Chemicals in Essential Oils of *Phlomoides rotata* (Benth. ex Hook. f.) Mathiesen and Their Antioxidant Activities Potential

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Article

Chemicals in Essential Oils of *Phlomoides rotata* (Benth. ex Hook. f.) Mathiesen and Their Antioxidant Activities Potential

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Abstract: Due to the low content, few studies are focused on the essential oils (EOs) of *Phlomoides rotata* (Benth. ex Hook. f.) Mathiesen (syn. *Lamiophlomis rotata* (Benth.) Kudô. This plant has been used to treat rheumatic arthritis and grasserie in China. However, such EOs may have important pharmacological activities such as anti-cancer. To identify the chemical markers (CM) in the EOs and evaluate their antioxidant activities (AAs), we firstly conduct a thoroughly investigation on their chemicals and AAs, to the best of our knowledge. Light yellow EOs with fresh and elegant smell are obtained by hydro-distillation with average yield 0.11% (volume mL/weight g). The crystals are separated from the EOs through cryoprecipitation, respectively. A total of 81 components are qualified and quantified in the EOs, crystals and EOs removed crystals, in which 44 ones are first reported. As for content, the main compounds are long-chain fatty acids (FAs) and their esters. The most abundant one *n*-hexadecanoic acid (palmitic acid, PA) accounting for 43.15-54.8%, 58.49-64.57% and 15.9-41.1%, in the EOs, crystals and EOs removed crystals, respectively. Seven compounds including PA, tetradecanoic acid, linoleic acid, oleic acid, methyl hexadecanoate, hexahydrofarnesyl acetone and phytol can be chosen a the CMs in these EOs. The AAs are evaluated through *in vitro* assays. PA presents pro-oxidant activities in a concentration dependence manner. Usually, the EOs removed crystals demonstrate stronger AAs, and the crystals demonstrate weaker AAs compared with that of the corresponding EOs, which is related to the different content of PA in these samples. This study can give some hints for the utilization of such EOs which are abundant in FAs such as PA.

Keywords: *Phlomoides rotata* (Benth. ex Hook. f.) Mathiesen; *Lamiophlomis rotata* (Benth.) Kudô; essential oils; fatty acids; *n*-hexadecanoic acid (palmitic acid); chemical markers; antioxidant activities

1. Introduction

Phlomoides rotata (Benth. ex Hook. f.) Mathiesen (*P. rotata*, PR), syn. *Lamiophlomis rotata* (Benth.) Kudô, a medicinal herb called "Duyiwei" (*Lamiophlomis* herba) in Chinese, belongs to the *Phlomoides* Moench of Lamiaceae, which grows at the high altitudes in China [1-4]. The entire aerial part of PR, as well as the root and rhizome can be used as medicine. The above-ground parts are used to treat grasserie, fracture, injuries from falls, osteomyelitis, gunshot injury, and edema pain. The function of the underground parts is to increase blood circulation, remove stasis and detumescence, and act as an analgesic [2, 5-7]. The whole plant can also be collected for medicinal use [2, 5-6]. In present, only the aerial part can be used indicated by the Pharmacopoeia of the People's Republic of China (Volume I) 2020 edition [4] and the digging for the root is banned because PR is now listed as a first-class endangered Tibetan medicine[6-7]. The surface of *Lamiophlomis* herba is yellowish-brown or sallow in color, bitter in taste, flat in nature [4-5, 7]. It was first recorded in the classical masterpiece of Tibetan Medicine, Somaratsa [7]. It has been used to treat traumatic injury, rheumatic arthritis and grasserie

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for more than 2000 years in the traditional Tibetan medicine known as "Daba" and "Dabuba" [4-7]. Generally, PR is used directly in the clinic without any prior processing, and commonly for pain relief [6]. Meanwhile, PR is also prescribed as a critical ingredient in combination with other Chinese herbs such as *Curcuma longa*, *Salvia miltiorrhiza* and *Pyrrosia lingua* [6-7]. As an ingredient, PR is used in many health products including health drinks, soap, wine, mouth rinses and biological toothpastes [6].

Due to the low content of volatile components, the researches are mainly focused on the involatile compounds. Some efficacious ingredients such as iridoids, flavonoids and phenylethanoids, have been found [6-7]. At least 223 chemical constituents have been isolated from PR, including iridoids, flavonoids, phenylethanoid glycosides, polysaccharides, organic acids, volatile oils, et al. [6-7]. The main compounds isolated from the aerial regions and rhizomes of PR are iridoid glycosides, which are. responsible for the analgesic effect [6-7], and are used as marker compounds to evaluate the quality of *Lamiophlomis* herba [7]. Meanwhile, fourteen organic acids including *n*-hexadecanoic acid (palmitic acid, PA) have been isolated and identified [6]. On the other hand, the petroleum ether extracted part has been reported to have the anti-tumor activities, which means its essential oils (EOs) may have such activities [8]. Up to now, there is only one paper reported the chemicals in such EOs extracted by steam distillation, and another paper reported the lipophilic composition in the CH_2Cl_2 extracted part, seen in supplemental Table 1, and no-evaluation on their antioxidant activities (AAs), to the best of our knowledge [9-10]. EOs with light yellow color, yields as 0.1% and 0.23% (volume mL/weight g) have been extracted by steam distillation from the above and below the ground components, respectively. A total of 17 components are identified and quantified. As for content, the main compounds are fatty acids (FAs), especially long-chain FAs (LCFAs) such as *n*-hexadecanoic acid (palmitic acid, PA), oleic acid and linoleic acid. The identification of linoleic acid ethyl ester is debatable considering its linear retention index (LRI) value [9]. A total of 67 components are qualified and quantified in the CH_2Cl_2 extracted part of flower, leaf and root of *L. rotata*. The major components are still FAs such as PA and linoleic acid [10]. Only three compounds including tetradecanoic acid, PA and linoleic acid are detected in both studies [9-10].

At the same time, studies on the more in-depth biological effects of volatile oils from PR have been very limited in recent years [7]. There is no evaluation on the AAs of EOs extracted from PR presently. However, the supplemental FAs have important meaning for keeping the balance between oxidation and antioxidation in cells [11-18]. The effects of FAs on oxidant injury appear to be related to the degree of their unsaturation[12]. Saturated fatty acids (SFAs) such as PA can increase oxidative stress in angiogenic mononuclear cells in a concentration dependant manner [19], but stearic acid is reported to protect pulmonary artery endothelial cell from oxidant injury. Usually, polyunsaturated fatty acids (PUFAs) can reduce oxidant injury [13-17], but there also has exception [12].

