Article

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Chemicals in Essential Oils of *Lamiophlomis rotata* (Benth.) Kudo and Their Antioxidant Activities

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Abstract: Due to the low content, very few studies were focused on the essential oils (EOs) of *Lamiophlomis rotata* (Benth.) Kudo (*L. rotata*), which has been used to treat rheumatic arthritis and grasserie in China. However, such EOs may have important pharmacological activities such as anti-cancer. To explore the potential of *L. rotata*, we firstly conducted a thoroughly investigation on the chemicals in its EOs and their antioxidant activities, to the best of our knowledge. Light yellow EOs with fresh and elegant smell were obtained by hydro-distillation with percentage yields of 0.08-0.13% (volume mL/weight g). The crystals were separated from these EOs further. Therefore, the EOs, crystals, and EOs removed crystals were studied in-depth. A total of 56 components were qualified and quantified, in which 22 ones were first reported in such EOs. The main compounds were long-chain fatty acids and their esters. Seven compounds included *n*-hexadecanoic acid (47.1-60.8%), tetradecanoic acid (2.8-3.9%), linoleic acid (2.7-7.7%), oleic acid (0-3.7%), hexadecanoic acid, methyl ester (1.5-3.9%), 2-pentadecanone, 6,10,14-trimethyl- (2.0-3.0%), and phytol (2.0-5.8%) were prominent. The crystals were mainly composed of *n*-hexadecanoic acid with content 61.3-69.2%. Their antioxidant activities were evaluated through three common methods such as DPPH (1,1-Diphenyl-2-picrylhydrazy1 radical), ABTS ((2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), and FRAP (ferric reducing/antioxidant power). Three kinds of EOs and EOs removed crystals present some antioxidant activities, but insignificant compared with that of ascorbic acid. The crystals showed nearly non antioxidant activities and even oxidant activities, and the same situation for palmitic acid. As a result, the EOs removed crystals usually showed some stronger antioxidant activities than that of corresponding EOs. This study will give some hints for the full usage of such EOs with lower extracted rate and mainly composed of compounds with high boiling point such as *n*-hexadecanoic acid, 6,10,14-trimethyl-2-pentadecanone, *etc.*.

Keywords: *Lamiophlomis rotata* (Benth.) Kudo; essential oils; *n*-hexadecanoic acid (palmitic acid); 6,10,14-trimethyl-2-pentadecanone (hexahydrofarnesyl acetone); chemical markers; antioxidant activities

1. Introduction

Lamiophlomis rotata (Benthl) Kudo (*L. rotata*), a medicinal herb, is the sole member of the *Lamiophlomis* Kudo of Lamiaceae, which grows at the high altitudes in China [1]. The portion that grows above the ground is collected as the "Duyiwei" (*Lamiophlomis* herba) with slight fragrance in Chinese materia medica (CMM) [1,2], also known as "Daba" and "Dabuba" in the traditional Tibetan system (TTS) [3]. It has been used to treat rheumatic arthritis and grasserie for more than 2000 years in TTS. According to the theory of Chinese medicine, the tropism of "Duyiwei" is sweet and bitter [2,4].

Due to the low content of volatile components, the researches are mainly focused on the involatile compounds of *L. rotata*, and some efficacious ingredients such as iridoids, flavonoids, phenylethanoids, have been found [5–9]. However, the petroleum ether extracted part of *L. rotata* was reported to have the anti-tumor activities [10]. Up to now, there is only one paper reported the chemicals in such EOs, and another paper reported the lipophilic composition in the CH_2Cl_2 extracted part, and no-evaluation of their antioxidant activities, to the best of our knowledge [11,12]. In previous report, EOs with light yellow color, yields as 0.1% and 0.23% (volume mL/weight g) had been extracted by steam distillation from the above and below the ground components of *L. rotata*, respectively. A total of 17 components were identified and quantified mainly composed of long-chain fatty acids, in which, 16 and 13 ones (92.9% and 95.5%) were identified in the aboveground and underground portions, respectively. *n*-Hexadecanoic acid (50.1% and 34.5%), oleic acid (13.4% and 11.1%), linoleic acid (7.6% and 23.9%), and linoleic acid ethyl ester, which identification is

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debatable, (1.7% and 14.4%) are the four prominent compounds in the aboveground and underground parts of the herb, respectively [11]. A total of 67 components were qualified and quantified in the CH_2Cl_2 extracted part of flower, leaf, and root of *L. rotata* collected from Tibet, Yunnan, and Qinghai of China. Among them, only 4 fatty acids accounting for 13.66-46.27%, 4 fatty acid esters accounting for 8.77-20.8%, and 35 alkanes ranged from 6.1% to 37.84% were detected. *n*-Hexadecanoic acid (7.08-18.54%), linoleic acid (2.75-19.11%), linolenic acid, methyl ester (8.77-20.8%), and *β*-sitosterol (13.05-18.00%) were the four prominent compounds [12]. The essential oils (EOs) from herbs are used as flavorings or fragrances in the food, perfumery industries, and for medicinal purposes in several regions [13].

Considering the importance of such EOs, and basing on the previously investigation [9], we have done an exhaustive exploration on the chemicals present in the EOs of *L. rotata*, and analyzed their antioxidant activities through DPPH, ABTS, and FRAP assay.

2. Results

2.1. Chemicals in the EOs of L. ratata

A light yellow EO with fresh and elegant smell like the lavender flavour was obtained from L8, L9, and L10, respectively. The yields (0.08-0.13%) were close to the yields (0.1%) reported previously [11]. In total, 56 compounds were qualified and quantified (Table 1).

The mass spectra of compounds 41, 42, and 43 were highly similar with those of linoleic acid, oleic acid, and octadecanoic acid, respectively, whereas their LRI^c values of 2884, 2770, and 2700, respectively, were significantly different from the corresponding LRI^d values of 3164, 3173, and 3136, respectively. Considering the MS oven temperature program of FFAP, the max calculated LRI^c was 2984, and the chemicals with $LRIs^d$ higher than 2984 such as linoleic acid, oleic acid, and octadecanoic acid, would not be eluted in the employed analytical conditions and would be eluted in the next chromatogram, which would significantly change their LRI^c values. In such a scenario, the compounds 41, 42, 43 were still identified as linoleic acid, oleic acid, and octadecanoic acid, respectively, which were also reported previously [11,12].

In addition, the compounds corresponding to the five peaks detected in the total ion chromatograms (TICs), which characteristic ion peaks could be seen in Table 2, could not be deduced just by mass spectra and LRI values, respectively. Unknown-1 was detected by mass spectrometry (MS) using DB-5 with a LRI^a of 1098, the mass spectrum of which could be seen in Figure 1. At the same time, the standard chemical such as linalool was detected by MS upon using DB-5 with a LRI^a of 1099, and linalool was detected by MS using FFAP, and undetected by MS using DB-5 in the samples. On the basis of these results, the corresponding peak identified by FID was confirmed as linalool

Table 1. The compounds qualified and quantified in EOs from *L. rotata*.

