Stability of Chemical Components and Antioxidant Activity of Volatile Oils from Some Medicinal Plants in Thailand

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ABSTRACT

The volatile oils from fresh leaves of ten medicinal plant species growing in northern Thailand were isolated by hydrodistillation method. These volatile oils were determined for their chemical components by gas chromatography coupled with mass selective detector (GC-MS) and antioxidant activity was investigated by ABTS assay, FRAP assay, lipid peroxidation assay and amount of total phenolic compounds. In addition, the stability of volatile oils in simulative condition was also studied. The major components of the extracted volatile oils were mainly terpenoid compounds, i.e., caryophyllene, D-germacrene, humulene and linalool. From the results, it can be concluded that the volatile oil of P. fruticosa showed the highest TEAC value of 279.79±0.35 µM trolox equivalents/mg sample while C. bejolghota oil showed the highest EC1 value (387.77±0.36 µM/mg sample). Moreover, the volatile oil of K. lenta possessed the highest lipid peroxidation value (83.43?±0.00%) and the highest amount of total phenolic compounds was found in the volatile oil of S. acmella (GAE value = 308.14±0.76 µg/ml sample). In the stability study, it was revealed that the antioxidant activity of the volatile oil from E. odoratum was more stable than S. acmella due to the stability of the chemical components towards oxidation reaction as well as humidity. It was also found that the chemical components of S. acmella oil were comparatively more stable than E. odoratum when they were preserved at -20, 30 and 45°C under the same conditions. The simulated storage condition in this study was effectively aimed to preserve the antioxidant activity and chemical components in the extracted volatile oils. The results of this investigation clearly suggested that the volatile oils of E. odoratum, S. acmella and P. fruticosa possessed the highest antioxidant activity and indicated that they proved to be a good source of antioxidants that might serve to protect health and fight against diseases in the future.

Key words: GC-MS; Volatile oil; Stability; ABTS; FRAP; Lipid peroxidation; Antioxidant

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), generated in the human body, can cause oxidative damages associated with many diseases such as atherosclerosis, coronary heart diseases, Parkinson's diseases, diabetes, neurodegenerative (Alzheimer's disease), aging and cancer (Madhavi et al., 1996: Finkel and Holbrook, 2000). The balance of free radical production and level of antioxidant defenses is essential for health. Too high level of free radicals and low amount of antioxidants can lead to a condition of oxidative stress and chronic injury. Antioxidants may act by decreasing oxygen concentration, intercepting singlet oxygen, preventing firstchain initiation by scavenging initial radicals, binding metal ion catalysts, decomposing primary products to non-radical compounds and chain breaking to prevent continued hydrogen abstraction from substrates (Saha et al., 2004). Therefore, it is very important to find out new sources of safe and inexpensive antioxidants of natural origin. Medicinal plants have long been used to treat human diseases. People are becoming increasingly interested in medicinal plants because of their good therapeutic performance and low toxicity. Some plant extracts are believed to have strong antioxidant effects. The leaves of medicinal plants that were selected in this study are used as food and traditional medicine in Thailand (The Institute of Thai Traditional Medicine, 1999). The extract from *C. odorata* was scientifically demonstrated to have a strong antioxidant effect (Phan et al., 2001). Its chemical components have been studied (Alexander et al., 1988; Kelm et al., 2000; Oyedeji and Afolayan, 2005) as well as pharmaceutical activities (Nghiem, 1992; Iwalewa et al., 2003; Jiang et al., 2005; Choochote et al., 2006). In this study, the investigation of chemical components in certain volatile oils was performed and the determination of ABTS assay, FRAP assay, lipid peroxidation assay and amount of total phenolic acids were used to measure the antioxidant property of the plant samples. Apart from that, the stability study of the chemical components which contained in the extracts was done in order to understand the decomposition reactions of the products usually occurring on storage to prevent the loss of the active ingredients.

MATERIALS AND METHODS

Materials

Fresh leaves of ten medicinal plant species of Thailand (Table 1) were selected from northern Thailand. Voucher specimens were deposited in the Pharmacy Herbarium, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand.