Until now, no single extract or compound from PR has been clinically applied to cure diseases, to the best of our knowledge. Therefore, it is necessary to study and develop potentially therapeutic extracts or compounds from PR in accordance with the previous studies [7]. Based on the previously study [20], in this study, we focus on the volatile chemicals and their AAs. Considering the complexity of EOs, it is necessary to do some separation work for the further study. As a result, we have first separated the extracted EOs from PR through cryoprecipitation, to the best of our knowledge. The EOs, crystals and EOs removed crystals are gotten, respectively. In order to identify the chemical markers (CMs) such as PA in these EOs and to evaluate their AAs, we have done an exhaustive exploration on the chemicals presented in these EOs, crystals and EOs removed crystals, 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, respectively.

2. Results

2.1. Extraction and separation

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 light yellow EOs with fresh and elegant smell is obtained from L8, L9 and L10, respectively. The average yield (0.11%) is close to the yield (0.1%) reported previously [9]. Crystals are separated from the EOs at 4 or -4 °C, respectively.

2.2. Chemicals in the EOs of L. ratata

In total, 81 compounds are qualified and quantified (Table 1, Figure 1).

Table 1. The compounds qualified and quantified (%) in EOs extracted from the aboveground parts of PR.

No.	Compounds	CAS No.	LRIs[b, d]	LRIs[a]	LRIs[c]	E8	C8	RC8	E9	C9	RC9	E10	C10	RC10
1	Propanoic acid (3:0)	79-09-4	700, 1535	-	1535	nd	0.01	0.10	nd	nd	nd	nd	0.01	nd
2	2-Hexanone	591-78-6	790, 1083	-	-	nd	nd	0.02	nd	0.01	nd	0.03	0.01	0.01
3	Hexanal	66-25-1	800, 1083	-	-	nd	0.07	0.21	nd	0.09	0.14	0.03	0.06	0.12
4	*β*-Pinene	127-91-3	970, 1112	-	1114	0.03	nd	nd	nd	nd	nd	nd	nd	nd
5	1-Octen-3-ol	3391-86-4	980, 1450	980	1454	nd	nd	nd	1.64	0.62	0.99	1.50	0.59	1.00
6	Hexanoic acid (6:0)	142-62-1	990, 1846	-	1838	nd	0.11	0.30	nd	0.18	0.38	0.12	0.18	0.38
7	*p*-Cymene	99-87-6	1011, 1272	-	1272	0.16	0.26	0.08	nd	0.01	0.03	0.11	nd	0.04
8	Limonene	138-86-3	1020, 1200	1026	1203	3.16	2.85	0.69	1.37	0.19	0.49	0.81	0.10	0.27
9	*γ*-Terpinene	99-85-4	1053, 1246	-	1247	0.14	0.16	nd	nd	nd	nd	nd	nd	nd
10	*cis*-Linalool oxide	5989-33-3	1074, 1444	-	1441	nd	nd	nd	nd	0.60	1.28	0.21	0.57	1.39
11	*trans*-Linalool oxide	34995-77-2	1102, 1452	-	1468	nd	nd	0.62	nd	0.47	1.11	0.19	0.60	1.04
12	Linalool	78-70-6	1082; 1547	1098	1552	2.27	0.67	3.58	3.79	1.04	1.88	3.65	1.13	1.88
13	Hotrienol	29957-43-5	1107, 1613	-	1612	nd	nd	nd	0.78	nd	nd	0.41	nd	nd
14	Terpinen-4-ol	562-74-3	1177, 1602	-	1595	0.11	0.03	0.15	0.17	0.04	0.06	0.10	0.02	0.04
15	*trans*-Linalool 3,7-oxide	39028-58-5	1173, 1739	-	1732	nd	0.02	0.07	nd	0.08	0.14	nd	0.07	0.17

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16	*cis*-Linalool 3,7-oxide	14009-71-3	1174, 1751	-	1759	nd	0.04	0.04	nd	0.07	0.16	nd	0.09	0.18
17	Caprylic acid (8: 0)	124-07-2	1180; 2060	-	2053	nd	0.10	0.25	nd	0.08	0.15	0.13	0.11	0.22
18	*α*-Terpineol	98-55-5	1189, 1697	1185	1690	2.76	1.09	4.21	3.69	1.19	1.82	3.12	1.12	1.83
19	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	13741-21-4	1190, 1945	-	1944	nd	0.07	0.18	nd	0.06	0.12	0.06	0.08	0.13
20	Benzoic acid, 4-methyl-, methyl ester	99-75-2	1215, 1740	-	1733	nd	0.02	0.11	nd	0.06	0.20	0.09	0.09	0.09
21	2-Hydroxycineol	18679-48-6	1228, 1845	-	1846	nd	0.23	0.08	nd	0.24	0.42	nd	0.26	0.35
22	*cis*-Geraniol	106-25-2	1228, 1797	-	1797	0.16	nd	0.10	nd	0.02	0.03	0.18	nd	0.02
23	Geraniol	106-24-1	1255, 1847	-	1846	0.53	nd	0.25	0.47	nd	nd	0.45	nd	nd
24	Nonanoic acid (9: 0)	112-05-0	1273, 2171	-	2156	0.07	0.13	0.37	nd	0.09	0.25	0.17	0.21	0.37
25	Geranyl formate	105-86-2	1300, 1695	-	1705	nd	nd	0.23	nd	nd	0.03	nd	nd	nd
26	Tridecane	629-50-5	1300, 1300	-	1300	nd	nd	0.01	nd	nd	nd	nd	nd	nd
27	*n*-Decanoic acid (10: 0)	334-48-5	1350, 2276	-	2262	0.17	0.20	0.42	0.43	0.37	0.74	0.33	0.33	0.61
28	Dehydro-ar-ionene	30364-38-6	1354, 1732	-	1729	0.05	nd	0.10	nd	nd	0.02	0.15	0.02	0.04
29	*trans-β*-Damascenone	23726-93-4	1386, 1823	-	1808	0.36	nd	0.17	0.49	0.02	0.04	0.47	0.02	0.04
30	Tetradecane	629-59-4	1400, 1400	-	1400	nd	nd	0.05	nd	nd	nd	0.02	nd	0.01
31	*β*-Caryophyllene	87-44-5	1419, 1595	-	1583	0.07	0.08	0.08	nd	0.04	0.04	0.13	0.01	0.03
32	Nonanoic acid, 9-oxo-, methyl ester	1931-63-1	1436, -	-	2041	nd	0.05	0.09	nd	0.10	0.17	0.14	0.11	0.21
33	trans-Geranylacetone	3796-70-1	1453, 1859	-	1849	0.11	0.05	0.23	nd	0.05	0.10	0.27	0.08	0.13
34	Undecanoic acid (11:0)	112-37-8	1475, 2400	-	2367	0.06	0.09	0.13	nd	0.23	0.43	0.12	0.16	0.30
35	trans-β-Ionone	79-77-6	1486, 1940	-	1920	0.26	nd	0.15	0.27	0.01	0.03	0.61	0.04	0.04