No.	Compounds	CAS	LRI[b, d]	LRI[a]	LRI[c]	E8			C8			RC8			E9			C9			RC9			E10			C10			RC10		
						FID	DB-5	FFAP	FID	DB-5	FFAP	FID	DB-5	FFAP	FID	DB-5	FFAP	FID	DB-5	FFAP	FID	DB-5	FFAP	FID	DB-5	FFAP	FID	DB-5	FFAP	FID	DB-5	FFAP
1	Hexanal	66-25-1	800, 1083	-	-	0.1	nd	nd	0.3	2.0	0.1	0.6	3.2	0.2	0.3	nd	nd	0.3	nd	0.1	0.6	7.5	0.2	0.3	nd	tr	0.3	nd	0.1	0.5	4.7	0.1
2	*β*-Pinene	127-91-3	970, 1112	-	1114	nd	nd	tr	0.5	nd	nd	1.1	nd	nd	4.3	nd	nd	1.3	nd	nd	2.8	nd	nd	4.9	nd	nd	1.5	nd	nd	2.6	nd	nd
3	1-Octen-3-ol	3391-86-4	980, 1450	980	1454	nd	nd	nd	0.2	nd	nd	0.3	nd	nd	nd	nd	1.8	0.3	nd	0.7	0.7	nd	1.1	nd	nd	1.6	0.3	3.7	0.6	0.7	nd	1.1
4	Hexanoic acid	142-62-1	990, 1846	-	1838	nd	nd	nd	nd	nd	0.1	tr	nd	0.3	nd	nd	nd	0.1	nd	0.2	0.2	nd	0.4	0.2	nd	0.1	0.1	nd	0.2	0.1	nd	0.4
5	*p*-Cymene	99-87-6	1011, 1272	-	1272	1.4	nd	0.2	0.3	nd	0.3	0.3	nd	0.1	1.2	nd	nd	0.1	nd	tr	0.3	nd	tr	0.4	nd	0.1	0.2	nd	nd	0.3	nd	tr
6	Limonene	138-86-3	1020, 1200	1026	1203	12.8	nd	3.4	4.6	36.4	3.0	2.3	5.8	0.8	12.0	nd	1.5	1.0	nd	0.2	3.0	8.1	0.5	3.2	7.3	0.9	1.3	nd	0.1	2.0	nd	0.3
7	*γ*-Terpinene	99-85-4	1053, 1246	-	1247	1.6	nd	0.1	0.5	nd	0.2	0.5	nd	nd	1.3	nd	nd	0.8	nd	nd	0.8	nd	nd	3.2	nd	nd	1.4	nd	nd	0.7	nd	nd
8	*cis*-Linalool oxide	5989-33-3	1074, 1444	-	1441	nd	nd	nd	0.9	nd	nd	1.2	nd	nd	0.5	nd	nd	1.1	nd	0.6	3.0	nd	1.4	0.5	nd	0.2	1.5	nd	0.6	3.0	nd	1.5
9	*trans*-Linalool oxide	34995-77-2	1102, 1452	-	1466	nd	nd	nd	0.8	nd	nd	1.0	nd	0.7	0.4	nd	nd	1.0	nd	0.5	2.5	nd	1.2	0.4	nd	0.2	1.3	nd	0.6	2.7	nd	1.1
10	Unknown-1			1098	-	un	nd	un	un	6.6	un	un	20.9	un	un	nd	un	un	11.7	un	un	18.0	un	un	22.3	un	un	22.4	un	un	16.3	un
11	Linalool	78-70-6	1082, 1547	-	1552	0.2	nd	2.4	2.2	nd	0.7	6.8	nd	4.0	9.1	nd	4.2	2.9	nd	1.1	5.5	nd	2.1	11.0	nd	4.0	3.4	nd	1.2	5.7	nd	2.0
12	Caprylic acid	124-07-2	1180, 2060	-	2053	nd	nd	nd	nd	nd	0.1	nd	nd	0.3	nd	nd	nd	nd	nd	0.1	nd	nd	0.2	nd	nd	0.1	nd	nd	0.1	nd	nd	0.2
13	*α*-Terpineol	98-55-5	1172, 1697	1185	1690	71.2	100.0	2.8	5.4	17.5	1.1	12.8	56.5	4.7	17.8	100.0	4.1	5.9	30.6	1.3	11.4	47.6	2.0	14.8	70.4	3.4	4.8	27.0	1.2	8.5	41.4	2.0
14	Tridecane	629-50-5	1300, 1300	-	1300	nd	nd	nd	nd	nd	nd	nd	nd	tr	nd	nd	nd	0.2	nd	nd	0.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
15	Tetradecane	629-59-4	1400, 1400	-	1400	nd	nd	nd	tr	nd	nd	0.2	nd	0.1	nd	nd	nd	nd	nd	nd	0.1	nd	nd	0.1	nd	tr	nd	nd	nd	0.1	nd	tr
16	*β*-Caryophyllene	87-44-5	1419, 1595	-	1583	1.5	nd	0.1	0.2	nd	0.1	0.3	nd	0.1	0.6	nd	nd	0.2	nd	tr	0.4	nd	tr	0.4	nd	0.1	0.2	nd	tr	0.4	nd	tr
17	Pentadecane	629-62-9	1500, 1500	-	1500	nd	nd	tr	0.1	nd	tr	0.2	nd	0.1	nd	nd	nd	tr	nd	tr	0.2	nd	0.1	0.2	nd	0.1	tr	nd	tr	0.1	nd	0.1
18	Dodecanoic acid	143-07-7	1556, 2498	-	2474	nd	nd	0.7	1.1	nd	0.9	1.2	nd	1.6	1.0	nd	1.1	1.3	nd	1.4	2.1	nd	2.5	0.7	nd	1.2	1.0	nd	1.3	1.7	nd	2.0
19	Cedrol	77-53-2	1598, 2116	-	2086	un	nd	tr	un	nd	0.1	un	nd	0.2	un	nd	nd	un	nd	0.1	un	nd	0.2	un	nd	0.1	un	nd	0.1	un	nd	0.1
20	Hexadecane	544-76-3	1600, 1600	-	1600	nd	nd	0.1	0.2	nd	0.1	0.3	nd	0.1	nd	nd	nd	0.1	nd	tr	0.3	nd	0.1	0.3	nd	0.1	0.2	nd	0.1	0.5	nd	0.2
21	Heptadecane	629-78-7	1700, 1700	-	1700	nd	nd	0.1	0.1	nd	0.1	0.2	nd	0.2	nd	nd	nd	tr	nd	0.1	0.2	nd	0.2	0.1	nd	0.1	0.1	nd	0.1	0.7	nd	0.3
22	Tetradecanoic acid	544-63-8	1748, 2694	-	2685	nd	nd	3.9	5.1	nd	5.6	3.6	nd	6.4	1.9	nd	2.8	3.5	nd	4.4	3.9	nd	5.4	2.3	nd	3.2	4.0	nd	5.3	4.7	nd	6.1
23	Octadecane	593-45-3	1800, 1800	-	1800	nd	nd	tr	0.1	nd	tr	nd	nd	nd	nd	nd	nd	tr	nd	nd	0.1	nd	0.1	0.1	nd	nd	0.1	nd	nd	0.1	nd	0.2

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24	2-Pentadecanone, 6,10,14-trimethyl- (hexahydrofarnesyl acetone)	502-69-2	1842, 2131	1843	2119	nd	nd	2.0	3.5	nd	2.7	5.0	7.1	6.2	2.7	nd	2.0	2.5	nd	2.3	6.4	nd	5.6	3.7	nd	3.0	3.8	1.9	3.5	7.9	17.0	7.0
25	* Pentadecanoic acid	1002-84-2	1823, 2822	-	2790	nd	nd	0.5	0.5	nd	0.7	0.2	nd	0.8	0.2	nd	nd	0.4	nd	0.8	0.3	nd	0.6	0.1	nd	0.4	0.4	nd	0.7	0.3	nd	0.7
26	Nonadecane	629-92-5	1900, 1900	-	1900	nd	nd	0.1	nd	nd	tr	nd	nd	0.1	nd	nd	nd	0.1	nd	nd	0.3	nd	nd	nd	nd	nd	nd	nd	tr	nd	nd	0.1
27	Farnesyl acetone	1117-52-8	1919, 2384	-	2362	nd	nd	0.7	nd	nd	0.1	0.4	nd	0.9	0.8	nd	nd	tr	nd	0.1	nd	nd	0.1	0.9	nd	0.8	tr	nd	0.1	0.1	nd	0.2
28	Hexadecanoic acid, methyl ester (Methyl palmitate)	112-39-0	1909, 2208	1924	2214	0.2	nd	1.5	1.8	nd	1.6	2.8	6.5	4.1	2.9	nd	2.8	2.5	nd	3.0	6.7	11.1	7.1	3.8	nd	3.9	3.6	3.3	4.1	7.8	18.4	8.3
29	9*E*-Hexadecenoic acid	2091-29-4	1942, 2954	-	2935	un	nd	0.9	un	nd	0.8	un	nd	2.7	un	nd	nd	un	nd	nd	un	nd	0.4	un	nd	0.3	un	nd	0.3	un	nd	0.4
30	Palmitoleic acid	373-49-9	1951, 2926	-	2926	nd	nd	1.8	0.6	nd	1.5	1.4	nd	4.3	0.3	nd	nd	0.4	nd	0.7	0.7	nd	1.3	0.6	nd	0.7	0.5	nd	0.8	0.9	nd	1.1
31	Dibutyl phthalate	84-74-2	1965, 2680	-	2675	nd	nd	nd	0.1	nd	0.3	0.2	nd	0.8	nd	nd	nd	0.1	nd	nd	0.2	nd	0.4	nd	nd	0.2	0.2	nd	0.2	0.3	nd	0.5
32	*n*-Hexadecanoic acid (Palmitic acid)	57-10-3	1972, 2931	1960	2894	0.9	nd	51.9	60.3	37.5	64.1	10.0	nd	17.8	23.8	nd	60.8	65.0	57.7	69.2	31.9	7.7	35.7	24.2	nd	47.1	62.1	41.8	61.3	31.7	2.1	44.8
33	Eicosane	112-95-8	2000, 2000	-	2000	0.1	nd	tr	0.1	nd	tr	0.3	nd	0.1	nd	nd	nd	nd	nd	nd	0.1	nd	0.1	nd	nd	nd	0.1	nd	tr	0.2	nd	0.1
34	Linoleic acid, methyl ester (Methyl linoleate)	112-63-0	2092, 2482	-	2485	un	nd	1.9	un	nd	0.3	un	nd	2.8	un	nd	4.4	un	nd	0.6	un	nd	0.9	un	nd	4.3	un	nd	0.4	un	nd	0.6
36	Oleic acid, methyl ester, *cis*- (Methyl oleate)	112-62-9	2091, 2434	-	2439	0.2	nd	0.9	1.0	nd	0.7	3.2	nd	2.5	nd	nd	2.0	1.7	nd	1.9	4.1	nd	4.8	nd	nd	2.2	1.9	nd	2.1	4.2	nd	4.4
35	Linolenic acid, methyl ester (Methyl linolenate)	301-00-8	2098, 2571	-	2552	un	nd	1.8	un	nd	nd	un	nd	1.2	un	nd	2.8	un	nd	nd	un	nd	nd	un	nd	3.4	un	nd	nd	un	nd	nd
37	Heneicosane	629-94-7	2100, 2100	-	2100	0.6	nd	nd	1.1	nd	tr	3.5	nd	0.1	5.7	nd	nd	1.7	nd	nd	4.3	nd	0.1	6.5	nd	0.1	2.0	nd	tr	4.3	nd	0.1