Table 1. Plants used in this study.

Scientific name	Local name	Family	Voucher specimen number
Emilia sonchifolia	Hang-Pla-Chon	Asteraceae	009813
Eupatorium odoratum	Sap-Suea	Asteraceae	009804
Spilanthes acmella	Puk-Khrat-Hua-Waen	Asteraceae	009806
Vernonia cinerea	Ya-La-Oong	Asteraceae	009809
Cinnamomum bejolghota	Op-Choei	Lauraceae	009818
Houttuynia cordata	Phlu-Khao	Saururaceae	009812
Knema lenta	Lueat-Khwai	Myristicaceae	009805
Piper sarmentosum	Cha-Phlu	Piperaceae	009815
Polygonum odoratum	Puk-Preaw	Polygonaceae	009816
Polyscias fruticosa	Leb-Krut	Araliaceae	009807

Chemicals

Acetic acid, ethanol, Folin-Ciocalteu reagent, 2,4,6-tripyridyl-s-triazine (TPTZ) and hydrochloric acid were purchased from Merck. 2,2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium-salt (ABTS), 2,6-di-tert-butyl-4-methylphenol (BHT), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and potassium persulfate were bought from Sigma. β-Carotene, gallic acid, linoleic acid, sodium sulfate anhydrous and chloroform were bought from Fisher. Dichloromethane was bought from J.T.Baker. Sodium carbonate was bought from Riedel-de Maen. Ferric chloride, ferrous sulfate, sodium acetate and Tween 20 were of analytical grade.

Isolation of volatile oils by hydrodistillation

Fresh leaves (2 kg) were placed in a boiling pot with a certain amount of water. Distillation was carried out for 5 h. The volatile oil was dried over sodium sulfate anhydrous and stored at low temperature (-20°C) for subsequent analyses.

Identification by GC-MS

The isolated oils were analyzed by GC-MS to determine the percentage total peak areas of chemical compounds. The GC-MS analysis was performed on Agilent 6890 gas chromatography coupled to electron impact (EI, 70 eV) with HP 5973 mass selective detector and fitted with a fused silica capillary column (HP-5MS) supplied by HP, USA (30.0 m × 250 µm, i.d. 0.25 µm film thickness). The liner velocity of the helium carrier gas was 1.0 ml/min. The injector and detector temperatures were 260°C and 280°C, respectively. The oven temperature was programmed from 3 min isothermal at 100°C (no peaks before 100°C after first injection), then at 3°C/min to 188°C and then at 20°C/min to 280°C (3 min isothermal). Each injection was done in triplicate. The programmed-temperature

Kováts retention indices (KI) were obtained by GC-MS analysis of an aliquot of the volatile oil spiked with a mixture of *n*-alkanes containing a homologue from *n*-C_{11} to *n*-C_{27}. Identification of the compounds was based on comparison of their mass spectra database (WILEY & NIST) and on spectroscopic data.

Antioxidant activity

1. ABTS assay (Roberta et al., 1999)

The antioxidant activity of the volatile oils was determined by the ABTS radical cation ($ABTS^{\bullet+}$) decolourisation assay involving preformed ABTS radical cation. ABTS was dissolved in water to yield a 7 mM concentration. ABTS radical cation was produced by reacting ABTS stock solution with potassium persulfate (2.45 mM) in water and allowing the mixture to stand in the dark at low temperature for 16-18 hours before use. The solution was diluted in ethanol (1:20 v/v) to give an absorbance at 750 nm of 0.700±0.200. In the test reaction, $ABTS^{\bullet+}$ working solution and sample (volatile oil) were mixed. The mixture was shaken vigorously and left to stand for 5 minutes, following with the measurement of the absorbance every 5 minutes for 30 minutes. The absorbance was then measured at 750 nm. Trolox was used for the preparation of the calibration curve. % Inhibition was calculated using the following equation: % inhibition = $\{(Abs_{control} - Abs_{sample}) / Abs_{control}\} \times 100$.