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36	Pentadecane	629-62-9	1500, 1500	-	1500	0.04	0.03	0.09	nd	0.01	0.05	0.06	0.03	0.07
37	Dodecanoic acid (12:0)	143-07-7	1556, 2498	-	2474	0.78	0.88	1.40	1.02	1.26	2.64	1.12	1.24	1.85
38	Cedrol	77-53-2	1598, 2116	-	2086	0.02	0.05	0.14	nd	0.09	0.18	0.07	0.08	0.10
39	Hexadecane	544-76-3	1600, 1600	-	1600	0.05	0.05	0.09	nd	0.04	0.11	0.06	0.06	0.15
40	Tridecanoic acid (13:0)	638-53-9	1666, 2617	-	2579	0.12	0.25	0.49	nd	nd	nd	nd	nd	nd
41	Heptadecane	629-78-7	1700, 1700	-	1700	0.10	0.10	0.20	nd	0.09	0.22	0.14	0.13	0.27
42	Methyl tetradecanoate	124-10-7	1725, 2005	-	2008	0.08	0.14	0.24	nd	0.13	0.32	0.23	0.20	0.42
43	Tetradecanoic acid (14:0)	544-63-8	1748, 2694	-	2685	3.69	5.36	5.67	2.51	4.10	4.85	2.89	5.10	5.60
44	Octadecane	593-45-3	1800, 1800	-	1800	tr	0.04	nd	nd	nd	0.09	nd	nd	0.15
45	Hexahydrofarnesyl acetone	502-69-2	1842, 2131	1843	2119	1.88	2.54	5.55	1.78	2.15	5.12	2.73	3.32	6.44
46	Pentadecanoic acid (15:0)	1002-84-2	1823, 2822	-	2790	0.50	0.66	0.73	nd	0.46	0.56	0.38	0.62	0.63
47	Diisobutyl phthalate	84-69-5	1870, 2536	-	2521	0.14	0.14	0.27	nd	0.14	0.29	0.17	0.14	0.27
48	Nonadecane	629-92-5	1900, 1900	-	1900	0.06	0.03	0.07	nd	nd	0.03	nd	0.02	0.06
49	Methyl palmitoleate	1120-25-8	1898, 2240	-	2239	0.07	0.10	0.24	nd	0.13	0.30	0.15	0.12	0.22
50	Farnesyl acetone	1117-52-8	1919, 2384	-	2362	0.65	0.09	0.77	nd	0.08	0.12	0.75	0.09	0.18
51	Methyl hexadecanoate	112-39-0	1926, 2208	1924	2214	1.44	1.55	3.69	2.51	2.79	6.45	3.54	3.90	7.58
52	Dibutyl phthalate	84-74-2	1965, 2680	-	2675	nd	0.29	0.70	nd	nd	0.32	0.19	0.21	0.42
53	Isophytol	505-32-8	1948, 2296	-	2290	0.31	0.37	0.90	nd	0.23	0.56	0.39	0.39	0.80
54	9*E*-Hexadecenoic acid (16:1, n-7)	2091-29-4	1942, 2954	-	2935	0.87	0.79	2.38	nd	nd	0.35	0.30	0.25	0.37
55	Palmitoleic acid (16:1, n-7)	373-49-9	1951, 2926	-	2926	1.68	1.39	3.83	nd	0.92	1.17	0.67	0.76	1.04
56	PA (16:0)	21096	1972, 2931	1960	2894	48.55	61.24	15.90	54.80	64.57	32.31	43.15	58.49	41.10

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57	Hexadecanoic acid, ethyl ester	628-97-7	1993, 2251	-	2253	0.04	0.09	0.23	nd	0.10	0.21	0.12	0.13	0.27
58	Eicosane	112-95-8	2000, 2000	-	2000	0.03	0.04	0.07	nd	nd	0.07	nd	0.03	0.08
59	Methyl linoleate	112-63-0	2071, 2482	-	2485	1.96	0.29	2.52	3.96	0.53	0.83	3.97	0.43	0.55
60	Methyl oleate	112-62-9	2091, 2434	-	2439	0.86	0.68	1.90	1.85	1.78	4.36	2.05	2.01	4.03
61	Methyl linolenate	301-00-8	2098, 2571	-	2552	1.83	nd	1.03	2.54	nd	nd	3.11	nd	nd
62	Heneicosane	629-94-7	2100, 2100	-	2100	nd	0.04	0.08	nd	nd	0.08	0.05	0.05	0.12
63	Unknown-1			-	2476	0.16	0.27	2.50	nd	0.15	tr	0.27	0.21	0.48
64	Phytol	150-86-7	2104, 2622	-	2607	5.45	1.43	6.21	1.76	0.62	1.16	4.02	1.28	1.87
65	Methyl stearate	112-61-8	2128, 2418	-	2420	0.22	0.30	0.61	nd	0.27	0.67	0.39	0.38	0.84
66	Linoleic acid (18:2, n-6)	60-33-3	2133, 3164	-	2884	7.90	1.01	8.62	5.60	0.90	1.27	4.71	0.56	nd
67	Oleic acid (18:1, n-9)	112-80-1	2141, 3173	-	2770	2.75	2.81	6.21	2.73	3.09	9.05	3.40	3.86	nd
68	Stearic acid (18:0)	21128	2172, 3136	-	2700	2.43	4.86	0.44	nd	3.58	2.05	1.18	3.63	nd
69	Docosane	629-97-0	2200, 2200	-	2200	0.05	0.09	0.10	nd	nd	0.15	nd	0.07	0.13
70	Phytol acetate	-	-, -	-	2512	0.09	0.16	0.52	nd	nd	0.37	0.16	0.10	0.16
71	Tricosane	638-67-5	2300, 2300	-	2300	0.16	0.20	0.45	nd	0.20	0.54	0.18	0.21	0.37
72	Tetracosane	646-31-1	2400, 2400	-	2400	nd	0.13	0.29	nd	0.11	0.30	nd	0.08	0.17
73	Pentacosane	629-99-2	2500, 2500	-	2500	0.13	0.25	0.50	nd	0.19	0.54	0.13	0.16	nd
74	Methyl 5,6-octadecadienoate	-	-, -	-	2515	0.14	0.08	0.32	0.53	0.85	1.26	0.35	0.37	0.81
75	Hexacosane	630-01-3	2600, 2600	-	2600	0.17	0.10	0.31	nd	0.05	0.12	0.26	0.06	0.14
76	Heptacosane	593-49-7	2700, 2700	-	2700	0.21	0.34	nd	nd	nd	1.35	0.22	0.18	0.40
77	Octacosane	630-02-4	2800, 2800	-	2800	0.25	0.28	nd	nd	nd	0.37	nd	0.12	0.25
78	Unknown-2			-	2817	0.81	0.99	2.69	nd	0.76	1.56	0.72	0.76	1.31

