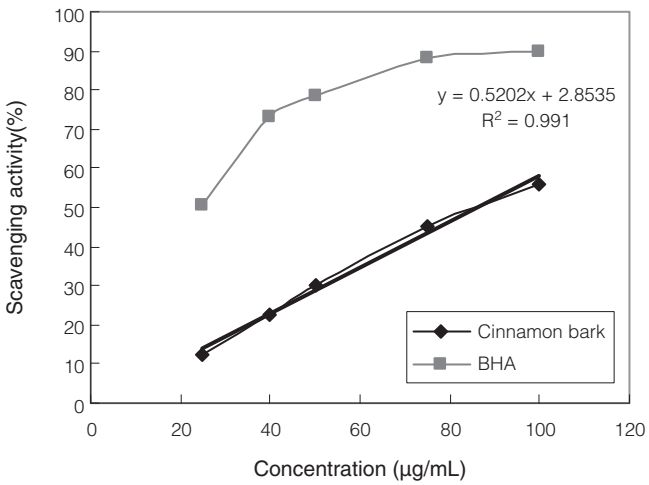

Figure 1. DPPH free-radical scavenging activity in different concentrations of cinnamon bark and BHA.

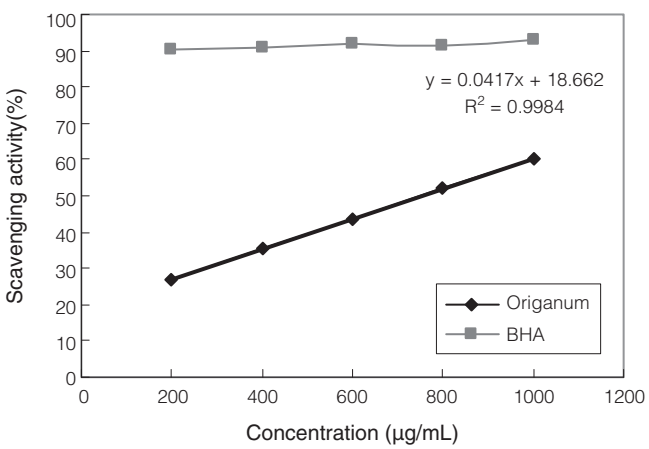

Figure 2. DPPH free-radical scavenging activity in different concentrations of origanum and BHA.

Table 2. DPPH free-radical scavenging activity and total phenolic contents of the forty-two essential oils