2. FRAP assay (Benzie and Strain, 1996)

FRAP reagent contained 5 ml of 10 mM TPTZ in 40 mM HCl plus 5 ml of 20 mM $FeCl_3 \bullet 6H_2O$ and 50 ml of 0.3 M acetate buffer (pH 3.6). FRAP reagent (180 µl) was mixed with 20 µl of sample and absorbance at 595 nm was measured at 30 min using a microplatereader. This test used ethanol for control. Ethanolic solutions of known Fe(II) concentration, ranging from 5-50 µM (final concentration) $FeSO_4 \bullet 7H_2O$, was used for the preparation of the calibration curve. The parameter Equivalent Concentration (EC_1) was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mM $FeSO_4 \bullet 7H_2O$. EC_1 was calculated as the concentration of antioxidant giving an increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mM concentration of Fe(II) solution, determined by using the corresponding regression equation.

3. Lipid peroxidation assay (β-carotene bleaching model) (Miller, 1971)

A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 2 ml of chloroform and mixed with linoleic acid (50 mg) and Tween 20 (50 mg). Two milliliters of this solution were transferred into a 100 ml roundbottom flask. After chloroform had been removed under vacuum, 50 ml of aerated distilled water were added to the flask with vigorous shaking. The same procedure was repeated without β-carotene as negative control. Aliquots (180 µl) of this emulsion were transferred into different plates containing different volatile oils (20 µl). As soon as the emulsion was added to each plate, the zero time absorbance was measured at 490 nm, using a microtiter plate reader. The plates were placed

at 45°C in an incubator and measurement of absorbance was recorded after 150 minutes; a blank, devoid of β-carotene, was prepared for background subtraction. The same procedure was repeated with the volatile oil (1 mg/ml), as positive control. Antioxidant activity was calculated using the following equation: % AA = $\{1 - [(Abs_{t0} - Abs_{t150})_{sample} / (Abs_{t0} - Abs_{t150})_{control}]\}$ x 100.

4. Amount of total phenolic compounds (Sato, 1996)

The total phenolic phytochemical concentration was measured using the Folin-Ciocalteu method. A Folin reagent was prepared by adding 2 % (0.6 g) sodium carbonate in water (30 ml) to 10 ml of Folin-Ciocalteu reagent. Afterwards, 180 µl of Folin-Ciocalteu reagent was mixed with 20 µl sample and the mixture was left to stand for 120 min at room temperature before the absorbance was measured spectrophotometrically at 750 nm, using a microplate reader. Gallic acid was used as the standard for the calibration curve. The total phenolic content of the sample is expressed in milligrams per serving of gallic acid equivalents (GAE). All samples were prepared in triplicate.

Stability of volatile oils (Ministry of Health, 2003)

The stability test was studied in *E. odoratum* and *S. acmella* Murr. only. Antioxidant activity was determined by ABTS assay and chemical components by GC-MS. According to a protocol previously described, the following stability conditions were studied (Table 2).

Table 2. Conditions for stability test.

Study	Storage condition	Minimum time period covered by data at submission
Longterm storage in a freezer	- 20°C ± 5°C	3 months
Longterm storage in an ambient condition	30°C ± 2°C	3 months
Accelerated storage in an incubator	40°C ± 2°C	3 months

RESULTS AND DISCUSSION

Volatile oil composition

The identification of the chemical components of volatile oils was performed by comparison of the mass spectra with literature data (NIST and WILEY) and by a comparison of their retention indices (RI) from Adam's book (Dool and Kratz, 1963; Davies, 1990; Adams, 2001). The GC-MS data of E. sonchifolia, *E. odoratum, S. acmella, V. cinerea, C. bejolghota, H. cordata, K. lenta, P. sarmentosum, P. odoratum* and *P. fruticosa* are summarized in Table 3. The major components of *E. sonchifolia* were caryophyllene (14.50%), germacrene-D (13.59%) and germacrene-B (13.06%). The major components of *E. odoratum*