79	Nonacosane	630-03-5	2900, 2900	-	2900	nd	nd	nd	nd	nd	0.96	nd	nd	nd
80	Unknown-3			-	2952	nd	nd	nd	4.02	0.97	0.45	nd	0.81	4.09
81	Unknown-4			-	2975	1.31	1.36	2.73	0.81	1.13	1.68	1.40	1.38	1.70
	Total (81)					98.47	98.22	94.75	99.50	98.42	96.67	97.29	98.06	96.45
	HMs (4)					3.48	3.27	0.77	1.37	0.20	0.53	0.91	0.10	0.31
	AMs (11)					3.08	1.05	5.06	5.21	2.63	5.19	5.25	2.81	5.20
	HSs (1)					0.07	0.08	0.08	0.00	0.04	0.04	0.13	0.01	0.03
	ASs (1)					0.02	0.05	0.14	0.00	0.09	0.18	0.07	0.08	0.10
	ADs (1)					5.45	1.43	6.21	1.76	0.62	1.16	4.02	1.28	1.87
	Aldehydes & ketones (8)					3.26	2.79	7.19	2.53	2.51	5.71	5.02	3.73	7.19
	FAs (16)					69.55	79.90	47.24	67.08	79.81	56.20	58.68	75.51	52.46
	LCFAs (12)					69.49	79.54	46.22	67.08	79.47	55.42	58.26	75.01	51.50
	SCFAs (4)					0.07	0.36	1.02	0.00	0.34	0.79	0.42	0.51	0.97
	SFAs (12)					56.36	73.90	26.20	58.75	74.91	44.35	49.59	70.08	51.05
	MUFAs (3)					5.29	4.99	12.41	2.73	4.01	10.57	4.38	4.87	1.41
	PUFAs (1)					7.90	1.01	8.62	5.60	0.90	1.27	4.71	0.56	0.00
	Esters (15)					6.89	3.89	12.71	11.39	6.88	15.77	14.67	8.20	15.90
	Phthalate (2)					0.14	0.43	0.97	0.00	0.14	0.61	0.36	0.35	0.69
	Esters of FAs (10)					6.65	3.28	10.88	11.39	6.68	14.57	14.06	7.65	14.96
	TOCs (54)					91.33	90.51	83.57	93.30	94.49	87.42	92.57	93.59	86.13
	n-Alkanes (17)					1.26	1.73	2.32	0.00	0.69	4.98	1.14	1.18	2.36
	Unknowns (4)					2.28	2.62	7.92	4.82	3.00	3.69	2.39	3.16	7.58

[1] Note: The data of content are gotten from the Gas Chromatography-Mass Spectrometer (GC-MS) detection using a free fatty acid phase (FFAP) column. E refers to EO, C refers to crystal isolated from the EO and RC refers to EO removed crystal; The numbers 8, 9 and 10 after E, C and RC refer to the voucher number of PR, respectively; un means uncertain; tr (trace) means the content is less than 0.005%. Unknown means the compound can not be elucidated by its mass spectrum. The same for the following Tables. LRIs[a] and LRIs[c] detected by DB-5 and FFAP are gotten in this experiment, respectively. The compounds denoted with red color are also reported in previous literatures [9-10]. The number in bracket means the sum of corresponding compounds.

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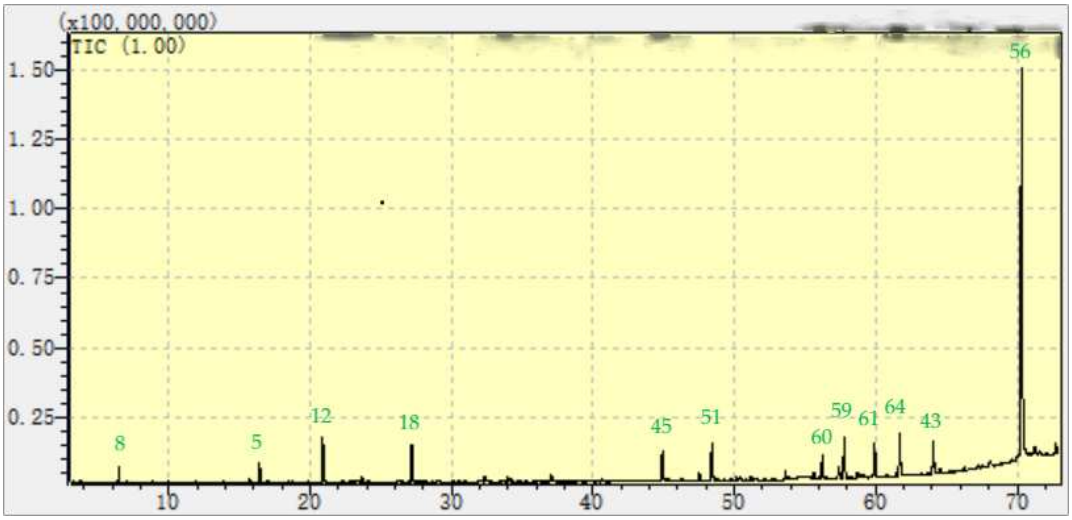

Figure 1. TIC of E10 detected by GC-MS using a FFAP column. Note: Compounds were listed by the corresponding No. in Table 1.

The mass spectra of compounds 66, 67 and 68 were highly similar with those of linoleic acid, oleic acid, and stearic acid, respectively, whereas their LRIs^{c} values of 2884, 2770, and 2700, are significantly different from the corresponding LRIs^{d} values of 3164, 3173, and 3136, respectively. Considering the MS oven temperature program of FFAP, the max calculated LRIs^{c} value is 2984, and the chemicals with LRIs^{d} higher than 2984 such as linoleic acid, oleic acid, and stearic acid, will not be eluted in the employed analytical conditions and will be eluted in the next chromatogram, which will significantly change their LRIs^{c} values. These compounds are not detected in the first detected sample as E8, which also proves this hypothesis. In such a scenario, the compounds 66, 67 and 68 are still identified as linoleic acid, oleic acid and stearic acid, respectively, which are also reported previously [9-10].

In addition, four compounds detected in the total ion chromatograms (TICs), which characteristic ion peaks can be seen in Table 2, can not be elucidated by mass spectra and LRIs values, respectively, based on the NIST 14, 17 or other database [21].

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.