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38	Phytol	150-86-7	2104, 2622	-	2607	un	nd	5.8	un	nd	1.5	un	nd	7.0	un	nd	2.0	un	nd	0.7	un	nd	1.3	un	nd	4.4	un	nd	1.3	un	nd	2.0
39	Unknown-2	-	-, -	-	2476	un	nd	0.2	un	nd	0.3	un	nd	2.8	un	nd	nd	un	nd	0.2	un	nd	0.3	un	nd	0.3	un	nd	0.2	un	nd	0.5
40	Methyl stearate	112-61-8	2128, 2418	-	2420	nd	nd	0.2	0.2	nd	0.3	0.2	nd	0.7	0.8	nd	nd	0.6	nd	0.3	1.4	nd	0.7	0.4	nd	0.4	0.2	nd	0.4	0.6	nd	0.9
41	Linoleic acid	60-33-3	2133, 3164	-	2884	un	nd	7.7	un	nd	1.1	un	nd	9.7	un	nd	2.7	un	nd	0.1	un	nd	0.7	un	nd	5.1	un	nd	0.6	un	nd	nd
42	Oleic acid	112-80-1	2141, 3173	-	2770	un	nd	2.9	un	nd	2.9	un	nd	7.0	un	nd	nd	un	nd	3.3	un	nd	10.0	un	nd	3.7	un	nd	4.0	un	nd	nd
43	Octadecanoic acid	57-11-4	2172, 3136	-	2700	un	nd	1.7	un	nd	4.3	un	nd	nd	un	nd	nd	un	nd	1.6	un	nd	1.5	un	nd	0.3	un	nd	3.8	un	nd	nd
44	Docosane	629-97-0	2200, 2200	-	2200	1.0	nd	0.1	0.2	nd	0.1	2.8	nd	0.1	nd	nd	nd	0.1	nd	nd	0.2	nd	0.2	0.2	nd	nd	0.1	nd	0.1	0.4	nd	0.1
45	Phytol, acetate	-	-, -	-	2512	un	nd	0.1	un	nd	0.2	un	nd	0.6	un	nd	nd	un	nd	0.1	un	nd	0.4	un	nd	0.2	un	nd	0.1	un	nd	0.2
46	Tricosane	638-67-5	2300, 2300	-	2300	1.5	nd	0.2	0.3	nd	0.2	5.1	nd	0.5	0.3	nd	nd	0.3	nd	0.2	0.6	nd	0.6	0.3	nd	0.2	0.3	nd	0.2	0.8	nd	0.4
47	Tetracosane	646-31-1	2400, 2400	-	2400	1.9	nd	nd	0.2	nd	0.1	6.8	nd	0.3	0.1	nd	nd	0.6	nd	0.1	0.3	nd	0.3	0.2	nd	nd	0.1	nd	0.1	0.5	nd	0.2
48	Pentacosane	629-99-2	2500, 2500	-	2500	1.5	nd	0.1	0.3	nd	0.3	6.4	nd	0.6	0.3	nd	nd	0.3	nd	0.2	0.5	nd	0.6	tr	nd	0.1	0.2	nd	0.2	0.6	nd	nd
49	Methyl 5,6-octadecadienoate		-, -	-	2515	un	nd	0.2	un	nd	0.1	un	nd	0.4	un	nd	0.5	un	nd	0.7	un	nd	1.4	un	nd	0.4	un	nd	0.4	un	nd	0.9
50	Hexacosane	630-01-3	2600, 2600	-	2600	1.2	nd	0.2	0.1	nd	0.1	5.2	nd	0.3	0.1	nd	nd	0.1	nd	tr	0.1	nd	0.1	nd	nd	0.3	tr	nd	0.1	0.3	nd	0.1
51	Heptacosane	593-49-7	2700, 2700	-	2700	nd	nd	0.2	nd	nd	0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.5	nd	nd	0.2	nd	nd	0.2	nd	nd	0.4
52	Octacosane	630-02-4	2800, 2800	-	2800	nd	nd	0.2	nd	nd	0.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.4	nd	nd	nd	nd	nd	0.1	nd	nd	0.3
53	Unknown-3			-	2817	un	nd	0.9	un	nd	1.0	un	nd	3.0	un	nd	nd	un	nd	0.8	un	nd	1.7	un	nd	0.8	un	nd	0.8	un	nd	1.4
54	Nonacosane	630-03-5	2900, 2900	-	2900	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.1	nd	nd	nd	nd	nd	nd	nd	nd	nd
55	Unknown-4			-	2952	un	nd	nd	un	nd	nd	un	nd	nd	un	nd	4.5	un	nd	1.0	un	nd	0.5	un	nd	nd	un	nd	0.8	un	nd	4.5
56	Unknown-5			-	2975	un	nd	1.4	un	nd	1.4	un	nd	3.1	un	nd	nd	un	nd	1.2	un	nd	1.9	un	nd	1.5	un	nd	1.4	un	nd	1.9
	Total (56)					98.3	100	100	99.6	100	100	98.8	100	100	100	100	100	99.8	100	100	99.9	100	100	97.4	100	100	100	100	100	99.4	100	100
	HM (4)					15.8	0	3.7	5.9	36.4	3.4	4.3	5.8	0.9	18.7	0.0	1.5	3.3	0	0.2	7.0	8.1	0.6	11.7	7.3	1.0	4.4	0	0.1	5.6	0	0.3
	AM (4)					71.4	100	5.2	9.5	17.5	1.8	21.7	56.5	9.4	27.8	100	8.3	10.9	30.6	3.5	22.4	47.6	6.7	26.8	70.4	7.8	11.1	27.0	3.6	20	41.4	6.7
	HS (1)					1.5	0	0.1	0.2	0	0.1	0.3	0	0.1	0.6	0	0	0.2	0	0	0.4	0	0	0.4	0	0.1	0.2	0	0	0.4	0	0
	AS (1)					0	0	0	0	0	0.1	0	0	0.2	0	0	0	0	0	0.1	0	0	0.2	0	0	0.1	0	0	0.1	0	0	0.1
	AD (1)					0.3	0	5.6	1.7	0	1.2	4.2	0	4.2	2.1	0	2.0	0.7	0	0.5	1.3	0	1.0	4.3	0	4.1	1.1	0	1.1	2.1	0	1.5

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Aldehyde & ketone (3)	0.1	0	2.7	3.7	2.0	2.8	6.0	10.3	7.3	3.8	0	2.0	2.8	0	2.5	7.0	7.5	5.9	4.9	0	3.8	4.2	1.9	3.6	8.4	21.7	7.4
Fatty acids (12)	1.0	0	62.7	69.7	37.5	77.1	22.2	0	41.9	30	0	64.7	72.5	57.7	80.1	43.1	7.7	56.7	31.1	0	57.1	69.7	41.8	74.2	43.1	2.1	56.3
Ester (8)	0.4	0	6.6	3.1	0	3.5	6.4	6.5	13.0	3.7	0	12.5	4.9	0	6.6	12.4	11.1	15.7	4.2	0	15.0	5.9	3.3	7.8	12.9	18.4	15.7
Phthalate (1)	0	0	0	0.1	0	0.3	0.2	0	0.8	0	0	0	0.1	0	0	0.2	0	0.4	0	0	0.2	0.2	0	0.2	0.3	0	0.5
Esters of fatty acid (7)	0.4	0	6.6	3.0	0	3.2	6.2	6.5	12.2	3.7	0	12.5	4.8	0	6.6	12.2	11.1	15.3	4.2	0	14.8	5.7	3.3	7.6	12.6	18.4	15.2
TOC (29)	73.8	100	92.5	91.8	57.0	91.9	66.6	73.3	87.6	79.9	100	94.0	94.3	88.3	95.7	89.4	73.8	89.5	83.9	70.4	95.0	94.1	77.6	95.4	89.1	83.7	88.8
n-Al (17)	7.2	0	1.3	1.8	0	1.8	27.6	0	2.6	0.8	0	0	2.0	0	0.7	3.1	0	5.5	1.4	0	1.2	1.3	0	1.2	4.4	0	2.6
Unknowns (5)	0	0	2.5	0	6.6	2.8	0	20.9	8.9	0	0	4.5	0	11.7	3.3	0	18.0	4.4	0	22.3	2.6	0	22.4	3.3	0	16.3	8.2

[1] Note: nd means not detected; un means uncertain; tr means the content is less than 0.05%. Unknown means the compounds can't be elucidated by MS. The same for the following Tables. LRI[b, d] (Linear retention indices) got from NIST (National Institute of Standards and Technology) 17 database means the linear retention index detected by semi-standard apolar column or polar column from NIST library, LRI[a] means it was gotten by DB-5 in this experiment, LRI[c] means it was gotten by FFAP in this experiment, respectively. The HM, AM, HS, AS, AD, TOC, *n*-Al equal to hydrocarbon monoterpene, alcohol monoterpene, hydrocarbon sesquiterpene, alcohol sesquiterpene, alcohol diterpene, total oxygenated compounds, *n*-alkane, respectively. The compounds denoted with red color means they were identified and quantified both in this study and previous literature.

