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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 such EOs, we firstly conducted a thoroughly investigation on the chemicals in the EOs and their antioxidant activities (AAs), to the best of our knowledge. Light yellow EOs with fresh and elegant smell were obtained by hydro-distillation with average yield as 0.11% (volume mL/weight g). The crystal was separated from the EO through cryoprecipitation, respectively. Therefore, the EOs, crystals, and EOs removed crystals were studied further. A total of 55 components were qualified and quantified, in which 21 ones were first reported in these EOs. In the EOs, crystals and EOs removed crystals, the main compounds were long-chain fatty acids (LCFAs) and their esters, whereas the crystals presented relatively higher content of LCFAs and the EOs removed crystals presented relatively lower content of LCFAs compared with that of corresponding EOs. The most abundant compound *n*-hexadecanoic acid (palmitic acid), a kind of LCFAs, presented 47.1-60.8%, 61.3-69.2% and 17.8-44.8%, in the EOs, crystals and EOs removed crystals, respectively. Their AAs of three kinds of EOs, crystals and EOs removed crystals and a chemical marker as *n*-hexadecanoic acid were evaluated through 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) assays, respectively. Three kinds of EOs and EOs removed crystals presented some AAs, but not so strong compared with that of ascorbic acid. Usually, the EOs removed crystals showed some stronger AAs compared with that of the corresponding EOs. It should be noteworthy that the crystals showed nearly non AAs and even pro-oxidant activities. The palmitic acid (PA) also presented pro-oxidant activities in a concentration dependant manner. It can be deduced that the monounsaturated FAs (MUFAs) including 9*E*-hexadecenoic acid, palmitoleic acid and oleic acid, and polyunsaturated FAs (PUFAs) only including linoleic acid detected in this study had some AAs. The FAs have important meaning for keeping the balance between oxidant and antioxidant which depends on their unsaturation extent. This study will give some hints for the full usage of such EOs with lower extracted rate and mainly composed of FAs such as PA.

Keywords: *Lamiophlomis rotata* (Benth.) Kudo; essential oils; fatty acids; *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 activity, which means its essential oil (EO) may have such activity [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, in which, 16 and 13 ones (92.9% and 95.5%) were identified in the aboveground and underground portions, respectively. The main compounds were fatty acids (FAs), especially long-chain FAs (LCFAs, when the chain is not less than ten crabons), *n*-Hexadecanoic acid (palmitic 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 debatable considering its linear retention index (LRI) value, (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 FAs accounting for 13.66-46.27%, 4 esters of FAs accounting for 8.77-20.8%, and 35 alkanes ranged from 6.1% to 37.84% were detected. Palmitic acid (PA) (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].

By now, there is no evaluation on the antioxidant activities (AAs) of EOs from *L. rotata*. However, supplemental FAs have important meaning for keeping the human health since they can keep the oxidant and antioxidant balance in cells [13–20]. The effects of FAs on oxidant injury appears to be related to the degree of FAs unsaturation[14]. Saturated fatty acids (SFAs) such as PA can increase oxidative stress in angiogenic mononuclear cells in a concentration dependant manner [21], but octadecanoic acid was reported to protect pulmonary artery endothelial cell from oxidant injury. Usually, polyunsaturated fatty acids (PUFAs) can reduce oxidant injury [15 +4, 16 +6-19 +9], but there also has exception [14]. The relationship between the degree of FAs unsaturation and susceptibility to oxidant injury remains controversial [13].

In order to study the chemicals in EOs of *L. rotata* further and try to explore the AAs of such EOs and its representative chemical PA, we have first done an exhaustive exploration on the chemicals present in these EOs and analyzed their AAs through DPPH (1,1-Diphenyl-2-picrylhydrazyl radical), ABTS ((2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), and FRAP (ferric reducing/antioxidant power) assays based on the previously investigation [9], to the best of our knowledge..

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 three samples named L8, L9, and L10 of *L. rotata*, respectively. The average yield (0.11%) was close to the yield (0.1%) reported previously [11]. In total, 55 compounds were qualified and quantified (Table 1, Figure 1).

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

No.	Compounds	CAS	LRIs[b, d]	LRIs[a]	LRIs[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	0.1	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 (6:0)	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	1030, 1200	1026	1203	12.8	nd	3.4	4.6	36.4	3	2.3	5.8	0.8	12	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	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	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	Linalool	78-70-6	1082, 1547	1098	1552	0.2	nd	2.4	2.2	6.6	0.7	6.8	20.9	4.0	9.1	nd	4.2	2.9	11.7	1.1	5.5	18.0	2.1	11.0	22.3	4	3.4	22.3	1.2	5.7	22.3	2
11	Caprylic acid (8:0)	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
12	*α*-Terpineol	98-55-5	1189, 1697	1185	1690	71.2	100	2.8	5.4	17.5	1.1	12.8	56.5	4.7	17.8	100	4.1	5.9	30.6	1.3	11.4	47.6	2	14.8	70.4	3.4	4.8	27	1.2	8.5	41.4	2
13	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
14	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
15	*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
16	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
17	Dodecanoic acid (12:0)	143-07-7	1556, 2498	-	2474	nd	nd	0.7	1.1	nd	0.9	1.2	nd	1.6	1	nd	1.1	1.3	nd	1.4	2.1	nd	2.5	0.7	nd	1.2	1	nd	1.3	1.7	nd	2
18	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
19	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
20	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
21	Tetradecanoic acid (14:0)	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