were germacrene-D (19.34%), geyrene (17.30%) and caryophyllene (11.66%). The major components of *S. acmella* were β-farnesene (54.38%), caryophyllene (14.58%) and β-elemene (4.53%). The major components of *V. cinerea* were caryophyllene (58.91%), β-humulene (10.89%) and β-muurolene (6.44%). The major components of *C. bejolghota* were linalool (35.56%), T-cadinol (6.65%) and caryophyllene oxide (6.61%). The major components of *H. cordata* were 2-undecanone (48.08%), caryophyllene (10.69%) and β-selinene (9.27%). The major components of K. lenta were linalool (35.56%), T-cadinol (6.65%) and caryophyllene oxide (6.61%). The major components of *P. sarmentosum* were caryophyllene (42.62%), β-selinene (7.92%) and β-selinene (7.50%). The major components of *P. odoratum* were dodecanal (45.79%), n-decanal (11.70%) and caryophyllene (10.41%). The major components of *P. fruticosa* were 2-tridecanone (32.93%), unidentified (25.44%) and germacrene-B (8.18%).

Table 3. Chemical components of volatile oils.

RT	KI	Compounds	*E. sonchifolia*	*E. odoratum*	*S. acmella*	*V. cinerea*	*C. bejolghota*	*H. cordata*	*K. lenta*	*P. sarmentosum*	*P. odoratum*	*P. fruticosa*
3.48	1053	trans-β-ocimene		1.51						4.36		
4.08	1092	n-undecene	2.61									
4.26	1103	linalool					35.56					
5.22	1154	geyrene		17.30								
6.46	1207	n-decanal									11.70	
8.23	1273	1-decanol									2.30	
8.80	1291	bornyl acetate						2.35				
8.97	1297	2-undecanone						48.08				
11.58	1378	decaoic acid						6.97				
11.72	1382	α-copaene		3.54		2.88	1.27					
12.25	1396	β-elemene	7.85	2.23	4.53	1.85	2.24	2.60	3.34	6.43		1.61
12.72	1410	dodecanal									45.79	
13.25	1427	caryophyllene	14.50	11.66	14.58	58.91	2.83	10.69	35.30	42.62	10.41	
13.66	1439	γ-elemene	4.93						1.61			3.96
14.41	1460	α-humulene	5.68	3.07	1.53	10.89	1.57		7.97	5.35	5.45	
14.99	1475	1-dodecanol									3.81	
15.37	1485	germacrene-D	13.59	19.34		1.54	1.33		1.94			7.07
15.45	1487	β-farnesene			54.38	1.25						
15.54	1490	β-selinene				1.11		2.37	1.12	7.50		
15.83	1497	2-tridecanone			1.25							32.93
15.85	1498	α-selinene						9.27		7.92		
15.93	1500	bicyclogermacrene		1.98	2.15				6.70			

RT	KI	Compounds	*E. sonchifolia*	*E. odoratum*	*S. acmella*	*V. cinerea*	*C. bejolghota*	*H. cordata*	*K. lenta*	*P. sarmentosum*	*P. odoratum*	*P. fruticosa*
16.04	1503	α-muurolene				6.44						
16.31	1511	β-bisabolene	9.66									
16.84	1527	δ-cadinene		6.48	1.09	2.69	4.36		1.64	1.26		
17.75	1552	elemol		2.24						2.03		
18.06	1561	germacrene-B	13.06						4.05			8.18
18.26	1566	trans-nerolidol								4.38	1.01	
18.97	1585	caryophyllene oxide	2.88	1.11	1.23	2.27	6.61	4.39		2.15	1.91	
19.04	1587	gamma-gurjunene							5.02			
19.32	1954	viridiflorol					3.87		3.71			
19.48	1598	unidentified						4.74				1.19
19.75	1608	unidentified					3.58					
21.10	1662	β-cadinol		1.84	1.57		5.86		2.35			
21.54	1678	valencene	1.21	2.35	2.14							
21.55	1679	T-cadinol					6.65		5.48			
22.09	1699	nor-copaanone			2.44						1.26	
29.51	1889	α-sinensal	2.51									
33.14	2043	unidentified										25.44
34.02	2114	neophytadiene	4.55		1.21	1.01						

Antioxidant activity

1. ABTS assay

The volatile oil of *P. fruticosa* showed the highest antioxidant activity with a TEAC value of 279.79±0.35 μM trolox equivalents/mg sample, followed by *E. odoratum, S. acmella* Murr. and *H. cordata* with TEAC values of 278.85±0.30, 277.93±0.17 and 234.12±0.36 μM trolox equivalents/mg sample, respectively (Figure 1). TEAC value was calculated from % inhibition value of sample prepared with standard curve of Trolox. The values are means ± standard deviation (n = 3).