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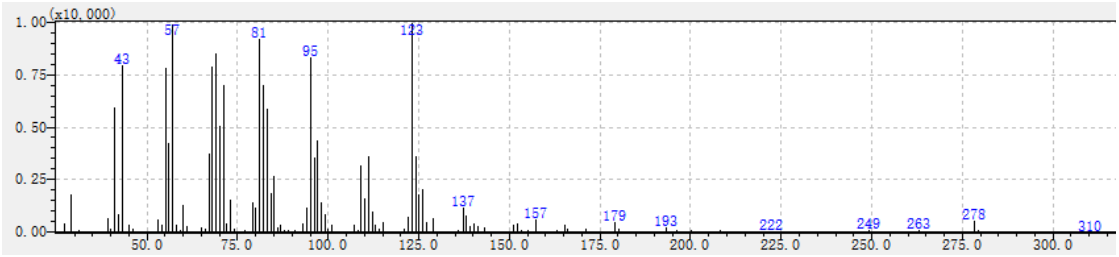

Figure 2. The mass spectrum of unknown-1 from RC8.

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.

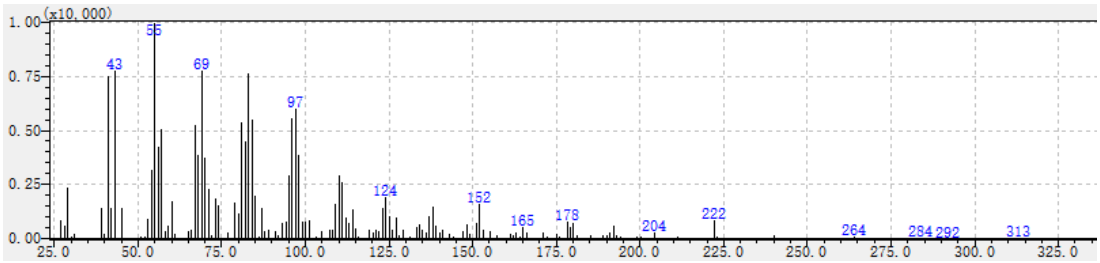

Figure 3. The mass spectrum of unknown-2 from RC9.

The most suitable match for unknown-3 is 1-cyclohexenylacetic acid with 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. Cyclohexenylacetic acid is reported as a compound in the CH_2Cl_2 extract of *L. rotata*, which should be corresponding to unknown-3 in this study [10].

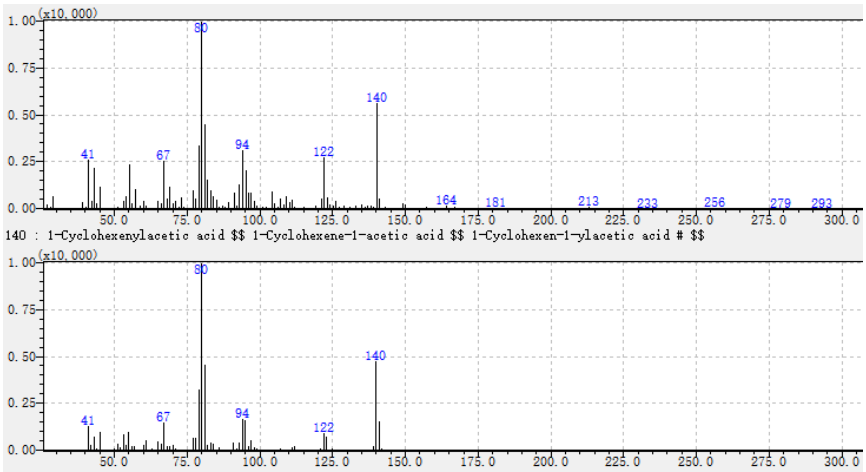

Figure 4. The mass spectra of unknown-3 from RC10 and the corresponding match 1-cyclohexenylacetic acid from NIST 14 library.

The most suitable match of unknown-4 is palmitoleic acid (Figure 5), whereas its LRI[c] 2975 is different from the LRI[d] 2926 of palmitoleic acid to some extent. Meanwhile, palmitoleic acid is 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.

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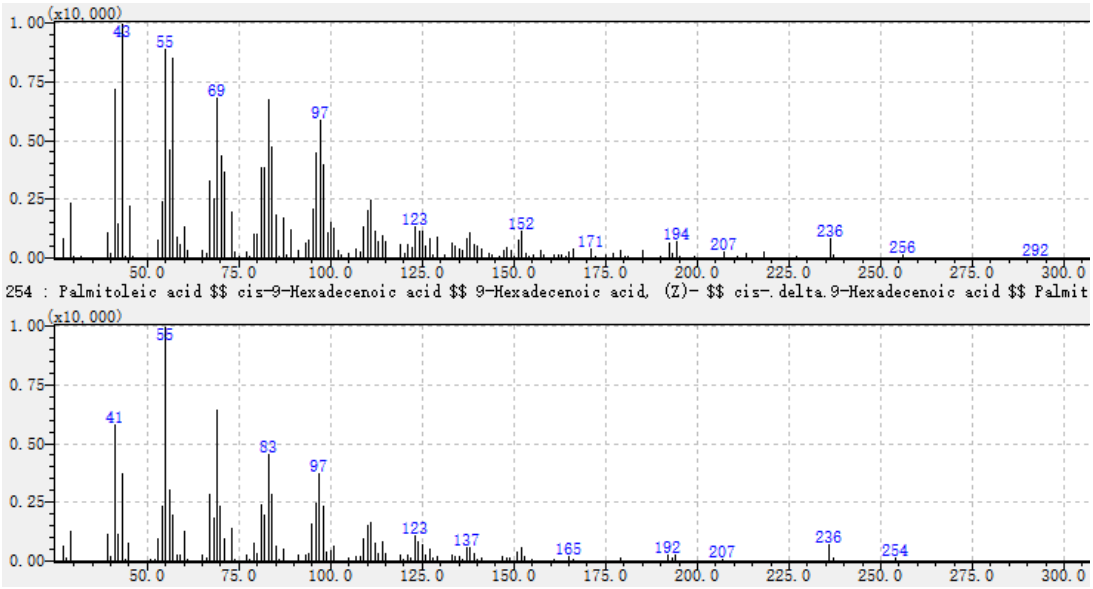

Figure 5. The mass spectra of unknown-4 from C10 and the corresponding match palmitoleic acid from NIST 14 library.