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Table 2. The characteristic peaks of unknown compounds.

Characteristic Ion Peaks (M/W, %)	Compounds
93 (100), 41 (67), 39 (60), 91 (59), 43 (53), 77 (50), 94 (43), 69 (37), 121 (36), 136 (9).	Unknown-1
123 (100), 57 (97), 81 (90), 43 (81), 69 (81), 95 (80), 68 (77), 55 (76), 82 (68), 278 (6).	Unknown-2
55 (100), 41 (77), 69 (76), 43 (74), 83 (73), 97 (59), 57 (57), 96 (56), 84 (56), 222 (11)	Unknown-3
80 (100), 140 (59), 81 (45), 94 (33), 79 (33), 122 (30), 67 (28), 41 (27), 43 (25), 149 (3).	Unknown-4
43 (100), 55 (81), 57 (80), 83 (67), 41 (65), 69 (62), 97 (58), 96 (45), 194 (8), 236 (8).	Unknown-5

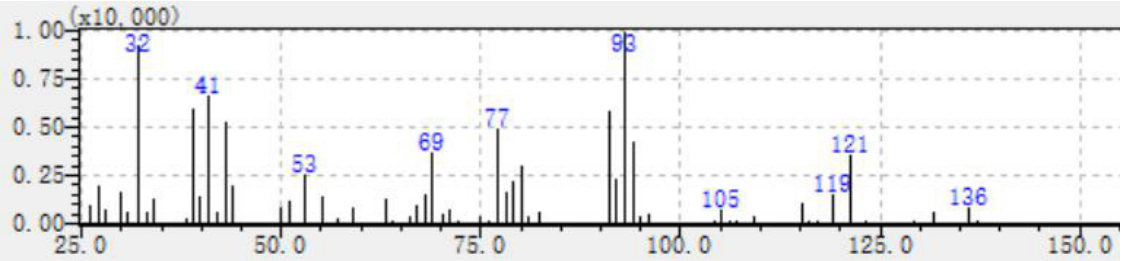

Figure 1. The mass spectrum of unknown-1 from RC8.

There was unsuitable match for unknown-2 in view of its mass spectrum (Figure 2) and LRI. It should be an analogue of phytol, acetate according to its characteristic ion peaks, .

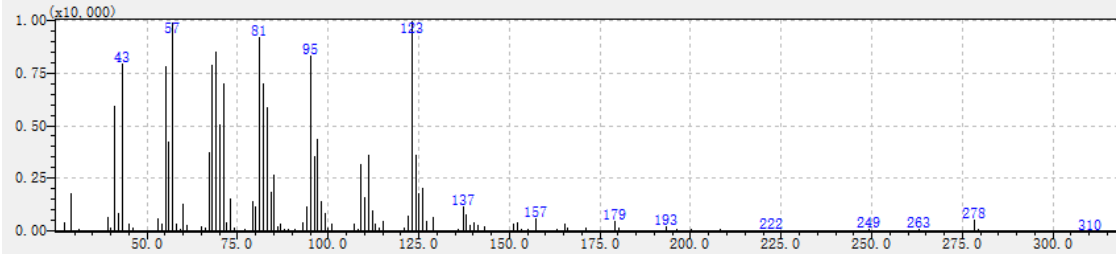

Figure 2. The mass spectrum of unknown-2 from RC8.

There was unsuitable match for unknown-3 based on its mass spectrum (Figure 3) and LRI. Based on the characteristic ion peaks, it should be an unsaturated long-chain fatty acid or the corresponding ester.

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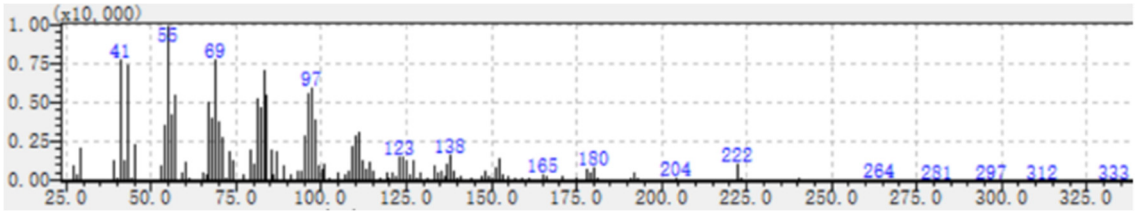

Figure 3. The mass spectrum of unknown-3 from RC9.

The most suitable match for unknown-4 was 1-cyclohexenylacetic acid, which has a M_w (molecular wight) of 140 (Figure 4), whereas its M_w should be beyond 140 because of the m/z 149 displayed as one of the characteristic ion peaks (Figure 5), which demonstrates that unknown-4 should be a derivative of 1-cyclohexenylacetic acid. Previously, cyclohexenylacetic acid was reported as a compound in the CH_2Cl_2 extract of *L. rotata*, which should be corresponding to unknown-4 in this study [12].

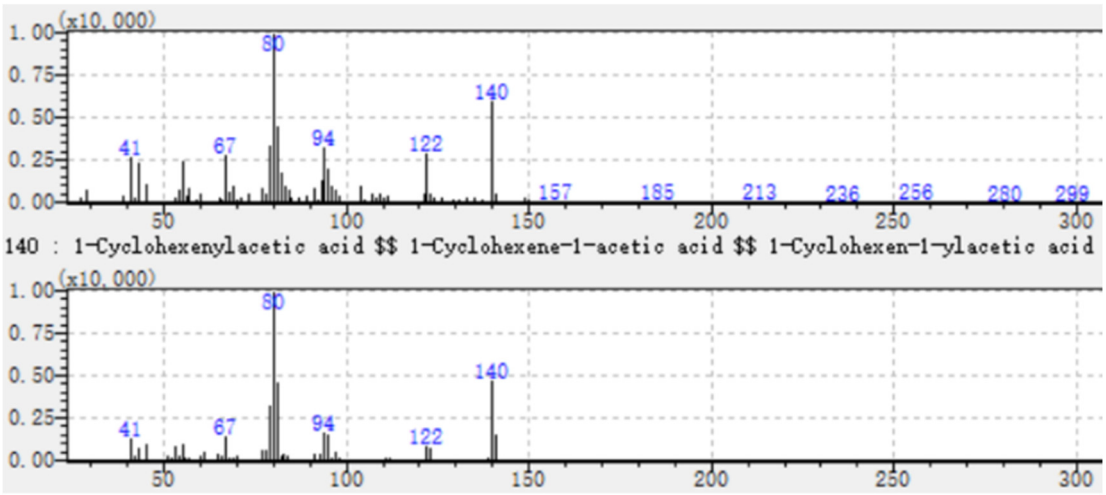

Figure 4. The mass spectra of unknown-4 from RC10 and the corresponding match 1-cyclohexenylacetic acid from NIST 14 library.

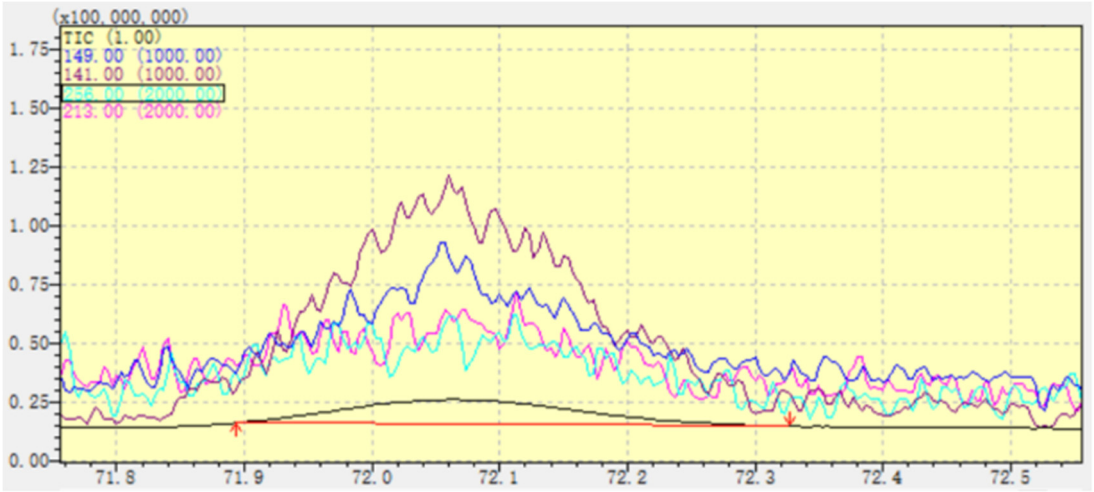

Figure 5. The characteristic ion peaks of unknown-4.