NO.	Name	Scientific name	DPPH free-radical scavenging activity (%)*	Total phenolic content (μg GAE/5 mg essential oil/mL EtOH)*
1	Highly Lavender	*Lavandula angustifolia*	15.18 ± 0.009	6.76 ± 0.611
2	Camphor	*Cinnamomum camphora*	10.08 ± 0.008	6.05 ± 0.98
3	Angelica root	*Angelica archangelica*	17.33 ± 0.004	11.75 ± 0.419
4	Patchouli	*Pogostemon cablin*	15.63 ± 0.009	20.50 ± 0.151
5	Lavendin	*Lavandula X.intermedia*	13.45 ± 0.018	5.11 ± 0.168
6	Palmarosa	*Cymbopogon martini var.motia*	14.67 ± 0.022	6.55 ± 0.118
7	Marioram	*Origanum maforana*	14.20 ± 0.013	11.41 ± 0.152
8	Origanum	*Origanum vulgare*	86.66 ± 0.008	1107.2 ± 0.768
9	Citronella Ceylon	*Cymbopogon nardus*	16.08 ± 0.020	9.90 ± 0.579
10	Cubeb	*Piper cubeba L.*	17.53 ± 0.030	31.05 ± 0.417
11	Galbanm	*Ferula galvaniflua*	12.45 ± 0.010	19.78 ± 0.606
12	Fir nedle Siberian	*Abies sibirica*	13.45 ± 0.010	5.91 ± 0.102
13	Litsea cubeba	*Litsea cubeba*	9.05 ± 0.007	13.27 ± 0.284
14	Melissa genuine	*Melissa officinalis*	9.39 ± 0.017	16.95 ± 0.413
15	Grapefruit	*Citrus paradisi*	6.3 ± 0.010	5.78 ± 0.354
16	Kanuka	*Kunzea ericiodes*	9.41 ± 0.007	7.47 ± 0.141
17	Elemi	*Canarium luzonicum*	11.83 ± 0.013	7.58 ± 0.530
18	Peru balsam	*Myroxylon pereirae*	24.83 ± 0.010	39.33 ± 0.563
19	Roman chamomile	*Anthemis nobilis*	13.68 ± 0.017	5.85 ± 0.450
20	Cabreuva	*Myrocarpus fastigiatus*	11.85 ± 0.020	4.05 ± 0.026
21	Cananga Java	*Cananga odorata*	26.47 ± 0.012	13.90 ± 0.296
22	Lime	*Citrus aurantifolia*	15.22 ± 0.011	7.89 ± 0.331
23	Lavender spike	*Lavandula latifolia*	10.97 ± 0.015	6.20 ± 0.111
24	Lemongrass	*Cymbopogon flexuosus*	34.67 ± 0.004	23.85 ± 0.862
25	Orange Bitter	*Citrus aurantium biagarade*	14.75 ± 0.020	7.87 ± 0.228
26	Spikenard	*Nardostacys jatamansi*	22.76 ± 0.008	19.73 ± 0.723
27	Tarragon	*Artemisia dracunculus*	15.86 ± 0.016	18.77 ± 0.591
28	Thyme linalool	*Thymus vulgaris*	31.62 ± 0.018	57.69 ± 0.649
29	Thyme wild	*Thymus serpyllum*	52.54 ± 0.016	275.50 ± 0.607
30	Dill weed	*Anethum graveolens*	14.79 ± 0.003	9.67 ± 0.369
31	Eucalyptus dives “C”	*Eucalyptus dives var.C*	12.57 ± 0.068	5.77 ± 0.250
32	Eucalyptus peppermint	*Eucalyptus dives”Type”*	6.85 ± 0.010	6.11 ± 0.401
33	Eucalyptus blue gum	*Eucalyptus globules*	12.53 ± 0.020	6.79 ± 0.168
34	Spearmint	*Minthe spicata*	9.37 ± 0.011	11.40 ± 0.188
35	Rosemary verbenone	*Rosmarinus officinalis*	7.71 ± 0.018	6.76 ± 0.232
36	Niaouli pacific islands	*Melaleuca quinqunervia*	4.29 ± 0.007	5.94 ± 0.022
37	Hyssop	*Hyssopus officinalis*	16.48 ± 0.020	11.12 ± 0.666

Table 2. Continued

NO.	Name	Scientific name	DPPH free-radical scavenging activity (%)*	Total phenolic content (μg GAE/5 mg essential oil/mL EtOH)*
38	Cinnamon bark	*Cinnamomum zeylanicum*	91.4 ± 0.002	658.4 ± 4.383
39	Caraway	*Elettaria cardamomum*	23.55 ± 0.015	20.98 ± 0.741
40	Carrot seed	*Daucrs carota*	9.01 ± 0.018	8.20 ± 0.180
41	Parsley herb	*Petroselinium crispum*	17.68 ± 0.007	21.12 ± 0.528
42	Celery seed	*Apium graveolens*	27.18 ± 0.016	17.74 ± 0.566

* Values are mean ± SD (n = 3)

Table 3. Antioxidant activity of cinnamon bark, origanum and thyme wild measured by using PCL methods

NO.	Name	Scientific name	ACL (mmol trolox/g)#
38	Cinnamon bark	*Cinnamomum zeylanicum*	133.9 ± 0.26
8	Origanum	*Origanum vulgare*	62.63 ± 1.73
29	Thyme wild	*Thymus serpyllum*	5.88 ± 0.16

#ACL: Antioxidant activity of lipid-soluble substance. Results are mean ± RSD.

EC_{50} values of BHA, cinnamon bark and origanum and their EC_{50} values were 25.11 μg/mL, 90.63 μg/mL and 751.51 μg/mL, respectively.