22	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
23	Hexahydrofarnesyl acetone	502-69-2	1842, 2131	1843	2119	nd	nd	2	3.5	nd	2.7	5	7.1	6.2	2.7	nd	2	2.5	nd	2.3	6.4	nd	5.6	3.7	nd	3.0	3.8	1.9	3.5	7.9	17	7
24	* Pentadecanoic acid (15:0)	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
25	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
26	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
27	Methyl hexadecanoate	112-39-0	1926, 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	6.7	11.1	7.1	3.8	nd	3.9	3.6	3.3	4.1	7.8	18.4	8.3
28	9*E*-Hexadecenoic acid (16:1, n-7)	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
29	Palmitoleic acid (16:1, n-7)	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
30	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
31	*n*-Hexadecanoic acid (16:0)	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
32	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
33	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
34	Methyl oleate	112-62-9	2091, 2434	-	2439	0.2	nd	0.9	1	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	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
36	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	nd	tr	4.3	nd	0.1
37	Phytol	150-86-7	2104, 2622	-	2607	un	nd	5.8	un	nd	1.5	un	nd	7	un	nd	2	un	nd	0.7	un	nd	1.3	un	nd	4.4	un	nd	1.3	un	nd	2
38	Unknown-1	-	-, -	-	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
39	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
40	Linoleic acid (18:2, n-6)	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
41	Oleic acid (18:1, n-9)	112-80-1	2141, 3173	-	2770	un	nd	2.9	un	nd	2.9	un	nd	7	un	nd	nd	un	nd	3.3	un	nd	10.0	un	nd	3.7	un	nd	4	un	nd	nd
42	Octadecanoic acid (18:0)	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

43	Docosane	629-97-0	2200, 2200	-	2200	1	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
44	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
45	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
46	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
47	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
48	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
49	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
50	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
51	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
52	Unknown-2			-	2817	un	nd	0.9	un	nd	1	un	nd	3	un	nd	nd	un	nd	0.8	un	nd	1.7	un	nd	0.8	un	nd	0.8	un	nd	1.4
53	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
54	Unknown-3			-	2952	un	nd	nd	un	nd	nd	un	nd	nd	un	nd	4.5	un	nd	1	un	nd	0.5	un	nd	nd	un	nd	0.8	un	nd	4.5
55	Unknown-4			-	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 (55)					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
	HMs (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
	AMs (4)					71.4	100	5.2	9.5	24.1	1.8	21.7	77.4	9.4	27.8	100	8.3	10.9	42.3	3.5	22.4	47.6	6.7	26.8	92.7	7.8	11.1	49.4	3.6	20	57.7	6.7
	HSs (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
	ASs (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
	ADs (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
	Aldehydes & ketones (3)					0.1	0	2.7	3.7	2	2.8	6	10.3	7.3	3.8	0	2	2.8	0	2.5	7	7.5	5.9	4.9	0	3.8	4.2	1.9	3.6	8.4	21.7	7.4
	FAs (11)					1	0	72	67.6	37.5	82.1	16.4	0	50.9	27.2	0	67.4	70.7	57.7	81.8	39.1	7.7	58.7	28.1	0	62.2	68.1	41.8	78.4	39.4	2.1	55.7
	LCFAs (9)					1	0	72	67.6	37.5	81.9	16.4	0	50.3	27.2	0	67.4	70.6	57.7	81.5	38.9	7.7	58.1	27.9	0	62	68	41.8	78.1	39.3	2.1	55.1
	SCFAs (2)					0	0	0	0	0	0.2	0	0	0.6	0	0	0	0.1	0	0.3	0.2	0	0.6	0.2	0	0.2	0.1	0	0.3	0.1	0	0.6
	SFAs (7)					0.9	0	58.7	67	37.5	75.8	15	0	27.2	26.9	0	64.7	70.3	57.7	77.7	38.4	77	46.3	27.5	0	52.4	67.6	41.8	72.7	38.5	2.1	54.2
	MUFAs (3)					0	0	5.6	0.6	0	5.2	1.4	0	14	0.3	0	0	0.4	0	4	0.7	0	11.7	0.6	0	4.7	0.5	0	5.1	0.9	0	1.5
	PUFAs (1)					0	0	7.7	0	0	1.1	0	0	9.7	0	0	2.7	0	0	0.1	0	0	0.7	0	0	5.1	0	0	0.6	0	0	0
	Esters (8)					0.4	0	6.6	3.1	0	3.5	6.4	6.5	13	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 FAs (7)					0.4	0	6.6	3	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
	TOCs (29)					73.8	100	92.5	91.8	63.6	91.9	66.6	94.2	87.6	79.9	100	94.0	94.3	100	95.7	89.4	73.8	89.5	83.9	92.7	95	94.1	100	95.4	89.1	100	88.8
	n-Alkanes (17)					7.8	0	1.3	2.8	0	1.8	31	0	2.6	6.5	0	0	3.5	0	0.7	7.4	0	5.5	8	0	1.2	3.2	0	1.2	8.6	0	2.6