The most suitable match of unknown-5 was palmitoleic acid (Figure 6), whereas its LRI[c] 2975 was different from the LRI[d] 2926 of palmitoleic acid to some extent. Meanwhile, palmitoleic acid was identified as compound 30 with LRI[c] 2926, and 9*E*-hexadecenoic acid was identified as compound 29

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with LRI^{c} 2935. Therefore, this compound should be an analogue of palmitoleic acid and not 9*E*-hexadecenoic acid.

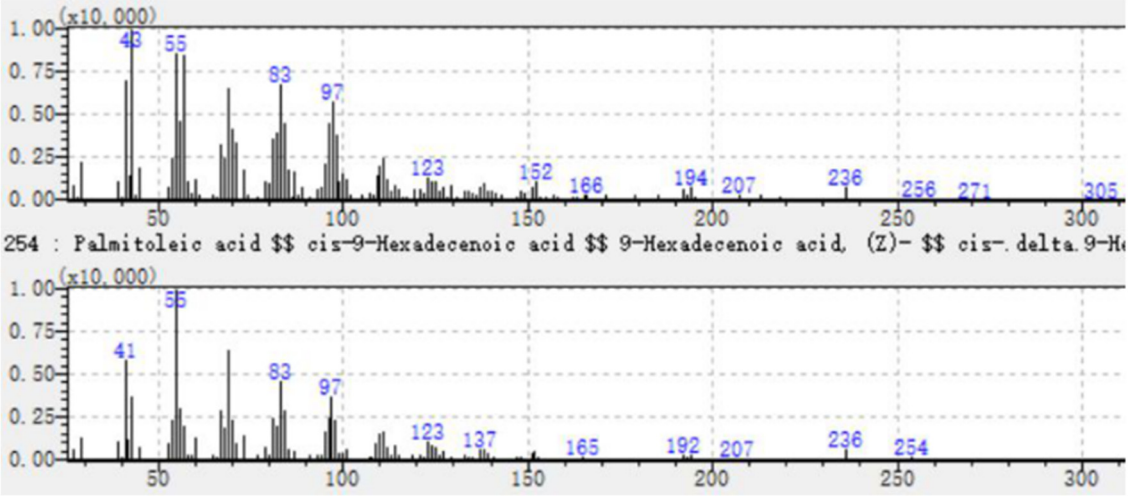

Figure 6. The mass spectra of unknown-5 from C10 and the corresponding match palmitoleic acid from NIST 14 library.

It should be noted that only eight compounds including hexanal, 1-octen-3-ol, limonene, unknown-1, *α*-terpineol, 2-pentadecanone, 6,10,14-trimethyl-, hexadecanoic acid, methyl ester, and *n*-hexadecanoic acid were detected by MS using DB-5 because of the low concentration of samples. Among them, the contents of limonene, *α*-terpineol, and *n*-hexadecanoic acid were relatively high. Whereas, limonene and *α*-terpineol were undetected previously [11,12]. Considering the EOs extracted from the peels of *Citrus reticulata Blanco* such as Nanfengmiju (*C. kinokuni* Hort. ex Tanaka) and *C. reticulata* 'Dahongpao' were also studied at the same time, and limonene and *α*-terpineol were the major compounds of such EOs, their has the possibility that these compounds were introduced from such EOs [14]. In such scenario, the quantitation results were based on the contents gotten from MS detected with FFAP column.

Twenty-two compounds including hexanal, *β*-pinene, 1-octen-3-ol, *p*-cymene, limonene, *γ*-terpinene, *cis*-linalool oxide, *trans*-linalool oxide, unknown-1, *α*-terpineol, *n*-tridecane, farnesyl acetone, hexadecanoic acid, methyl ester, palmitoleic acid, linoleic acid, methyl ester, oleic acid, methyl ester, unknown-2, methyl stearate, phytol, acetate, methyl 5,6-octadecadienoate, unknown-3, and unknown-5 were first reported from the EOs of *L. rotata*.

Among the 56 compounds, 7 compounds such as *n*-hexadecanoic acid, tetradecanoic acid, linoleic acid, oleic acid, hexahydrofarnesyl acetone, hexadecanoic acid, methyl ester, and phytol were prominent in view of their content.

The EOs, crystals, and EOs removed crystals were mainly consisted of long-chain fatty acids (57.1-64.7%, 74.2-80.1%, 41.9-56.7%; the content in sequence were denoted for EOs, crystals, and EOs removed crystals, the same for following), and their esters (6.6-14.8%, 3.2-7.6%, 12.2-15.3%). Among 12 fatty acids, the most outstanding one is *n*-hexadecanoic acid (47.1-60.8%, 61.3-69.2%, 17.8-44.8%), which was in line with the reported results [11,12]. The other prominent acids were tetradecanoic acid (2.8-3.9%, 4.4-5.6%, 5.4-6.4%), linoleic acid (2.7-7.7%, 0.1-1.1%, 0-9.7%), which were also reported previously [11,12], and oleic acid (0-3.7%, 2.9-4.0%, 0-10.0%) which was also reported previously [11]. It is reported that the fatty acids with odd carbons such as pentadecanoic acid (0-0.5%, 0.7-0.8%, 0.6-0.8%) detected in this study has the anti-tumor activities [15]. Among the 7 esters of fatty acids, linolenic acid, methyl ester (1.8-3.4%, 0%, 0-1.2%) was reported as the main component previously [12]. The other six esters were first reported, in which, hexadecanoic acid, methyl ester was prominent representing 1.5-3.9%, 1.6-4.1%, 4.1-8.3%. In addition, 6, 10, 14-trimethyl-2-pentadecanone (2.0-3.0%, 2.3-3.5%, 5.6-7.0%) was prominent. Linoleic acid ethyl ester reported as a major component in EO of

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L. rotata previously, which identification is unreliable considering its LRI value [11], was undetected in this study.

The *n*-alkanes with the largest number including 17 ones (C_{13}-C_{29}), representing 0-1.3%, 0.7-1.8%, 2.6-5.5%. Tridecane with content 0, 0, 0-tr, was first reported in the EOs of *L. rotata*. Previously, 22 *n*-alkanes (C_{11}-C_{12} and C_{14}-C_{33}) with content 4.6-37.8%, and 13 branched alkanes with content 0.55-6.75% were detected [12]. Two prominent ones were tricosane (0-0.2%, 0.2%, 0.4-0.6%), and pentacosane (0-0.1%, 0.2-0.3%, 0-0.6%). Four HMs including β-pinene, *p*-cymene, limonene, and γ-terpinene were detected with minor content. Previously, only one HM such as α-pinene was detected in flower and leaf of *L. rotata* with content 0-0.22% and 0-0.23%, respectively [11,12]. Four AMs including *cis*-linalool oxide, *trans*-linalool oxide, linalool, and α-terpineol were detected reprsenting 5.2-8.3%, 1.8-3.6%, 6.7-9.4%, in which, α-terpineol (2.8-4.1%, 1.1-1.3%, 2.0-4.7%) is prominent, and only linalool was detected previously [12].

The 1-octene-3-ol (0-1.8%, 0-0.7%, 0-1.1%) was reported to have a typical mushroom flavor [16].

2.2. Antioxidant Activities of These EOs

In beginning, three different concentrations such as 5, 15, and 25 μg·mL^{-1} of samples and chemical standards such as n-hexadecanoic acid and α-terpineol, were tested the DPPH free radical scavenging ability. The results showing that the clearance rates of most samples and standards at these concentrations were very weak. Following, the concentrations of samples and chemical standards were increased and the volume of DPPH working solution was reduced accordingly. The DPPH results (Figure 7) showed that all samples presented a certain DPPH free radical scavenging rate. Among them, the RC10 showed antagonistic effect with C10, and their clearance rate was higher when they existed alone than when they coexisted.

However, the crystals of L8 and L9 demonstrated a synergistic effect with the corresponding EO removed crystals of L8 and L9. The highest DPPH RSA value was 14.7% found in the RC10 at 110 μg·mL^{-1}, whereas the IC_{50} of ascorbic acid was 7.7 μg·mL^{-1}, which indicated that its RSA was much lower compared with that of ascorbic acid. Interestingly, the clearance rate of crystals all decreased when the concentration was 80 μg·mL^{-1} compared with 50 μg·mL^{-1}, and then began to rise, indicating that some compounds in the crystals which can act as an antagonistic anti-oxidation may reach the effective concentration at 80 μg·mL^{-1}. The standard chemical α-terpineol as a control was tested at three concentrations such as 30, 60, 90 mg·mL^{-1}, and the clearance rate was only 8.21% at 90 mg·mL^{-1}. Palmitic acid was tested at three concentrations such as 1.5, 3, 4.5 mg·mL^{-1}. The highest clearance rate was 4.1% at 1.5 mg·mL^{-1}, and was gradually decreased with the increase of concentration.