II. *Total Phenolic Contents*

The total phenolic contents (TPC) of forty-two essential oils were expressed as equivalents of gallic acid (GAE/5 mg of essential oil). As shown in Table 2, the essential oils were found to have various phenolic levels, range from 5.11 to 1107.20 μg GAE/5 mg essential oil. For each 5 mg essential oil, origanum had the highest contents of total phenolic (1107.20 ± 0.768 μg GAE), followed by cinnamon bark (658.40 ± 4.383 μg GAE) and thyme wild (275.50 ± 0.607 μg GAE), whereas, the least one was cabreuva (4.05 ± 0.026 μg GAE), followed by lavendin (5.11 ± 0.168 μg GAE).

III. *Antioxidant Capability*

According to the results of DPPH free-radical scavenging activity and TPC, essential oils with desired properties and ranked within the first three places were employed to measure antioxidative capabilities of lipid-soluble substances (ACL) by PCL assay. As shown in Table 3, cinnamon bark had the strongest antioxidant capability 133.9 ± 0.26 μmol trolox/g, followed by origanum (62.63 ± 1.73 μmol trolox/g) and thyme wild (5.88 ± 0.16 μmol trolox/g).

IV. *Analysis of Chemical Composition by GC-MS*

Cinnamon bark, origanum and thyme wild were the first three essential oils with strongest DPPH free-radical scavenging activity and highest total phenolic contents. The chemical compositions of the three essential oils were analyzed by GC-MS and were listed in Table 4. As shown in Table 4, *ρ*-cymene, *D*-limonene, β-phellandrene, β-linalool, α-terpineol, *trans*-cinnamaldehyde, methyl cinnamate, eugenol, copaene, β-caryophyllen, cinnamyl acetate, α-caryophyllene, eugenol acetate, caryophyllene oxide and benzyl benzoate were detected and there is 8.53% of eugenol in cinnamon bark. Four major components and cinnamon bark essential oil were employed to investigate the DPPH free-radical scavenging activity. At a concentration of 0.5 mg/mL, the DPPH free-radical scavenging activity of eugenol, β-linalool, β-caryophyllene, *trans*-cinnamaldehyde and cinnamon bark essential oil was shown in Figure 3 as follows: eugenol > cinnamon bark > β-caryophyllen > β-linalool. It is clear that the eugenol was the major component responsible for the DPPH free-radical scavenging activity of the cinnamon bark essential oil.

There were eleven components in origanum: 3-carene, *ρ*-cymene, D-limonene, eucalyptol, α-terpinene, camphor, borneol, terpene-4-ol, carvacrol, thymol and β-caryophyllen. As shown in Figure 4, the DPPH free-radical scavenging activity of origanum and it's components are in the following order: thymol > origanum > carvacrol > *ρ*-cymene. Thymol was the major component

Table 4. Chemical compositions of cinnamon bark, origanum and thyme wild essential oils

R_t [a]	Compound [b]	Percent in samples (%)		
		Cinnamon bark	Origanum	Thyme wild
6.96	3-Thujene	-	-	1.03
7.15	1R-α-Pinene	-	-	1.02
7.52	Camphene	-	-	1.6
7.95	β-Phellandrene	-	-	0.43
8.09	(-)-β-Pinene	-	-	0.69
8.22	β-Myrcene	-	-	2.99
8.85	Terpinolen	-	-	0.77
8.85	3-Carene	-	0.48	-
9.04	ρ-Cymene	1.26	5.42	18.84
9.12	D-Limonene	0.27	0.44	0.58
9.18	β-Phellandrene	0.56	-	-
9.21	Eucalyptol	-	1.31	1.26
9.69	α-Terpinene	-	1.59	1.91
9.97	cis-β-Terpineol	-	-	0.88
10.46	β-Linalool	2.18	-	21
10.6	Dihydrocarveol	-	-	0.41
11.61	Camphor	-	1.71	-
12.05	Borneol	-	0.6	2.7
12.16	Terpene-4-ol	-	0.48	1.66
12.44	α-Terpineol	0.34	-	6.89
12.88	*cis*-Geraniol	-	-	0.57
13.04	Thymol methyl ether	-	-	4.68
13.3	Nerol acetate	-	-	0.66
14.03	*trans*-Cinnamaldehyde	75.32	-	-
14.08	Carvacrol	-	2.56	10.88
14.21	Methyl cinnamate	1.02	-	-
14.25	Thymol	-	83.87	3.53
14.86	L-Carveol	-	-	2.83
15.02	α-Terpineol acetate	-	-	3.3
15.23	Eugenol	8.53	-	-
15.57	Copaene	0.73	-	-
15.73	β-Bourbonene	-	-	0.5
16.36	β-Caryophyllene	4.52	1.34	3.07
16.77	Cinnamyl acetate	1.14	-	-
16.96	α-Caryophyllene	0.75	-	-
17.35	ç-Muurolene	-	-	1.36
17.61	β-Bisabolene	-	-	2.22