Unknowns (4)	0	0	2.5	0	0	2.8	0	0	8.9	0	0	4.5	0	0	3.3	0	0	4.4	0	0	2.6	0	0	3.3	0	0	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 its mass spectrum. The same for the following Tables. LRIs$^{b, d}$ detected by semi-standard apolar or polar column were gotten from NIST (National Institute of Standards and Technology) 17 library, respectively, LRIs^{a} and LRIs^{c} detected by DB-5 and free fatty acid phase (FFAP) were gotten in this experiment, respectively. The HMs, AMs, HSs, ASs, ADs, TOCs, SCFAs equal to hydrocarbon monoterpenes, alcohol monoterpenes, hydrocarbon sesquiterpenes, alcohol sesquiterpenes, alcohol diterpenes, total oxygenated compoundss, short-chain FAs (when the chain is less than 10 carbons), respectively. The compounds denoted with red color means they were identified and quantified both in this study and previous literature. FAs are represented by number of carbon atoms in fatty carboxyl chain:number of double bonds. The n- designates the location of the double bond nearest the methyl terminus.

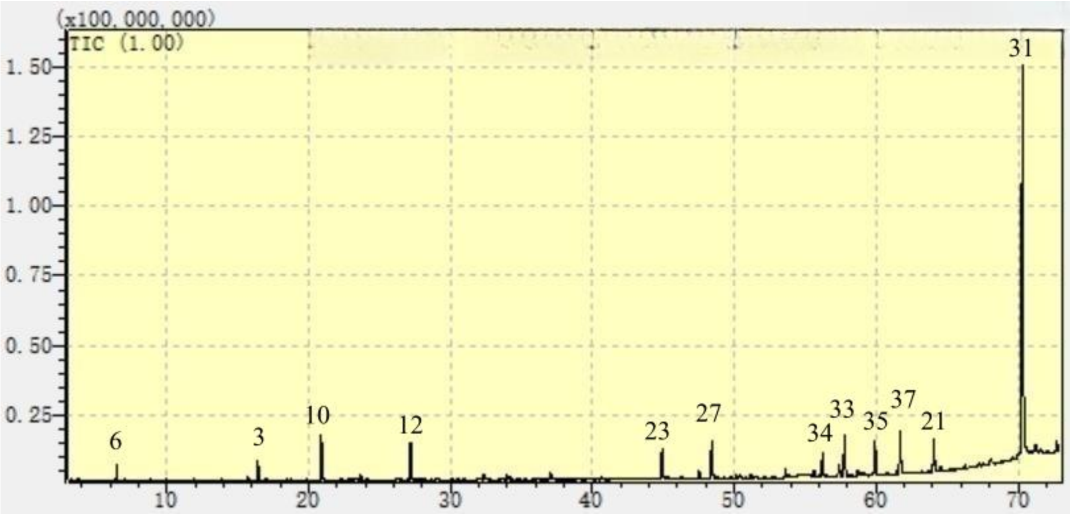


Figure 1. TIC of E10 detected by Gas Chromatography-Mass Spectrometer (GC-MS) using a FFAP column. Note: Compounds were listed by the corresponding numbers in Table 1. The denoted main compounds were 6 limonene, 3 1-octen-3-ol, 10 linalool, 12 α-terpineol, 23 hexahydrofarnesyl acetone, 27 methyl hexadecanoate, 34 methyl oleate, 33 methyl linoleate, 35 methyl linolenate, 37 phytol, 21 tetradecanoic acid, 31 *n*-hexadecanoic acid.

The mass spectra of compounds 40, 41, and 42 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 40, 41, 42 were still identified as linoleic acid, oleic acid, and octadecanoic acid, respectively, which were also reported previously [11,12].

In addition, four compounds detected in the total ion chromatograms (TICs), which characteristic ion peaks could be seen in Table 2, could not be elucidated by mass spectra and LRI values, respectively, based on the NIST 14, 17 or other database [22].

Table 2. The characteristic peaks of unknown compounds.

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

Unknown-1 should be an analogue of phytol acetate according to its characteristic ion peaks (Table2, Figure 2) and LRI value.

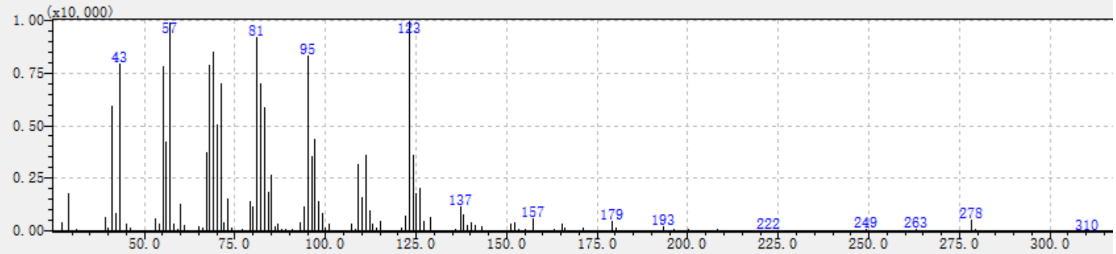


Figure 2. The mass spectrum of unknown-1 from RC8.

Unknown-2 should be an unsaturated long-chain fatty acid (ULCFC) or the corresponding ester based on its characteristic ion peaks (Table2, Figure 3) and LRI value.