The RSA values of the samples detected by ABTS (Figure 8) showed that the crystals may contain more substances that promote oxidation. After removing the crystals, the scavenging rate increased to some degrees. The highest clearance rate was 23.89% still from RC10 at 110 μg·mL^{-1}. It is worth noting that most of the samples showed better antioxidant activities compared with those in the DPPH experiment, which should be due to the higher reactivities of ABTS radical cations [17]. The scavenging rate of palmitic acid was negative in the ABTS experiment. The scavenging rate of α-terpineol was 27.73% at 110 μg·mL^{-1}.

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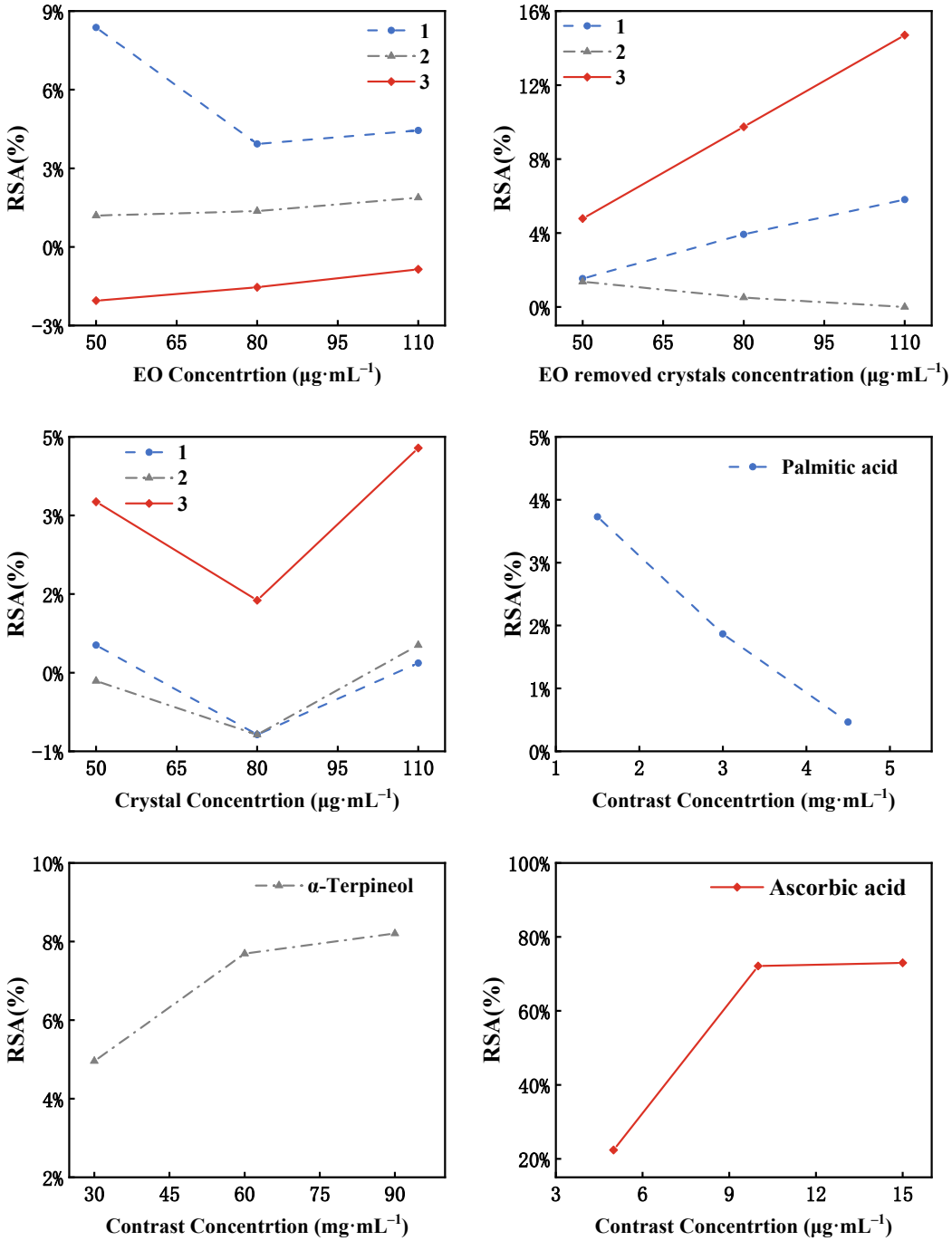

Figure 7. The results of DPPH assay. RSA (radical scavenging activity); 1, 2, 3 corresponding to L8, L9, L10, respectively.

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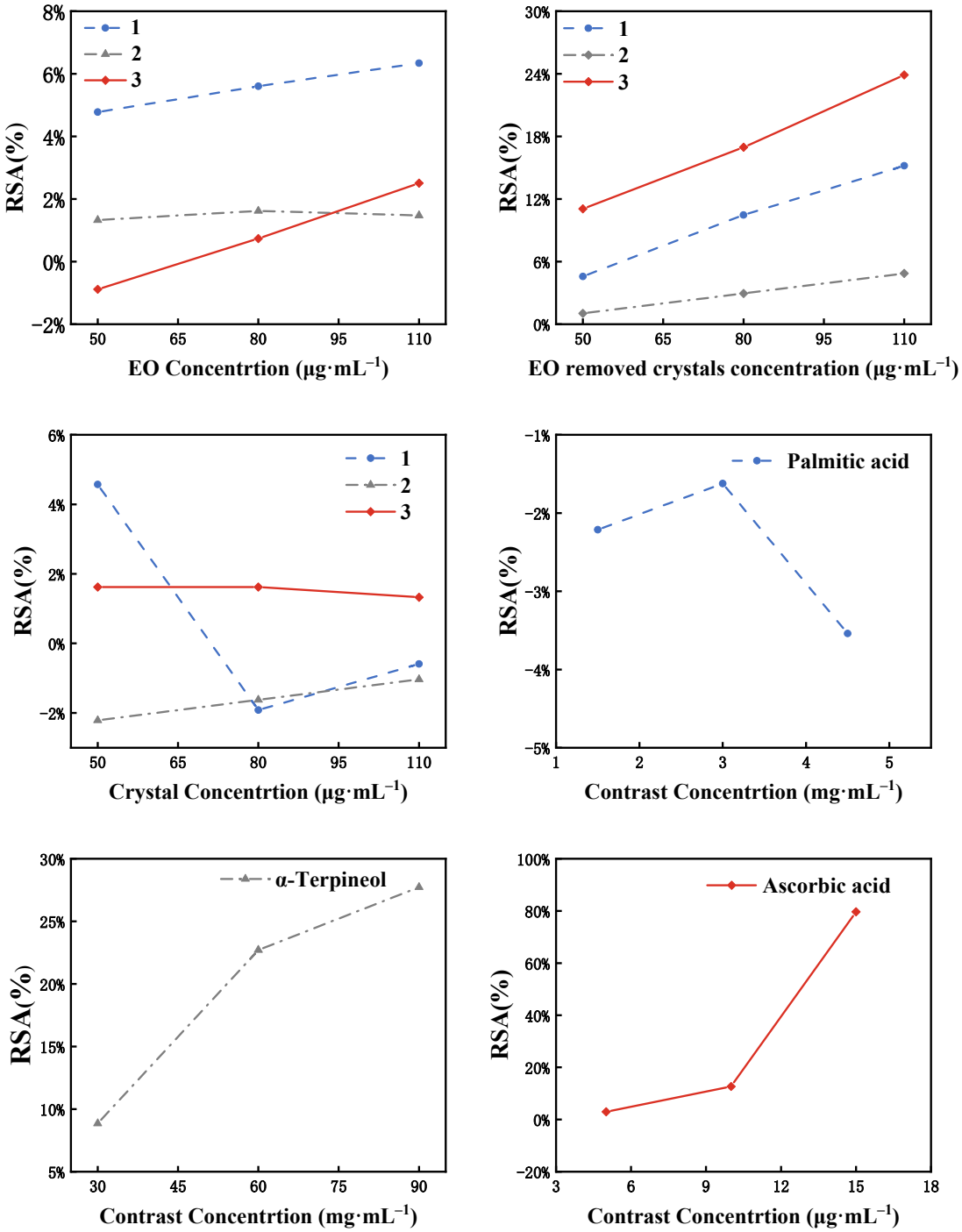

Figure 8. The RSA of ABTS assay.

The FRAP values of the samples and the standard as α-terpineol were nearly the same as that of ascorbic acid at 5 μg·mL-1 (Figure 9), which indicated that the tested samples had partial electron transfer ability. Interestingly, the standard as palmitic acid had a stronger FRAP value compared with that of ascorbic acid. However, the mixture solution of palmitic acid with the FRAP working solution is milky white turbid liquid, and there is no dark blue unique to ferrous ions. Since the FRAP working solution was composed mainly by pure water accounts, and the solubility of palmitic acid in water is relatively less, it would result in the partial precipitation of palmitic acid.