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Table 4. Continued

R_t [a]	Compound [b]	Percent in samples (%)		
		Cinnamon bark	Origanum	Thyme wild
17.78	Eugenol acetate	1.22	-	-
17.85	(+)- δ -Cadinene	-	-	0.61
18.99	Nerolidol	-	-	0.27
18.99	Caryophyllene oxide	0.51	-	-
21.73	Benzyl Benzoate	1.21	-	-
-	Total identified	99.55	99.8	98.15

[a] R_t: Retention time (min)

b The components were identified by the mass spectra and retention indicators (RIs) and the Wiley and NIST mass spectral databases and the previously published RIs.

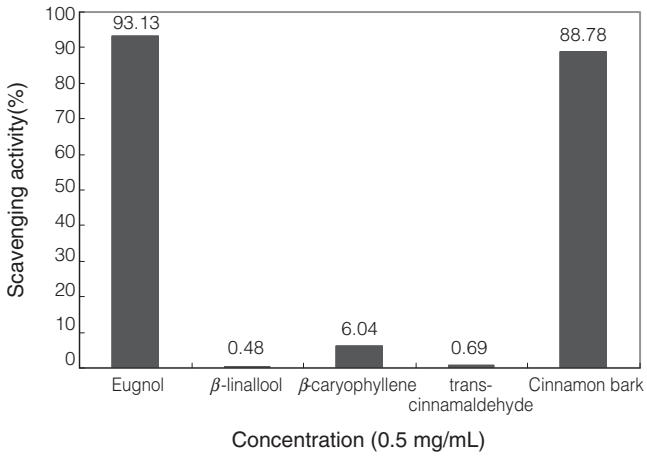

Figure 3. DPPH free-radical scavenging activity of four pure chemical components of cinnamon bark compared with its essential oils at a concentration of 0.5 mg/mL.

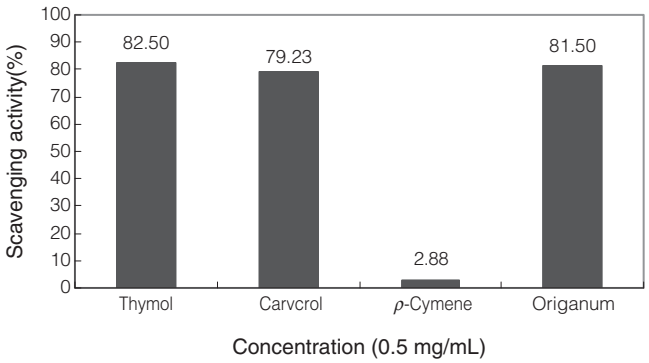

Figure 4. DPPH free-radical scavenging activity of three pure chemical components of origanum compared with its essential oils at a concentration of 5 mg/mL.

contributing to the DPPH free-radical scavenging activity of the origanum essential oil. Thymoquinone (40.2%), benzyl alcohol (8.9%), eugenol (7.5%), 2-phenyl-ethanol (5.6%), thymol (3.5%), 3-hexen-1-ol (3.4%) and carvacrol (2.4%) were the major components of *Origanum vulgare* L. ssp. *hirtum* reported by Milos *et al*. Thymoquinone and the other glycosidically bound volatiles in the spice plant oregano were found to be potent antioxidants, comparable in activity with its essential oil as well as to widely used natural anti-oxidant α -tocopherol[26] Kouri *et al*. reported carvacrol (45.0%), thymol (2.6%) and methyl-1, 4-benzoquinone (24.7%) were the major components of *Origanum dictamnus* identified by GC-MS analysis. The conclusion showed that *Origanum dictamnus* contains phenolic compounds, mainly flavonoids and phenolic acids, with hydrogen-donating capacity and ability to protect oil against oxidation[27].