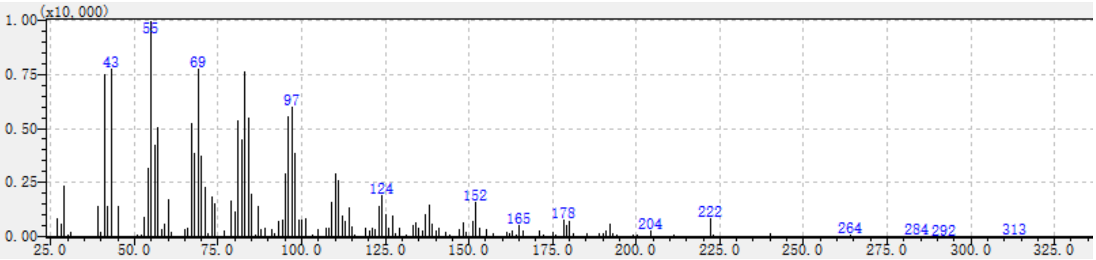


Figure 3. The mass spectrum of unknown-2 from RC9.

The most suitable match for unknown-3 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 its characteristic ion peaks, 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-3 in this study [12].

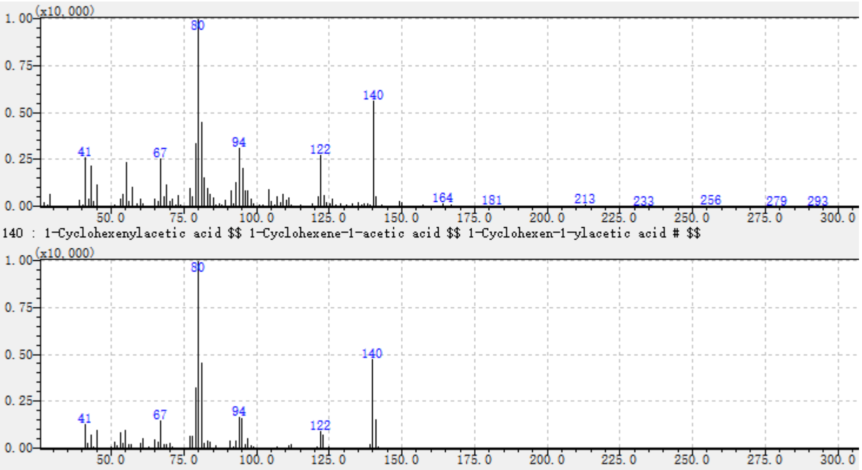


Figure 4. The mass spectra of unknown-3 from RC10 and the corresponding match 1-cyclohexenylacetic acid from NIST 14 library.

The most suitable match of unknown-4 was palmitoleic acid (Figure 5), 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 29 with LRI^c 2926, and 9*E*-hexadecenoic acid was identified as compound 28 with LRI^c 2935. Therefore, this compound should be an analogue of palmitoleic acid and not 9*E*-hexadecenoic acid.

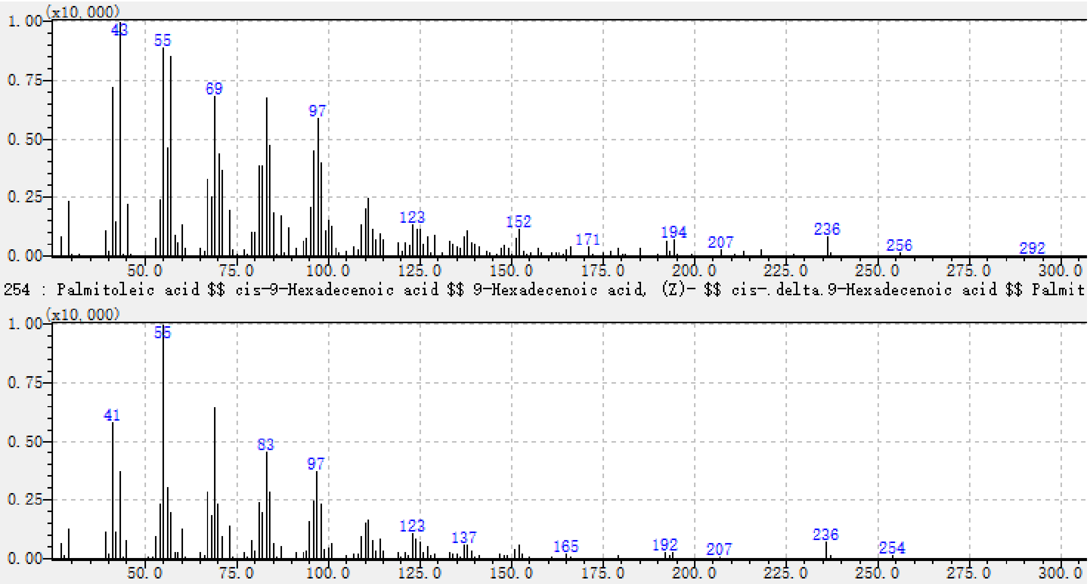


Figure 5. The mass spectra of unknown-4 from C10 and the corresponding match palmitoleic acid from NIST 14 library.

It should be noted that only eight compounds including hexanal, 1-octen-3-ol, limonene, linalool, α-terpineol, hexahydrofarnesyl acetone, methyl hexadecanoate 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 [23]. In such scenario, the quantitation results were based on the contents gotten from MS detected with FFAP column.