2. FRAP assay

The volatile oil of *C. bejolghota* showed the highest antioxidant activity with an EC1 value of 387.77±0.36 μM/mg sample, followed by *P. fruticosa, P. sarmentosum* and *V. cinerea* with EC1 values of 367.18±2.93, 365.83±0.34 and 352.99±0.33 μM/mg sample, respectively (Figure 2). EC1 value was calculated from absorbance/mg of sample prepared with standard curve of $FeSO_4 \cdot 7H_2O$. The values are means ± standard deviation (n = 3).

3. Lipid peroxidation assay

The volatile oil of *K.lenta* showed the highest antioxidant activity with %AA value of 83.43?0.00%, followed by *S. acmella, P. sarmentosum* and *V. cinerea* with % AA values of 28.10±0.05, 20.55±0.13 and 14.53±0.08%, respectively (Figure 3). The antioxidant activities of volatile oils were studied at the same concentration of 4 mg/ml in this assay. The values are means ± standard deviation (n = 3).

4. Amount of total phenolic compounds

The volatile oil of *S. acmella* showed the highest amount of total phenolic compounds with a GAE value of 308.14?0.76 µg/ml sample, followed by *E. odoratum, P. fruticosa* and *P. sarmentosum* with GAE values of 170.93±1.93, 107.11±5.25 and 63.51±1.28 µg/ml sample, respectively (Figure 4). GAE value was calculated from absorbance/mg of sample prepared with standard curve of gallic acid. The values are means ± standard deviation (n = 3).

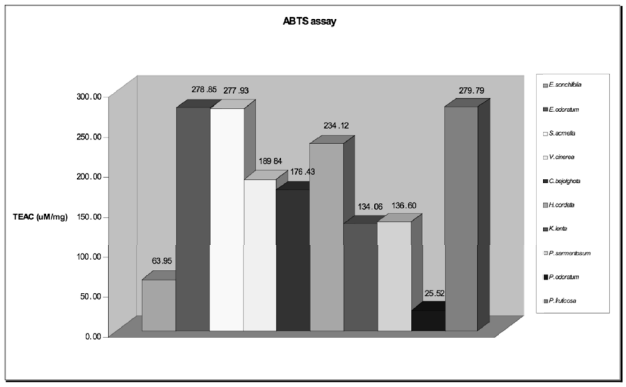

Figure 1. TEAC values of antioxidant activity of volatile oils.

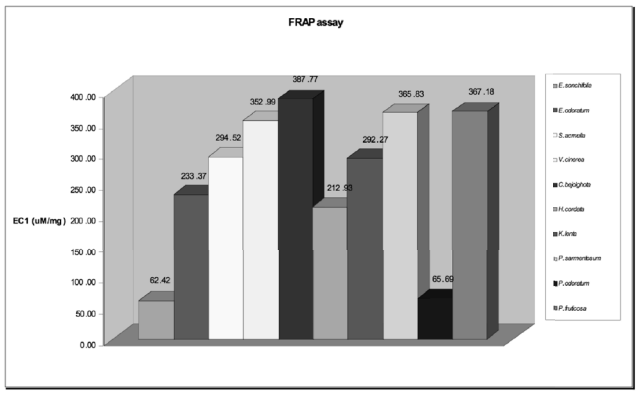

Figure 2. EC1 values of antioxidant activity of volatile oils.