It should be noted that only eight compounds including hexanal, 1-octen-3-ol, limonene, linalool, *α*-terpineol, hexahydrofarnesyl acetone, methyl hexadecanoate and PA are detected by MS using DB-5 because of the low concentration of samples. Among them, the contents of limonene, *α*-terpineol, and PA are relatively high. However, limonene and *α*-terpineol are undetected in previously studies [9-10]. Considering the EOs extracted from the peels of *Citrus reticulata Blanco* such as Nanfengmiju (*C. kinokuni* Hort. ex Tanaka) and *C. reticulata* 'Dahongpao' are also studied at the same time, their has the possibility that these compounds are introduced from such EOs which are abundant in limonene and *α*-terpineol [22]. In such scenario, the quantitation results are based on the data gotten from MS detected with FFAP column.

The 9-hexadecenoic acid reported previously [9] is most probably corresponding to 9*E*-hexadecenoic acid detected in this study based on their LRIs values. As a result, fourty-four are first reported from the EOs of *L. rotata*.

The EOs, crystals and EOs removed crystals are mainly consisted of FAs, especially LCFAs. PA is the most outstanding one, which is in line with the reported results [9-10]. Then, tetradecanoic acid, oleic acid and linoleic acid are also prominent, which are also reported previously [9-10]. The content of PA is relatively higher in crystals, but relatively lower in EOs removed crystals compared with that in the corresponding EOs.

As for esters of FAs, the major compounds are methyl hexadecanoate and methyl linolenate [10]. Among the aldehydes & ketones, hexahydrofarnesyl acetone is prominent. Tricosane and pentacosane are two highlight *n*-alkanes. As for AMs, linalool and *α*-terpineol are prominent. Phytol as a major compound is the only one of ADs.

2.3. AAs of EOs, crystals, EOs removed crystals and PA

The deduced IC_{50} (inhibitory concentration of 50% radical scavenging activity (RSA)) of each sample (the IC_{50} of reference substance ascorbic acid is detected) through DPPH and ABTS, and Ferric reducing ability through 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·mL^{-1})		Ferric reducing ability (mmol·L^{-1})
	DPPH	ABTS	

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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
PA	ND	ND	0.25
Ascorbic acid	0.0077	0.013	0.098

The results of DPPH assay show that RC8 and RC10 present some stronger RSA compared with the other samples. The PA even demonstrates pro-oxidation activity in a concentration dependent manner. The antagonistic effect between RC10 and C10 should be noted. Interestingly, the RSA of C8, C9 and C10 is minimum at the concentration 80 $\mu g \cdot mL^{-1}$, respectively, which indicates that some compounds in the crystals acting as pro-oxidation function may reach the effective concentration.

The RSA values of the samples detected by ABTS demonstrate that the crystals may contain more substances to promote oxidation. After removing the crystals, the RSA values are increased some. The highest RSA value is 23.89% still from RC10 at 110 $\mu g \cdot mL^{-1}$. It is worth noting that most of the samples showed better RSA values compared with those detected by the DPPH assay, which should be due to the higher reactivities of ABTS radical cations [23]. The RSA values of PA are negative.

The FRAP values of the samples are nearly the same as that of ascorbic acid at 5 $\mu g \cdot mL^{-1}$, which indicates that the tested samples have partial electron transfer ability. Interestingly, the PA has a larger value compared with that of ascorbic acid. However, the mixture solution of PA and FRAP working solution is milky white turbid liquid, and there is no dark blue unique to ferrous ions. Since the FRAP working solution is mainly composed of pure water, and the solubility of PA in water is relatively less, the partial precipitation would be resulted.

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-oxidation activities. The PA usually presents pro-oxidation 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 are mainly composed of LCFAs, which was in agreement with the previous reports [9-10]. The crystals and EOs removed crystals are also mainly composed of LCFAs. The crystals have relatively higher content of PA, while the EOs removed crystals have relatively lower content of PA, compared with that of EOs. Pentadecanoic acid, the only one FAs with odd carbons, is reported to have anti-tumor activities [24]. 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. As for HMs, only α-pinene is reported in flower and leaf of PR in previously researches [10]; As for AMs, only linalool is reported in leaf of PR previously [10]. The 1-octene-3-ol is reported to have a typical mushroom flavor [25].

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Seven compounds including PA, tetradecanoic acid, linoleic acid, oleic acid, methyl hexadecanoate, hexahydrofarnesyl acetone and phytol, are identified as the major chemicals according to content, which can be chosen as the CMs in these EOs. Compared with previously studies [9-10], methyl hexadecanoate, hexahydrofarnesyl acetone and phytol, are three new CMs.

The EOs extracted from PR present some similarities with the EOs extracted from *M. sylvestris* [26], *Cirsium japonicum* var. *ussurience* Kitamura, *Ixeris dentate* and *I. stolonifera* [27-28], because they are all represented with the high BP compounds such as PA and hexahydrofarnesyl acetone as the major components.

As for AAs assays, small differences in the experiment process may lead to large differences in results. The results are closely related to the environment such as the ratio of working solution to sample solution, the concentration of the samples, the intrinsic reactivity to free radicals and other reactive oxygen species (ROS) of an antioxidant, climate and temperature [29]. It is hardly to get the same result under the "equal condition". 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.

Previously studies also demonstrate that PA has pro-oxidation activity. For example, PA increases oxidative stress in cells in a concentration dependent manner [19, 30], because it can react with cells to generate ROS, reduce the content of NO, and make cells more prone to oxidative stress[30].

The crystals usually present weaker AAs, whereas, the EOs removed crystals present stronger AAs, compared with those of corresponding EOs. It should be related to the different content of PA in these samples,

FAs may constitute an important strategy for protecting cells against oxidant injury [11]. The oxidant injury can be alternately enhanced or reduced by supplemental FAs, depending on the degree of unsaturation rather than the fatty carboxyl chain length or position of the double bond systems [12]. Some investigators have shown that enrichment with SFAs such as PA enhances oxidant injury [14-17, 19, 30]. Usually, PUFAs can reduce oxidant injury [13-17], 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 [11, 18]. From this study, we can deduce that the MUFAs and PUFAs have AAs. However, another study shows that SFAs such as stearic 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 [12]. The relationship between the degree of unsaturation and susceptibility to oxidant injury remains controversial [11].

4. Materials and Methods

4.1. Plant Materials, Reagents and Chemicals

The information of three populations of the aboveground portion of *L. rotata*, named L8, L9 and L10, which were corresponding to the same No. samples in previous research [20], were presented in Table 4. 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 previous study [20]. The voucher samples L8, L9 and L10 were deposited in the college of ethnic medicine (CUTCM, Chengdu, China) and the Chongqing academy of Chinese materia medica (Chongqing, China).

Table 4. The origins of the materials and GenBank accession numbers of ITS2 sequences [8].