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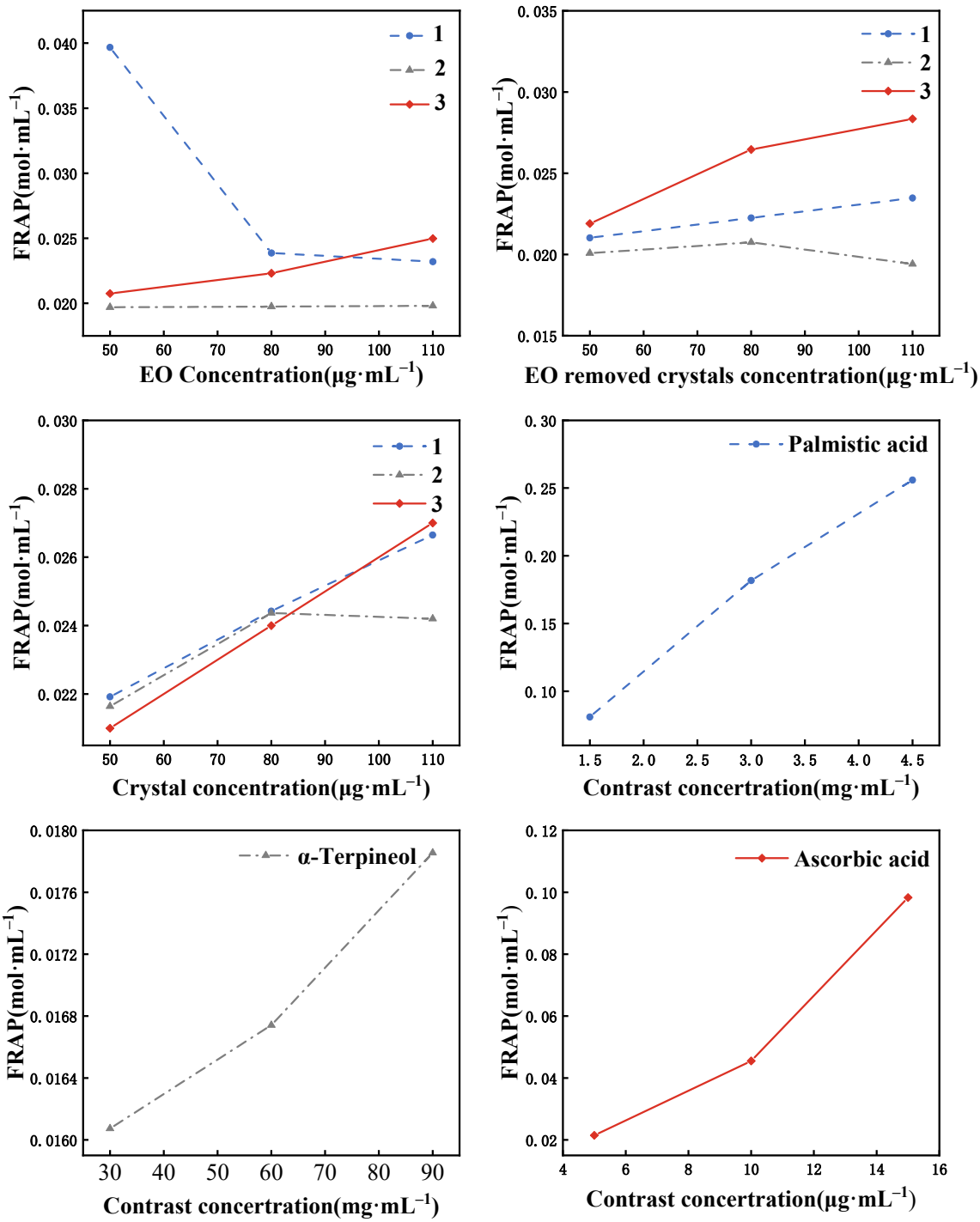

Figure 9. The results of FRAP assay.

3. Discussion

n-Hexadecanoic acid (10.1-49.3%) and 6, 10, 14-trimethyl-2-pentadecanone (3.2-13.7%) were also reported as the major components in the EOs from the flowers of *Malva sylvestris* L. [13], *Cirsium japonicum* var. *ussurience* Kitamura, *Ixeris dentate* (Thunb.) Nakai, and *I. stolonifera* A. Gray, respectively [18,19].

Besides limonene and *α*-terpineol, there also has the possibility that three HMs including *β*-pinene, *p*-cymene, and *γ*-terpinene and two AMs including *cis*-linalool oxide and *trans*-linalool oxide were introduced from the peels EOs of Nanfengmiju (*C. kinokuni*) and *C. reticulata* 'Dahongpao' [14].

In some studies [20], palmitic acid is used to induce oxidative damage in cells. Palmitic acid can react with cells to generate reactive oxygen species, reduce the content of NO, and make cells more

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prone to oxidative stress, which proves the antioxidant experimental results from another aspect, that is palmitic acid has nearly no antioxidant activity or even has oxidant activity.

The samples were micro-detected in the DPPH, ABTS, FRAP experiment with the ratio of 100 μL sample solution to 100 μL working solution. The advantage of micro-detection is fast and convenient, reducing reagent consumption, ensuring that the samples were simultaneously detected, and therefore ensuring the accuracy of the experimental results. However, there are high requirements for sample addition in micro-detection. As a result, small differences in the experiment process may lead to large differences in results. The results of three detection methods are closely related to the environment such as the ratio of working solution to sample solution, the concentration of the samples, and the intrinsic reactivity to free radicals and other reactive oxygen species [21]. After querying some literatures using the same sample concentration, the same ratio, and the same reference substance, it was found that the IC50 values obtained by them were still different, which indicated that the results were also effected by other factors from the environment such as the climate, temperature, etc.. Only the data obtained from the environment at that time can be used to draw conclusions after comparing with the positive reference substance.

4. Materials and Methods

The Materials and Methods should be described with sufficient details to allow others to replicate and build on the published results. Please note that the publication of your manuscript implicates that you must make all materials, data, computer code, and protocols associated with the publication available to readers. Please disclose at the submission stage any restrictions on the availability of materials or information. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited.

Research manuscripts reporting large datasets that are deposited in a publicly available database should specify where the data have been deposited and provide the relevant accession numbers. If the accession numbers have not yet been obtained at the time of submission, please state that they will be provided during review. They must be provided prior to publication.

Interventionary studies involving animals or humans, and other studies that require ethical approval, must list the authority that provided approval and the corresponding ethical approval code.*4.1. Plant Materials, Reagents, and Chemicals*

Three populations of the aboveground portion of *L. rotata*, named L8, L9, L10, which were corresponding to the same No. samples in previous research [9], were collected from the BianBa, LeiWuQI, and NaQu counties of Tibet, at the GPS coordinates E:93° W:31°. The collected populations were authenticated by Professor Yi Zhang (Chengdu University of Traditional Chinese Medicine (CUTCM), Chengdu, China), and voucher samples were deposited in the College of Ethnic Medicine (CUTCM, Chengdu, China) and the Chongqing Academy of Chinese Materia Medica (Chongqing, China). Some non-volatile components of these samples were analyzed previously [9].

n-Hexane for high-performance liquid chromatography (HPLC), linalool (98%+), *p*-cymene (99%+), *α*-terpineol (98%+), and nonane (98%) were produced by Adamas Reagent Company Ltd. *d*-Limonene (96%) was produced by Acros organics, USA. *γ*-Terpinene (97%) was produced by Wako pure chemical industries, Ltd., Japan. *n*-Hexadecanoic acid was produced by CATO. *n*-Alkanes standard solution of C_{10}–C_{25}, produced by Dr. Ehrenstorfer Inc, Germany, and *n*-octacosane (99%) produced by Aldrich, were used to determine LRIs. The above reagents, and chemicals were all supplied by Shanghai Titan Scientific Co.,Ltd., China.

DPPH, Ascorbic acid, ABTS powder, potassium persulfate ($K_2S_2O_8$), were all supplied by Shanghai Titan Scientific Co.,Ltd., China.

4.2. Extraction and Separation

The L8, L9, and L10 were crushed to a powder, and weighed 315 g, respectively. The powders were swollen with 3150 mL of pure water (10 volumes) in a round-bottomed flask, and were soaked for 0.5 h at 40 °C, respectively. The EOs were extracted thrice from each of the powders for 5 h by hydrodistillation through Clevenger-type apparatus with n-hexane as the collecting solvent. The

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water in the light yellow EOs was removed using anhydrous Na_2SO_4. A total of 0.29, 0.26, and 0.19 g, corresponding to 418, 405, and 238 μL, with densities of 0.69, 0.64, and 0.80, yields as 0.13, 0.13, and 0.08 (%, v/w) of the EO was obtained from L8, L9, and L10, respectively.

The EOs of L8, L9, and L10 were stored at 4, -4, and -80 °C, respectively, to evaluate crystallization. Crystals were obtained at 4 and -4 °C for L8, L9, and L10, respectively. At -80 °C, the EOs removed crystals in 4, and -4 °C were all being solid state. As a result, there were three samples as EO, crystal, and EO removed crystal for L8, L9, L10, respectively, corresponding to E8, E9, E10, C8, C9, C10, and RC8, RC9, RC10. The samples were stored in separate screw-capped vials at 4 °C.