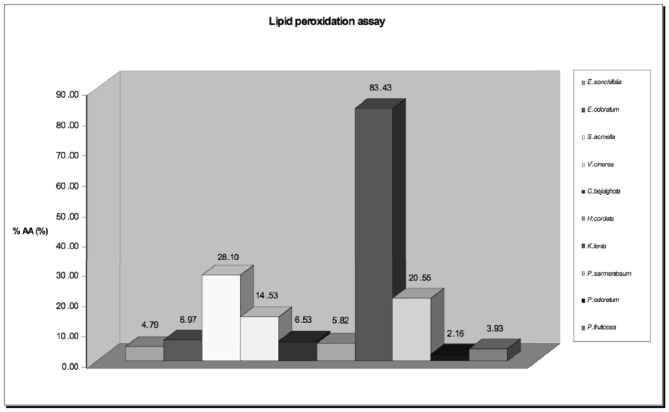

Figure 3. % AA values of antioxidant activity of volatile oils.

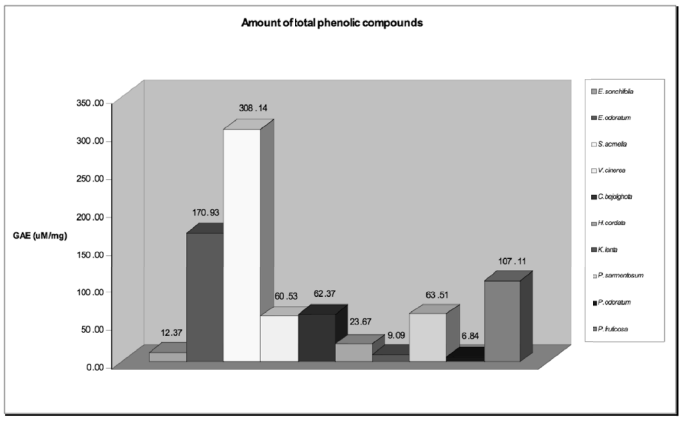

Figure 4. GAE values of antioxidant activity of volatile oils.

Stability of volatile oils

1. Stability of antioxidant activity by ABTS assay

It can be summarized that the longer the volatile oil was kept, the lower the chemical component stability was detected in any storage temperature by the reason that the TEAC value was decreased at any temperature at 5, 15, 30, 60 and 90 days (Figure 5). When the % remaining of TEAC value was assessed at -20, 30 and 45°C (Table 4), it also decreased in the same way. However, it was remarkable notices that the chemical components in *S. acmella* and *E. odoratum* oils were preserved from the oxidation reactions at -20°C (90 days) better than at 30 and 45°C, respectively.

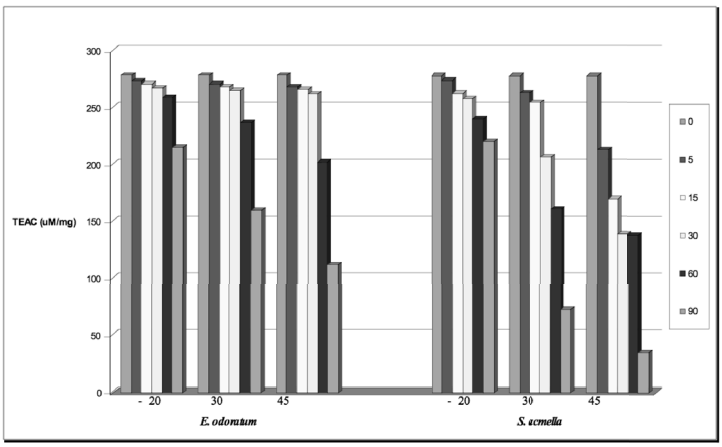

Figure 5. Stability test of *E. odoratum* and *S. acmella* oils by ABTS assay.

Table 4. % Remaining of TEAC values at various temperatures at 90 days.