Voucher	Sources	GPS Coordinates	GenBank Accession Number
L8 L9 L10	BianBa, LeiWuQi and NaQu counties of Tibet	E: 93° W: 31°	KP699743/45-4750-51/54

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*-

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Limonene (96%) was produced by Acros organics, USA. γ-Terpinene (97%) was produced by Wako pure chemical industries, Ltd., Japan. PA 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 wighed powders 315 g of L8, L9 and L10 was swollen with 3150 mL of pure water (10 volumes) in a round-bottomed flask, respectively. Then, they 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 by anhydrous Na_2SO_4.

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

4.3. Sample Preparation

The samples of E8, E9, E10, C8, C9, C10, RC8, RC9 and 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 a 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 standard of PA, were diluted in methanol (MeOH) 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, RC8, RC9 and RC10, were diluted in MeOH to the concentrations such as 50, 80 and 110 μg·mL^{-1} for DPPH, ABTS and FRAP detection, respectively. The PA was diluted in MeOH to the concentrations such as 1.5, 3 and 4.5 mg·mL^{-1} for DPPH, ABTS and FRAP detection, respectively. The ascorbic acid as a positive reference substance was diluted in MeOH to the concentrations such as 5, 10 and 15 μg·mL^{-1} for DPPH, ABTS and FRAP detection, respectively.

4.4. 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, respectively. The splitting ratio was 5: 1. The injection volume was 1 μL.

GC–MS analyses were carried out by a GCMS-TQ8040 (Shimadzu, Japan) matched with a NIST 14 MS database and a DB-5 column or a FFAP column. 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 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

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The peaks in the TICs obtained by GC-MS were identified by probability-based matching 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 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} and the detected C_{26}-C_{27}, C_{29}) (t_n, t_{n+1}) and detected compound x (t_x, $t_n \leq t_x \leq t_{(n+1)}$) by the equation proposed by Van Den Dool and Kratz [31-32].

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

The calculated LRI was compared with the $LRI^{b,d}$ of the corresponding chemical.

4.5.2. Quantitation

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

4.6. AAs

Eleven samples including E8, E9, E10, C8, C9, C10, RC8, RC9, RC10, PA and ascorbic acid were tested the AAs. In beginning, these samples diluted in MeOH in three different concentrations such as 5, 15 and 25 μg·mL^{-1} were tested the DPPH free radical scavenging ability, respectively. However, the results demonstrated that the clearance rates of these samples except ascorbic acid were minute. Following, their concentrations were increased and the volumes of DPPH, ABTS and FRAP working solution were reduced, respectively. Nine samples including E8, E9, E10, C8, C9, C10, RC8, RC9 and RC10 were tested at 50, 80 and 110 μg·mL^{-1}, respectively. PA was tested at 1.5, 3 and 4.5 mg·mL^{-1}, respectively.

4.6.1. DPPH Assay

A slight improvement was made according to the literature method [33]. The sample 100 μL at different concentrations was placed in a 96-well microplate and then supplemented with 100 μL DPPH (100 μmol·L^{-1}) solution also diluted by MeOH. After incubation for 30 min in darkness at room temperature, the absorbance was measured at 517 nm using a microplate reader. Each sample was set up 3 holes. MeOH was served as the blank control. RSA was calculated by the following equation:

$$RSA\ (\%)=[(A_{Blank}-A_{Sample})/A_{Blank}]*100\% \quad (2)$$

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 substance.

4.6.2. ABTS Assay

A slightly modification was made based on the previously method [34]. 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 MeOH (about 30-50 times) to absorbance value as 0.7±0.02 at 734 nm. Each sample 100 μl was added to 100 μl of ABTS radical solution, which was mixed totally at room temperature for 6 min. Then, the absorbance at 734 nm was measured by a microplate reader. The calculation method for RSA was consistent with that in DPPH assay.

4.6.3. FRAP Assay

A slight modification was made based on the literature method [33]. Each sample 100 μl was added to 100 μl of FRAP working solution, which was consisted of acetic acid buffer (0.3 mol·L^{-1}), TPTZ (2, 4, 6-Tris (2-pyridyl)-1, 3, 5-triazine) solution (10 mM) and $FeCl_3$ (20 mM) solution at a volume ratio of 10: 1: 1. The mixture was left in darkness at 37 °C for 30 min. Then, it was immediately placed in a microplate reader to measure the increase of absorbance value at 593 nm.

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A calibration curve was found through mixing the obtained 0.1 ml Fe(II) aqueous solutions in the 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 ($mmol \cdot L^{-1}$) of $FeSO_4$ was calculated by the absorbance value demonstrated in the standard curve after reaction, which was denoted as the value of FRAP. The higher FRAP value means the stronger AAs.