Twenty-one compounds including hexanal, β-pinene, 1-octen-3-ol, *p*-cymene, limonene, γ-terpinene, *cis*-linalool oxide, *trans*-linalool oxide, α-terpineol, *n*-tridecane, farnesyl acetone, hexahydrofarnesyl acetone, methyl hexadecanoate, palmitoleic acid, hexahydrofarnesyl acetone, methyl hexadecanoate, unknown-1, methyl stearate, phytol acetate, methyl 5,6-octadecadienoate, unknown-2 and unknown-4 were first reported from the EOs of *L. rotata*.

The EOs, crystals, and EOs removed crystals were mainly consisted of FAs (62.2-72%, 78.4-82.1%, 50.9-58.7%; the content in sequence were denoted for EOs, crystals, and EOs removed crystals, the same for following) including 11 compounds, especially LCFAs (62-72%, 78.1-81.9%, 50.3-58.1%) incldduing 9 compounds, and their esters (6.6-14.8%, 3.2-7.6%, 12.2-15.3%) including 7 compounds. According to the unsaturation, SFAs (52.4-64.7%, 72.7-77.7%, 27.2-54.2%) including 7 compounds were major. *n*-Hexadecanoic acid (47.1-60.8%, 61.3-69.2%, 17.8-44.8%) is the most outstanding one, which was in line with the reported results [11,12]. Then, tetradecanoic acid (2.8-3.9%, 4.4-5.6%, 5.4-6.4%) was also reported previously [11,12]. In addition, pentadecanoic acid (0-0.5%, 0.7-0.8%, 0.6-0.8%) with odd carbons detected in this study was reported to have anti-tumor activities [24]. MUFAs (0-5.6%, 4-5.2%, 1.5-14%) including 9*E*-hexadecenoic acid, palmitoleic acid and oleic acid, and PUFAs (2.7-7.7%, 0.1-1.1%, 0-9.7%) only including linoleic acid were minor. The prominent ones were oleic acid (0-3.7%, 2.9-4.0%, 0-10.0%) also reported previously [11], and linoleic acid (2.7-7.7%, 0.1-1.1%, 0-9.7%) also reported previously [11,12].

The content of PA is relatively higher in crystal, but relatively lower in EO removed crystal compared with that in the corresponding EO, whereas the content of MUFAs or PUFAs is usually relatively lower in crystal, but relatively higher in EO removed crystal compared with that in the corresponding EO.

Among the 7 esters of FAs, methyl linolenate (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, methyl hexadecanoate (1.5-3.9%, 1.6-4.1%, 4.1-8.3%) was prominent. In addition, hexahydrofarnesyl acetone (2-3%, 2.3-3.5%, 5.6-7%) was prominent.

The *n*-alkanes (0-1.3%, 0.7-1.8%, 2.6-5.5%) with the largest number include 17 ones (C_{13}-C_{29}). Among them, tridecane (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%). HMs (1-3.7%, 0.1-3.4%, 0.3-0.9%) including four compounds such as β-pinene, *p*-cymene, limonene, and γ-terpinene were detected. Previously, only α-pinene was detected in flower and leaf of *L. rotata* with content 0-0.2% and 0-0.2%, respectively [11,12]. AMs (5.2-8.3%, 1.8-3.6%, 6.7-9.4%) including four compounds such as *cis*-linalool oxide, *trans*-linalool oxide, linalool and α-terpineol were detected, in which, α-terpineol (2.8-4.1%, 1.1-1.3%, 2.0-4.7%) was prominent, and only linalool was detected in leaf of *L. rotata* collected in Tibet with content 0.2% previously [12].

The 1-octene-3-ol (0-1.8%, 0-0.7%, 0-1.1%) was reported to have a typical mushroom flavor [25].

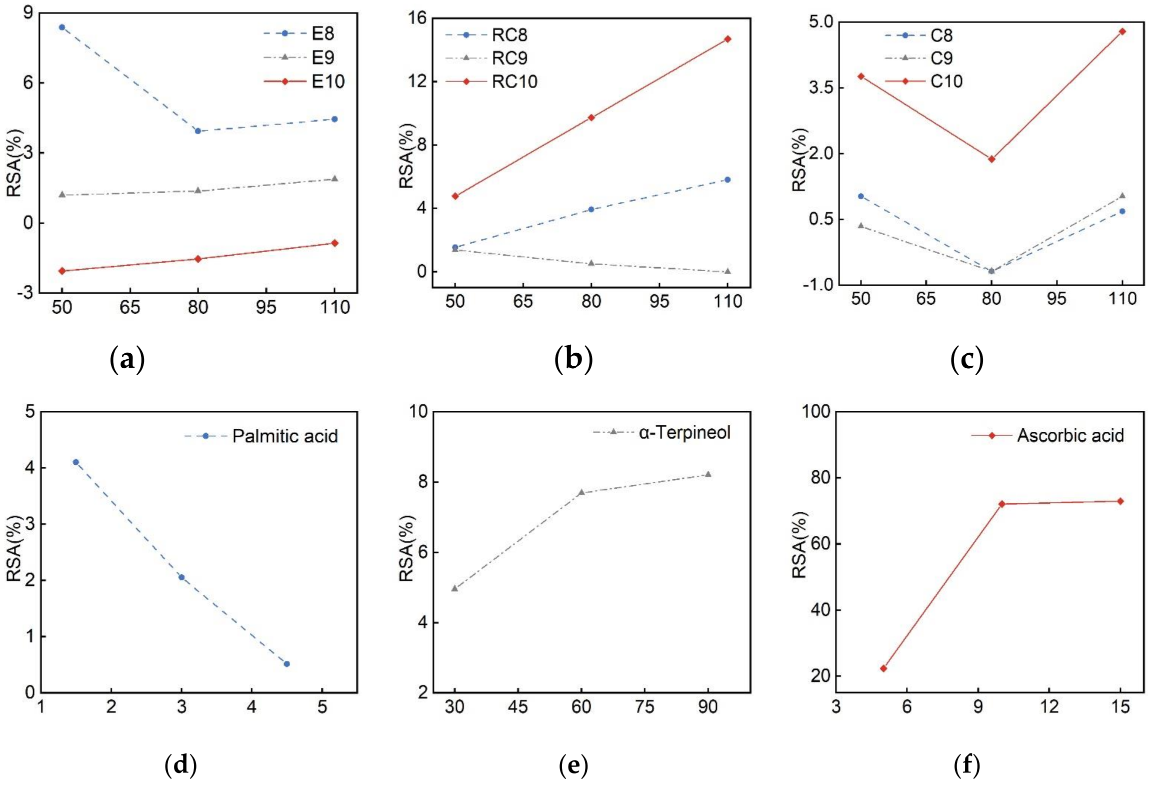
2.2. AAs of these EOs and their representative chemicals