4.3. Sample Preparation

The samples of E8, E9, E10, C8, C9, C10, and RC8, RC9, RC10 were diluted in the ratio V_{sample}: $V_{n\text{-hexane}}$ (HPLC) 1: 1000 (0.1%) for the GC-FID and GC-MS detection using the DB-5 column (30 m×0.25 mm i.d., 0.25 μm film thickness), and were diluted in the ratio V_{sample}: $V_{n\text{-hexane}}$ (HPLC) 1: 250 (0.4%) for GC-MS detection using a free fatty acid phase (FFAP) column (30 m × 0.32 mm×0.5 μm).

First, the samples of E8, E9, E10, C8, C9, C10, RC8, RC9, RC10, and chemical standards such as *α*-terpineol and *n*-hexadecanoic acid, were diluted in methanol to the concentrations such as 5, 15, and 25 μg·mL^{-1} for DPPH, ABTS, and FRAP detection, respectively. Then, the samples of E8, E9, E10, C8, C9, C10, and RC8, RC9, RC10, were diluted in methanol to the concentrations such as 50, 80, and 110 μg·mL^{-1} for DPPH, ABTS, and FRAP detection, respectively. The samples of chemical standards such as *α*-terpineol was diluted in methanol to the concentrations such as 30, 60, and 90 mg·mL^{-1} for DPPH, ABTS, and FRAP detection, respectively. The samples of chemical standards such as *n*-hexadecanoic acid was diluted in methanol to the concentrations such as 1.5, 3, and 4.5 mg·mL^{-1} for DPPH, ABTS, and FRAP detection, respectively. The samples of ascorbic acid were diluted in methanol to the concentrations such as 5, 10, and 15 μg·mL^{-1} for DPPH, ABTS, and FRAP detection, respectively.

4.4. GC (Gas Chromatography) Analyses

GC–FID (Flame Ionization Detector) analyses were obtained on a GC-2010 (Shimadzu, Japan) with a DB-5 column. The oven temperature was programmed from 60 (3-min hold) to 250 °C at 2.5 °C·min^{-1}, and then held for 2 min. The carrier gas was nitrogen at a constant flow of 1.7 mL·min^{-1}. The injector and detector were maintained at 250 °C both. The splitting ratio was 5: 1, and the injection volume was 1 μL.

GC–MS analyses were carried out using a GCMS-TQ8040 (Shimadzu, Japan) matched with a NIST 14 MS database, a DB-5 column, and an FFAP column, respectively. The oven temperature for DB-5 was programmed from 60 (3-min hold) to 280 °C at 2.5 °C·min^{-1}, and then held for 2 min. The oven temperature for FFAP was programmed from 60 (3-min hold) to 230 °C at 2.5 °C·min^{-1}, and then held for 2 min. The following parameters were the same for DB-5 and FFAP. The carrier gas was helium, at a constant flow of 1 mL min^{-1}. The splitting ratio was 100: 1. The solvent delay was 3.0 min. The injector, ion-source, and interface were maintained at 250, 200, and 250 °C, respectively. Electron impact mass spectra were acquired at 70 eV at a scan rate of 3.9 scans·s^{-1} from m/z 25-450 amu. The injection volume was 1 μL.

4.5. Identification and Quantitation

4.5.1. Identification

The peaks in the TICs obtained by GC-MS were identified by probability-based matching (PBM) first. Since overlapped and embedded peaks typically exist in the TICs, the identification results may be incorrect. In such situations, the characteristic ion peaks were selected and compared with the NIST (National Institute of Standards and Technology) 14 or 17 database or the mass spectra of the standards. The LRIs were calculated relative to the retention time of the *n*-alkanes (C_{10}-C_{25}) with the *n*-octacosane standard and compared with the LRIs of the corresponding chemicals provided in the NIST 17 database, literatures, or standards.

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4.5.2. Quantitation

The peak area normalization was used to calculate the relative area percentage of each compound.

4.6. Antioxidant activities

4.6.1. DPPH Assay

A slight improvement was made according to the literature method [22]. The 0.0394 g DPPH was weighed, dissolved in moderate methanol and metered to 10 mL in a volumetric flask, and then shaken fully as stock solution. 0.1 mL DPPH stock solution was metered to 10 mL to get the DPPH solution (100 μmol·L^{-1}). All solutions were kept in dark until used. Samples 100 μL at different concentrations diluted in methanol were placed in a 96-well microplate and then supplemented with 100 μL of DPPH (100 μmol·L^{-1}) solution. Then, the solution was shaken fully. After incubation for 30 min in the dark at room temperature, the absorbance was measured at 517 nm using a microplate reader. Each sample and standard set up 3 holes. Methanol was served as the blank control. Radical-scavenging activity was calculated as a percentage of DPPH discoloration using the following equation:

$$RSA\ (\%)=[(A_{Blank}-A_{Sample})/A_{Blank}]*100. \quad (1)$$

In this equation, A_{Sample} is the absorbance of the reaction mixture containing the sample, and A_{Blank} is the absorbance of the blank control. Ascorbic acid was used as the positive control. Calculated the inhibition rate in a series of concentration (50-110 μg·mL^{-1}, diluted in methanol).

4.6.2. ABTS Assay

A slightly modification was made based on the literature description method [23]. ABTS powder 0.0441g was weighed accurately, and then dissolved in 11.482 mL ultrapure water to 7 mM concentration; Potassium persulfate ($K_2S_2O_8$) 378.4 mg was dissolved in 10 mL distilled water to make the 140 mM $K_2S_2O_8$, which was stored in glass bottle. The ABTS radical cation ($ABTS^{\bullet+}$) solution was prepared by reaction of 5 mL of a 7 mM aqueous ABTS solution and 88 μL of a 140 mM (final concentration 2.45 mM) $K_2S_2O_8$ aqueous solution, which was kept in darkness at room temperature for 16 h. Then, radical cation was diluted with methanol (about 80-100 times) to absorbance value as 0.7±0.02 at 734 nm. Samples 100 μl dissolved in methanol (50–110 μg·ml^{-1}) was added to 100 μl of ABTS radical solution and mixed totally at room temperature for 6 min, and then, the absorbance at 734 nm was measured through using the microplate reader. Ascorbic acid (5–15 μg·ml^{-1}) was used as the standard control. The calculation method of radical scavenging activity was consistent with DPPH assay.

4.6.3. FRAP Assay

A slight modification was made based on the literature method [22]. Sample solution 0.1 ml dissolved by methanol (50-110 μg·ml^{-1}) was added to 0.1 ml of FRAP working solution, which was consisted of acetic acid buffer (0.3 mol·L^{-1}), TPTZ solution (10 mM), and $FeCl_3$ (20 mM) solution in this order at a volume ratio of 10: 1: 1 corresponding to 25, 2.5, 2.5 mL, respectively. The mixture was left in the darkness at 37 ℃ for 30 min, which was then immediately placed in a microplate reader to measure the increase of absorbance value at 593 nm.

A calibration curve was found through mixing the obtained 0.1 ml Fe(II) aqueous solutions in concentration range 0.1-2.5 mM·dm^{-3} with 0.1 ml FRAP reagent. In this measuring system, the total antioxidant capacity was calculated by the Fe (II) equivalents. The concentration (mmol·L^{-1}) of $FeSO_4$ was calculated by the absorbance value after reaction demonstrated in the standard curve, which was denoted as the value of FRAP. The higher FRAP value means the stronger antioxidant activity.

5. Conclusions

The EOs were mainly composed of long-chain fatty acids and their ester, which was in agreement with the previous report [11,12]. The crystals separated from the EOs were mainly composed of palmitic acid. The chemicals, which have high boiling point (BP), lead to the lower extraction rate compared with that of the EOs from other plants, such as *Citrus* L. Twenty-two compounds were first reported from the EOs of *L. rotata*. Seven compounds including *n*-hexadecanoic acid, tetradecanoic acid, linoleic acid, oleic acid, hexadecanoic acid, methyl ester, hexahydrofarnesyl acetone, and phytol, were identified as the major chemicals in these EOs, which could be chosen as the chemical markers of such EOs. The EOs extracted from *L. rotata* presented some similarities with the EOs extrated from *M. sylvestris* [13], *C. japonicum* var. *ussurience* Kitamura, *I. dentate*, and *I. stolonifera* [18,19], since they were all represented with the high BP compounds such as *n*-hexadecanoic acid, hexahydrofarnesyl acetone, *etc.*, as the major components. This study can give some hints for the full usage of such EOs.

Three kinds of EOs and EOs removed crystals, respectively, present some antioxidant activities, but insignificance compared with that of ascorbic acid. It should be noteworthy that the crystals present nearly non antioxidant activities and maybe even have oxidant activities, which is the same for standard palmitic acid. At the same time, the EOs removed crystals usually present some stronger antioxidant activities compared with that of EOs.

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References

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