In Figure 5, twelve components of thyme wild were used in an experiment on the DPPH free-radical scavenging activity with the order: thymol > carvacrol > thyme wild > thymol methyl ether. Thymol and carvacrol were the major components attributing the DPPH free-radical scavenging activity in the thyme wild essential oil.

In previous study[20], we reported DPPH free-radical scavenging activity and TPC of forty-five different kinds of essential oils. The free radical scavenging ability and TPC of cinnamon leaf and clove bud essential oils were the best two in those essential oils. A half milliliter of cinnamon leaf and clove bud essential oils (10 mg/mL EtOH) presented 96.7% and 96.1% of the DPPH (2.5 mL, 1.52×10^{-4} M) free radical scavenging ability, with effective concentration (EC_{50}) at 53 μg/mL and 36 μg/mL, respectively. At the concentration of 1mg/mL, cinnamon leaf, clove bud and thyme red essential oils were shown to contain 420, 480 and 270 (μg/g of GAE) of TPC. Eugenol took account of 82.9% and 82.3% of TPC in cinnamon leaf and clove bud essential oils

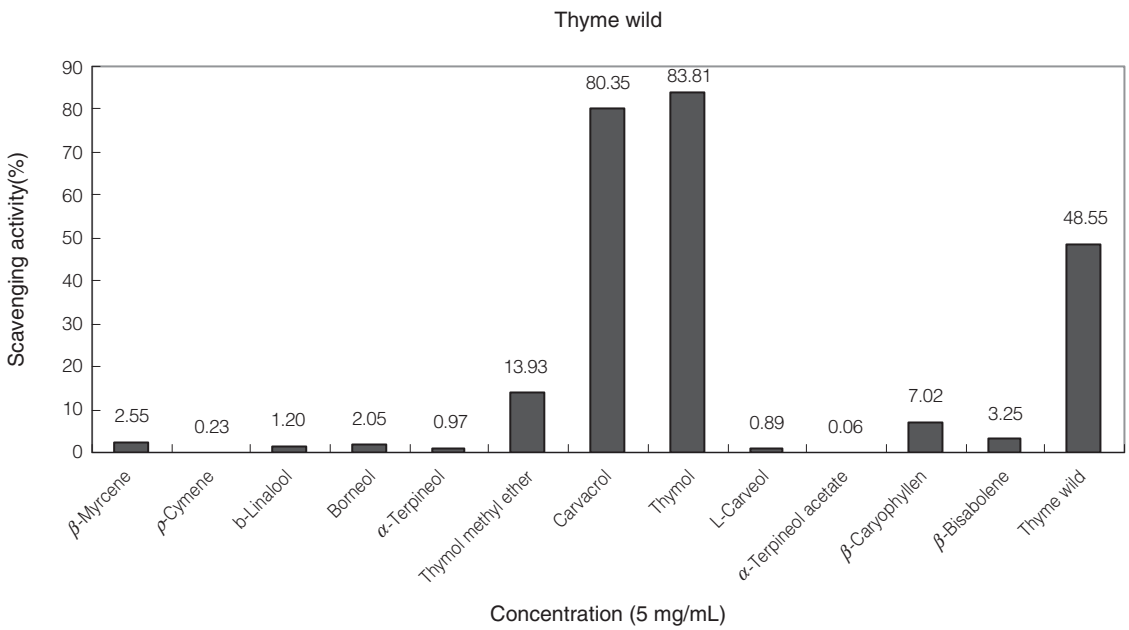

Figure 5. DPPH free-radical scavenging activity of twelve pure chemical components of thyme wild compared with its essential oils at a concentration of 5 mg/mL.

as determined by GC-MS, respectively. In this study, three phenols eugenol, thymol and carvacrol existed in cinnamon bark, origanum and thyme wild essential oils. These phenols have shown better DPPH free-radical scavenging activity and higher TPC.

From the experimental results, it is clear that cinnamon leaf and clove bud essential oils are better than cinnamon bark, origanum and thyme wild essential oils with respect to the DPPH free-radical scavenging activity and total phenolic contents.

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