Nine samples including E8, E9, E10, C8, C9, C10, RC8, RC9 and RC10 and two chemical standards such as n-hexadecanoic acid and α-terpineol were tested the AAs. First, α-terpineol was chosen as a representative chemical due to its high content detected by GC-MS using DB-5, whereas, it should not be a compound with high content as the analyses above. The data of α-terpineol was still listed. In beginning, the samples and chemical standards in three different concentrations such as 5, 15, and 25 $\mu g \cdot mL^{-1}$ were tested the DPPH free radical scavenging ability, respectively. However, the results showing that the clearance rates of these samples and standards at these concentrations were minute. Following, the concentrations of samples and chemical standards were increased and the volume of DPPH, ABTS and FRAP working solution was reduced accordingly. The IC_{50} (inhibitory concentration of 50% radical scavenging activity (RSA)) of each sample detected or deduced by DPPH, ABTS, and FRAP, respectively, can be seen in Table 3.

Table 3. The IC_{50} and Ferric reducing ability of each sample. Ferric reducing ability: FRAP value of each sample in the maximum concentration. ND: not determined.

Samples	**IC_{50} ($mg \cdot mL^{-1}$)**		**Ferric reducing ability ($mmol \cdot L^{-1}$)**
	DPPH	**ABTS**	
E8	ND	133.1	0.023
E9	764.96	ND	0.02
E10	ND	0.227	0.025
RC8	0.629	0.323	0.023
RC9	ND	0.541	0.019
RC10	0.344	0.293	0.028
C8	ND	ND	0.027
C9	ND	ND	0.024
C10	ND	ND	0.026
Palmitic acid	ND	ND	0.025
α-Terpineol	747.9	197.65	0.018
Ascorbic acid	0.0077	0.0127	0.098

The DPPH results (Figure 6) 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.



(**a**) (**b**) (**c**)

(**d**) (**e**) (**f**)

Figure 6. The results of DPPH assay. (**a**) RSA of EO at 50-110 μg·mL^{-1}; (b) RSA of EO removed crystal at 50-110 μg·mL^{-1}; (c) RSA of Crystal at 50-110 μg· mL^{-1}; (d) RSA of PA at 1.5-4.5 mg·mL^{-1}; (e) RSA of α-terpineol at 30-90 mg·mL^{-1}; (f) RSA of ascorbic acid at 5-15 μg·mL^{-1}. The same sequence and concentration for Figures 7 and 8.

However, the C8 and C9 demonstrated a synergistic effect with the corresponding RC8 and RC9. The highest DPPH RSA value was 14.7% found in the RC10 at 110 μg·mL^{-1}, and the IC$_{50}$ of ascorbic acid was 7.7 μg·mL^{-1}. Interestingly, the clearance rate of C8, C9 and C10 was minimum when the concentration was 80 μg·mL^{-1}, respectively, which indicated that some compounds in the crystals acting as a pro-oxidation function may reach the effective concentration. The clearance rate of α-terpineol was only 8.21% at 90 mg·mL^{-1}. The highest clearance rate of PA 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 7) showed that the crystals may contain more substances to promote oxidation. After removing the crystals, the scavenging rate was 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 AAs compared with those in the DPPH experiment, which should be due to the higher reactivities of ABTS radical cations [26]. The scavenging rate of PA was negative in the ABTS experiment. The maximum scavenging rate of α-terpineol was 27.73% at 110 μg·mL^{-1}.