Plant	% Remaining of TEAC (µM/mg)		
	-20°C	**30°C**	**45°C**
E. odoratum	78.54	59.10	41.79
S. acmella	80.33	27.62	16.45

2. Stability of chemical components by GC-MS

The chemical components of *E. odoratum* and *S. acmella* volatile oils are shown in Table 5 and Table 6. When the main chemical components of E. odoratum volatile oil at different temperatures (-20, 30 and 45°C) were compared, it was found that the % area of geyrene, caryophyllene and germacrene-D was decreased but % area of beta-cubebene, caryophyllene oxide and valencene was increased. When the main chemical components of *S. acmella* volatile oil were compared, it was found that the % area of germacrene-D was decreased but % area of β-elemene, caryophyllene, caryophyllene oxide, β-oplopenone and valencene was increased.

Therefore, we can conclude that the degradation of the chemical components in *E. odoratum* and *S. acmella* volatile oils are mainly due to oxidation and reduction mechanisms.

Table 5. Stability of chemical components of *E. odoratum.*

Temperature	Main chemical compounds	Time (days)				
		5	15	30	60	90
-20°C	geyrene	28.35	26.45	23.86	15.03	14.62
	caryophyllene	16.68	13.86	13.45	13.09	10.00
	β-cubebene	1.61	1.73	3.29	5.61	10.72
	germacrene-D	28.13	27.84	27.47	23.50	1.22
	caryophyllene oxide	0.00	0.58	0.72	2.39	21.53
	valencene	2.39	2.45	2.64	3.97	7.16
30°C	geyrene	23.88	20.09	19.99	18.35	16.74
	caryophyllene	15.59	15.41	15.23	14.62	9.05
	β-cubebene	5.55	6.52	9.52	14.42	16.30
	germacrene-D	22.40	20.96	15.20	6.01	0.96
	caryophyllene oxide	1.26	1.60	1.69	3.06	16.01
	valencene	3.09	3.23	3.97	6.66	7.49
45°C	geyrene	20.54	16.27	15.06	14.15	10.21
	caryophyllene	15.55	13.91	10.66	5.23	3.78
	β-cubebene	10.11	10.72	16.97	20.37	22.77
	germacrene-D	12.77	11.15	2.59	1.03	0.76
	caryophyllene oxide	4.23	4.30	12.20	18.71	22.27
	valencene	4.92	5.20	8.07	9.82	10.49

Table 6. Stability of chemical components of *S. acmella*.

Temperature	Main chemical compounds	Time (days)				
		5	15	30	60	90
-20°C	β-elemene	3.71	3.92	3.99	4.00	4.30
	caryophyllene	13.71	14.03	14.13	14.14	14.41
	germacrene-D	67.09	67.03	67.01	66.79	62.96
	caryophyllene oxide	0.44	0.46	0.47	0.54	0.74
	β-oplopenone	0.75	0.77	0.77	0.96	1.08
	valencene	2.04	2.05	2.06	2.06	2.42
30°C	β-elemene	5.39	5.50	7.31	10.05	11.60
	caryophyllene	17.93	18.15	22.35	24.11	28.30
	germacrene-D	57.46	56.90	44.12	18.98	9.87
	caryophyllene oxide	1.72	1.94	3.29	10.77	15.48
	β-oplopenone	1.16	1.28	2.01	3.36	4.78
	valencene	2.93	3.06	4.36	7.84	9.73
45°C	β-elemene	6.18	7.21	9.56	9.82	10.33

Temperature	Main chemical compounds	Time (days)				
		5	15	30	60	90
	caryophyllene	10.14	20.53	20.93	22.65	28.05
	germacrene-D	49.62	43.17	20.21	7.41	2.27
	caryophyllene oxide	2.45	2.58	8.73	20.27	35.66
	β-oplopenone	1.59	1.70	3.43	5.32	8.04
	valencene	4.50	5.13	7.33	11.42	13.49

CONCLUSION

The major components of the extracted volatile oils were mainly composed of terpenoid compounds, i.e., caryophyllene, germacrene-D, humulene and linalool. The results of this investigation significantly showed that the volatile oils of *E. odoratum, S. acmella* and *P. fruticosa* possessed the highest antioxidant activity. The degradation of the chemical components in *E. odoratum* and *S. acmella* volatile oils are mainly due to oxidation and reduction mechanisms. Their antioxidant activity certainly proved them to be a good source of antioxidants that might serve to protect health and fight against diseases in the future.

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