5. Conclusions

Forty-four chemicals are first reported from the EOs of PR. As for content, seven compounds including PA, tetradecanoic acid, linoleic acid, oleic acid, methyl hexadecanoate, hexahydrofarnesyl acetone and phytol can be chosen as the CMs in these EOs. The most outstanding PA presents higher content in crystals but lower content in EOs removed crystals compared with that in EOs. PA presents pro-oxidation activity in a concertration dependence manner. Usually, the EOs removed crystals demonstrate stronger AAs and the crystals dmonstrate weaker AAs compared with that of EOs, which is related to the different content of PA in these samples. This study advances the study in the EOs of PR and can give some hints for the utilization of such EOs which are abundant in FAs.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mathiesen, C.; Scheen, A.C.; Lindqvist, C. Phylogeny and biogeography of the lamioid genus *Phlomis* (Lamiaceae). *Kew Bull.* **2011**, *66*, 83–99.
2. The editorial board of Flora of China of Chinese Academy of Sciences. *Flora of China* (in Chinese, Volume 65 issue 2). Science press; Beijing, China, 1977; p. 1, 480.
3. Li, H.; Hedge, I.C. *Flora of China* (*Lamiaceae*) (Volume 17). Science press; Beijing, China, 1994; p. 50, 52, 156-157.
4. Pharmacopoeia committee of the People's Republic of China. *Pharmacopoeia of the People's Republic of China* (Volume I). China Medical Science and Technology Press; Beijing, China, 2020; p. 274.
5. Nanjing University of Chinese medicine. *The dictionary of Chinese materia medica* (Volume 2) - 2nd edition. Shanghai scientific and technical publishers, China. 2006; p. 2390-2391.
6. Cui, Z.H.; Qin, S.S.; Qin, E.H.; Qin, C.; Gao, L.; Li, Q.C.; Wang, Y.L.; Huang, X.Z.; Zhang, Z.Y.; Li, M.H. Traditional uses, phytochemistry, pharmacology and toxicology of *Lamiophlomis rotata* (Benth.) Kudo: a review. *RSC Adv.* **2020**, *10*, 11463.
7. Li, Y.; Li, F.; Zheng, T.T.; Shi, L.; Zhang, Z.G.; Niu, T.M.; Wang, Q.Y.; Zhao, D.S.; Li, W.; Zhao, P. Lamiophlomis herba: A comprehensive overview of its chemical constituents, pharmacology, clinical applications, and quality control. *Biomed. Pharmacother.* **2021**, *144*, 112299.
8. Jia, Z.P.; Li, M.X.; Zhang, R.X.; Wang, J.H.; Wang, M.; Guo, X.N.; Shen, T. Vitro screening of the effective antitumor components of Herba *Lamiophlomis rotata*. *Med. J. Nation. Defend Force Northwest Chin.* **2005**, *26*, 173-175.
9. Liu, H.F.; Li, X.; Deng, Y.; Song, X.; Li, H. Study on the chemical constituents of the essential oil from *Lamiophlomis rotata*. *Chin. J. Pharm. Anal.* **2006**, *26*, 1794-1796.
10. Liu, J.; Nan, P.; Wang, L.; Wang, Q.; Tsering, T.; Zhong, Y. Chemical variation in lipophilic composition of *Lamiophlomis rotata* from the Qinghai-Tibetan plateau. *Chem. Nat. Compd.* **2006**, *42*, 525-528.
11. Hart, C.M.; Tolson, J.K.; Block, E.R. Fatty acid supplementation protects pulmonary artery endothelial cells from oxidant injury. *Am. J. Respir. Cell Mol. BioI.* **1990**, *3*, 479-489.
12. Hart, C.M.; Tolson, J.K.; Block, E.R. Supplemental fatty acids alter lipid peroxidation and oxidant injury in endothelial cells. *Am. J. Physiol.* **1991**, *260*, L481-L488.
13. Dormandy, T.L. Biological rancidification. *Lancet* **1969**, *2*, 684-688.
14. Kehrer, J.P.; Autor A.P. The effect of dietary fatty acids on the composition of adult rat lung lipids: relationship to oxygen toxicity. *Toxicol. Appl. Pharmacol.* **1978**, *44*, 423-430.
15. Kennedy, J.I.; Chandler, D.B.; Fulmer, J.D.; Wert, M.B.; Grizzle, W.E. Dietary fish oil inhibits bleomycin-induced pulmonary fibrosis in the rat. *Exp.Lung Res.* **1989**, *15*, 315-329.
16. Sosenko, I.R.S.; Innis, S.M.; Frank, L. Polyunsaturated fatty acids and protection of newborn rats from oxygen toxicity. *J. Pediatr.* **1988**, *112*, 630-637.
17. Sosenko, I.R.S.; Innis, S.M.; Frank, L. Menhaden fish oil, n-3 polyunsaturated fatty acids, and protection of newborn rats from oxygen toxicity. *Pediatr. Res.* **1989**, *25*, 399-404.
18. Horton, A.A.; Fairhurst S. Lipid peroxidation and mechanisms of toxicity. *CRC Crit. Rev. Toxicol.* **1987**, *18*, 27-79.
19. Favre, J.; Yıldırım, C.; Leyen, T.A.; Chen, W.J.Y.; Genugten, R.E.; Golen, L.W.; Garcia-Vallejo, J.J.; Musters, R.; Baggen, J.; Fontijn, R.; Pouw Kraan, T.; Serné, E.; Koolwijk, P.; Diamant, M.; Horrevoets, A.J.G. Palmitic acid increases pro-oxidant adaptor protein p66Shc expression and affects vascularization factors in angiogenic mononuclear cells: Action of resveratrol. *Vasc. Pharmacol.* **2015**, *75*, 7-18.
20. Wang, J.; Gao, Y.L.; Chen, Y.L.; Chen, Y.W.; Zhang, Y.; Xiang, L.; Pan, Z. *Lamiophlomis rotata* identifification via ITS2 barcode and quality evaluation by UPLC-QTOF-MS couple with multivariate analyses. *Molecules* **2018**, *23*, 3289.
21. Adams, R.P. *Identification of essential oil components by gas chromatography/mass spectrometry*, ed. 4.1. Allured publishing; Illinois, America, 2017; p. 1-804.
22. Wang, J. Alkanes and chemical markers identified in the essential oil from pericarp of Nanfengmiju (*Citrus kinokuni* Hort. ex Tanaka). *J. Mex. Chem. Soc.* **2023**, *67*, 82-93.
23. Teow, C.C.; Truong, V.D.; Mcfeeters, R.F.; Thompson, R.L.; Pecota, K.V.; Yencho, G.C. Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. *Food Chem.* **2007**, *103*, 829-838.
24. Feng, X.Q. Fatty acids and volatile compounds of meat from *Cervus elaphus* subspecies hybrid offspring. Gansu agricultural university, Gansu, China, 2008.

25. Zhao, Q.Y.; Yousaf, L.; Xue, Y.; Shen, Q. Changes in flavor of fragrant rice during storage under different conditions. *J. Sci. Food Agric.* **2020**, *100*, 3435-3444.
26. Usami, A.; Kashima, Y.; Marumoto, S.; Miyazawa, M. Characterization of aroma-active compounds in dry flower of *Malva sylvestris* L. by GC-MS-O analysis and OAV calculations. *J. Oleo Sci.* **2013**, *62*, 563-570.
27. Choi, H.S. GC-MS analyses of the essential oils from *Ixeris dentate* (Thunb.) Nakai and *I. stolonifera* A. Gray. *Korean J. Food Nutr.* **2012**, *25*, 274-283.
28. Choi, H.S. Chemical composition of *Cirsium japonicum* var. *ussurience* Kitamura and the quantitative changes of major compounds by the harvesting season. *Korean J. Food Nutr.* **2016**, *29*, 327-334.
29. Munteanu, I.G.; Apetrei, C. Analytical methods used in determining antioxidant activity: A review. *Int. J. Mol. Sci.* **2021**, *22*, 3380.
30. Ke, J.; Wei, R.; Liu, Y. Metformin combined with liraglutide has a synergistic protective effect on palmitic acid-induced oxidative damage of endothelial cells. *Chin. J. Diabetes Mellitus* **2014**, *6*, 312-316.
31. Van Den Dool, H.; Kratz, P.D. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463-471.
32. Zhao, C.; Liang, Y.; Hu, Q.; Zhang, T. Review on gas chromatographic retention index. *Chinese J. Anal. Chem.* **2005**, *33*, 715-721.
33. Zengin, G.; Sarikurkcu, C.; Uyar, P.; *et al.* ,. Aktumsek, A.; Uysal, S.; Kocak, M.S.; Ceylan, R. *Crepis foetida* L. subsp. *rhoeadifolia* (Bieb.) Celak. as a source of multifunctional agents: Cytotoxic and phytochemical evaluation. *J. Funct. Foods* **2015**, *17*, 698-708.
34. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio. Med.* **1999**, *26*, 1231-1237.