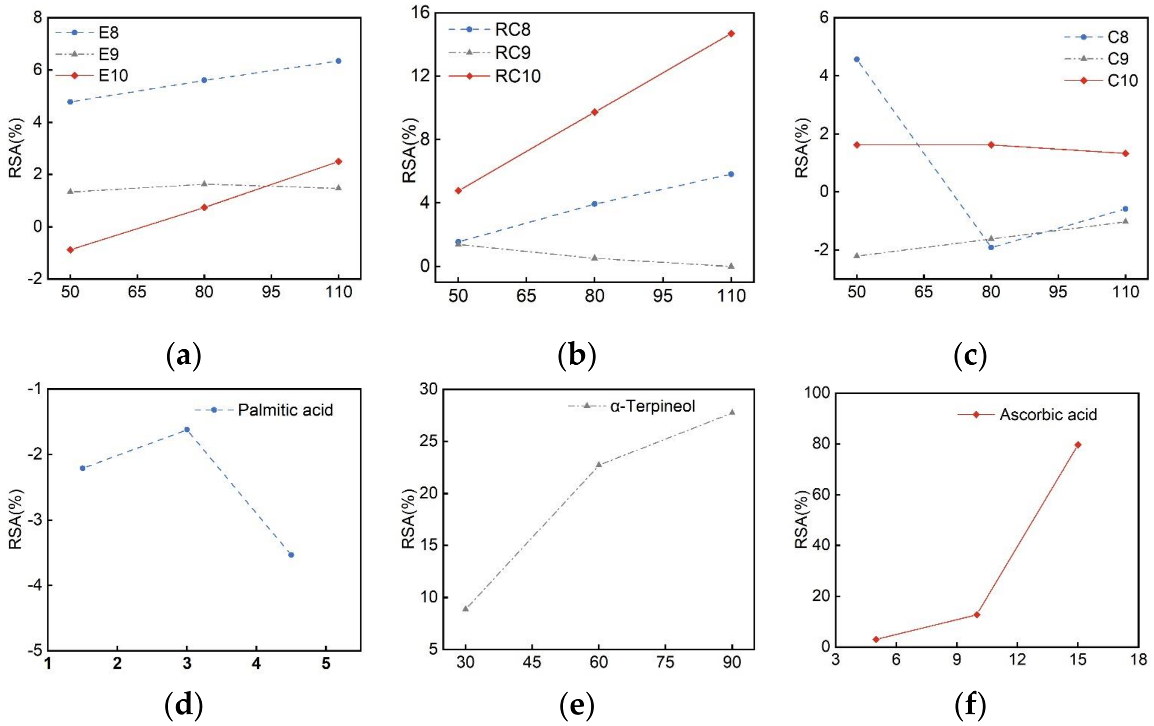


Figure 7. The RSA of ABTS assay.

The FRAP values of the samples and the α-terpineol were nearly the same as that of ascorbic acid at 5 μg·mL^{-1} (Figure 8), which indicated that the tested samples had partial electron transfer ability. Interestingly, the PA had a stronger FRAP value compared with that of ascorbic acid. However, the mixture solution of PA 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 mainly composed of pure water, and the solubility of PA in water is relatively less, it would result in the partial precipitation of PA.

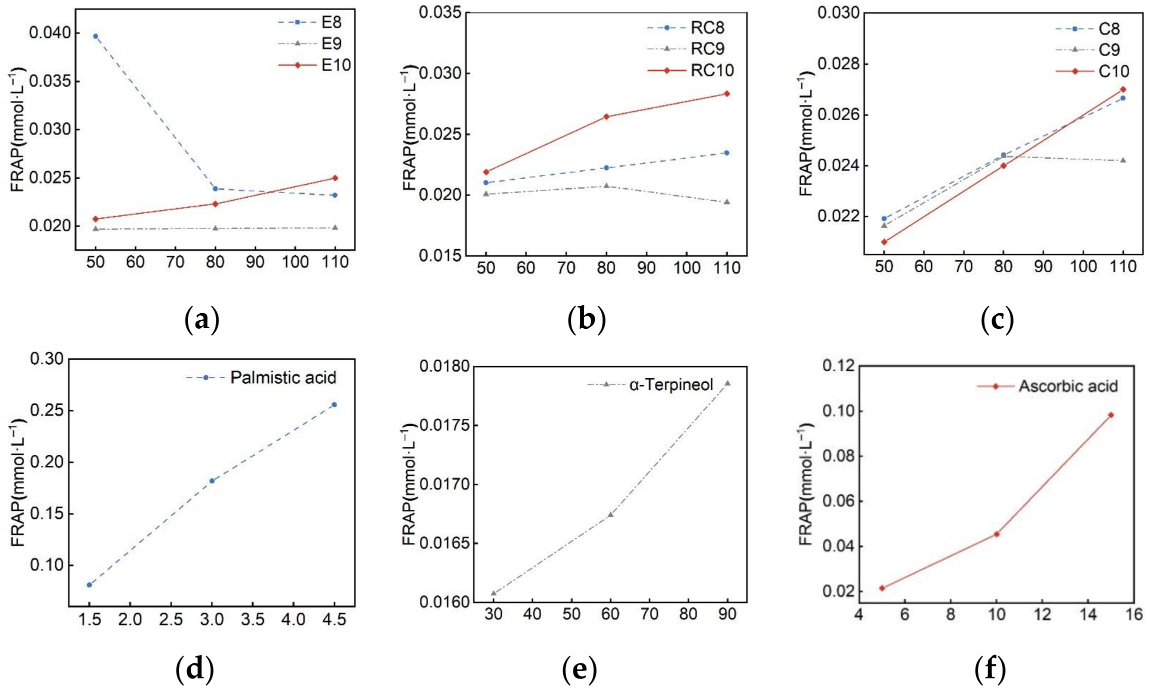


Figure 8. The results of FRAP assay.

Three kinds of EOs and EOs removed crystals present some AAs, respectively, but not so strong compared with that of ascorbic acid. It should be noteworthy that the crystals usually present weaker AAs compared with that of EOs or EOs removed crystals, and sometimes even present pro-oxidant activities. The PA usually presents pro-oxidant activities and in a concentration dependent manner. At the same time, the EOs removed crystals usually present some stronger AAs compared with that of the corresponding EOs.

3. Discussion

The EOs were mainly composed of LCFAs and their ester, which was in agreement with the previous report [11,12]. The crystals were mainly composed of PA, but had relatively higher content compared with that of EOs. The chemicals, which have high boiling point (BP) such as FAs and their ester, lead to the lower extraction rate compared with that of the EOs from other plants, such as *Citrus* L. Twenty-one compounds were first reported from the EOs of *L. rotata*. Seven compounds including *n*-hexadecanoic acid, tetradecanoic acid, linoleic acid, oleic acid, methyl hexadecanoate, hexahydrofarnesyl acetone, and phytol, were identified as the major chemicals in these EOs, which could be chosen as the chemical markers in such EOs. The EOs extracted from *L. rotata* presented some similarities with the EOs extracted from *M. sylvestris* [27], *Cirsium japonicum* var. *ussurience* Kitamura, *Ixeris dentate* and *I. stolonifera* [28,29], since they were all represented with the high BP compounds such as *n*-hexadecanoic acid, hexahydrofarnesyl acetone, *etc.*, as the major components.

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' [23].

As for AAs assays, 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 (ROS) of an antioxidant [30]. It is hardly to get the same result under the "equal condition" since the result was also effected by environmental factors 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 those of the positive reference substance.

FAs may constitute an important strategy for protecting cells against oxidant injury [13]. The oxidant injury can be alternately enhanced or reduced by supplemental FAs, depending on their degree of unsaturation [14]. Some investigators have shown that enrichment with SFAs enhances oxidant injury [16–19]. For example, PA increases oxidative stress in cells in a concentration dependent manner [21,32]. PA can react with cells to generate ROS, reduce the content of NO, and make cells more prone to oxidative stress[32]. Usually, PUFAs can reduce oxidant injury[15–19], since the ROS tend to react with the loosely bound electrons of carbon double bonds found in abundance in the fatty acyl chains of cell membrane lipid bilayers [13,20]. In addition, MUFAs such as *cis*-vaccenic acid (l8:1, n-7) can also decrease the susceptibility of cultured endothelial cells to oxidant injury [13]. It can be deduced from the result in this study that the MUFAs and PUFAs also have AAs.

However, another study showed that SFAs such as octadecanoic acid protected pulmonary artery endothelial cell from oxidant injury, but PUFAs such as linolenic acid (l8:3, n-6) and eicosatrienoic acid (20:3, n-3) enhanced oxidant injury [14].

The effects of FAs on oxidant injury appear to be related to the degree of FAs unsaturation rather than fatty carboxyl chain length or the position of the double bond system[14]. The relationship between the degree of FAs unsaturation and susceptibility to oxidant injury remains controversial [13].

4. Materials and Methods

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 internal transcribed spacer 2 (ITS2) DNA barcodes in our previous study [9], and voucher samples L8, L9, L10 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.

4.1. 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 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.

4.2. 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 (Flame Ionization Detector) 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 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.

4.3. GC Analyses

GC–FID 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.

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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.

4.4. Identification and Quantitation

4.4.1. 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 (t) of the n-alkanes (C_{10}-C_{25}, C_{28}) (t_n, t_{n+1}) and detected compound x (t_x, $t_n \le t_x \le t_{(n+1)}$) by the equation proposed by Van Den Dool and Kratz [33,34].

$$LRI=100n+100[(t_x-t_n)/(t_{(n+1)}-t_n)] \quad (1)$$

The calculated LRIs were compared with the LRIs of the corresponding chemicals provided in the NIST 17 database, literatures, or standards.

4.4.2. Quantitation

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

4.5. Antioxidant activities

4.5.1. DPPH Assay

A slight improvement was made according to the literature method [35]. Samples 100 µL at different concentrations diluted in methanol (MeOH) were placed in a 96-well microplate and then supplemented with 100 µL of DPPH (100 $µmol·L^{-1}$) solution also diluted by MeOH. 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).

4.5.2. ABTS Assay

A slightly modification was made based on the literature description method [36]. The ABTS radical cation ($ABTS^{•+}$) 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) potassium persulfate ($K_2S_2O_8$) aqueous solution, which was kept in darkness at room temperature for 16 h. Then, radical cation was diluted with methanol (about 30-50 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.

4.5.3. FRAP Assay

A slight modification was made based on the literature method [35]. 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 °C 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 0.01-0.2 mM 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

This study first analyzed the chemcials in EOs, crystals separated from the EOs, and EOs removed crystals of *L. rotata* and made an evaluation of the AAs of these samples and the representative compound PA, to the best of our knowledge. The EOs and separated crystals were both mainly composed by PA, whereas the latter had higher content. PA presents pro-oxidant activity in a concertration dependence manner, which is in accordance with the former research [21,32]. FAs have important meaning for keeping the oxidant and anti-oxidant balance in cells. This study can give some hints for the full usage of such EOs abundance in FAs.

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