Article

Not peer-reviewed version

Characteristic Volatile Compounds of *Phlomoides rotata* (Benth. ex Hook. f.) Mathiesen and Their Potential Anti-oxidant ActivitiesCitation: To be added by editorial staff during production

Zheng Pan , Chen Xie , Xiao Tong Yan , Yong Mei Su , Anwar Ul Haq , Jian Wang *

Posted Date: 31 October 2023

doi: 10.20944/preprints202310.1959.v1

Keywords: Phlomoides rotata (Benth. ex Hook. f.) Mathiesen (Lamiophlomis rotata (Benth.) Kudô); essential oils; chemical markers; fatty acids; palmitic acid; C13-norisoprenoids; trans-β-damascenone; anti-oxidant activities; pro-oxidant activities

Article

Characteristic Volatile Compounds of *Phlomoides rotata* (Benth. ex Hook. f.) Mathiesen and Their Potential Anti-Oxidant Activities

Zheng Pan [2,3], Chen Xie [2,3], Xiaotong Yan [2,3], Yongmei Su [2,3], Anwar Ul Haq [4] and Jian Wang [1,2,3,*]

[1] Department of Chinese Materia Medica, Chongqing College of Traditional Chinese Medicine, Chongqing 402760, China
[2] College of Chinese Medicine, Chongqing medical university, Chongqing 400016, China
[3] Chongqing Key Laboratory of Chinese Medicine for Prevention and Cure of Metabolic Diseases, Chongqing Medical University, Chongqing 400016, China
[4] Department of Pharmacy, Shaheed Benazir Bhutto University, Sheringal Dir (Upper) Khyber Pakhtunkhwa 18000, Pakistan
* Correspondence: wj_2000_abc@cqmu.edu.cn

Abstract: Due to the low content, few studies are focused on the volatile compounds of *Phlomoides rotata* (Benth. ex Hook. f.) Mathiesen (PR, syn. *Lamiophlomis rotata* (Benth.) Kudô). However, such volatile compounds may have important pharmacological activities such as antioxidant, anticancer. In order to identify the chemical markers (CMs) in the essential oils (Es) and explore the potential bioactivities, we first conduct a thoroughly investigation on the volatile compounds and evaluate their anti-oxidant activities (AOAs), to the best of our knowledge. Light yellow Es with a fresh and elegant smell are obtained by hydro-distillation with an average yield of 0.11% (mL/g). After separation through cryoprecipitation, the crystals (Cs) and EOs removed crystals (RCs) were gotten, respectively. A total of 81 components are qualified and quantified in the Es, Cs and RCs, in which 44 ones are first reported from PR. As for content, the main compounds are long-chain fatty acids (LCFAs) and their esters. Among them, palmitic acid (PA), myristic acid (MA), linoleic acid (LA), oleic acid (OA) and methyl palmitate are highlighted, Besides, hexahydrofarnesyl acetone and phytol are also prominent Four kinds of C_{13}-norisoprenoids are first reported from PR, in which, *trans*-β-damascenone is potent in flavour. In sum, these eight representative compounds can be chosen as the CMs. The AOAs of Es, Cs, RCs and four CMs such as PA, MA, LA and OA are evaluated by *in vitro* assays. In general, PA and MA present pro-oxidant activities (POAs) or weak AOAs. LA and OA show POAs or weak AOAs in lower concentration group, whereas demonstrate medium or strong AOAs in higher concentration group. Usually, the RCs demonstrate stronger AOAs and the Cs present weaker AOAs compared with those of the corresponding Es, which should be related to their different content of PA. This study can give some hints on the utilization of such Es which are abundant in LCFAs.

Keywords: *Phlomoides rotata* (Benth. ex Hook. f.) Mathiesen (*Lamiophlomis rotata* (Benth.) Kudô); essential oils; chemical markers; fatty acids; palmitic acid; C_{13}-norisoprenoids; *trans*-β-damascenone; anti-oxidant activities; pro-oxidant activities

1. Introduction

Phlomoides rotata (Benth. ex Hook. f.) Mathiesen (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]. Traditionally, the root and rhizome or the whole herb are used as medicine [5,6]. Nowadays, only the aerial parts are used [4] and the digging for the root is banned because PR is now listed as a first-class endangered Tibetan medicine [6,7]. The function of the underground parts is to increase blood circulation, remove stasis and detumescence, and act as an analgesic. The above-ground parts are used to treat grasserie, fracture, injuries from falls, osteomyelitis, gunshot injury, and edema pain. [2,5–7]. The *Lamiophlomis* herba is yellowish-brown or sallow, bitter and

 doi:10.20944/preprints202310.1959.v1

flat in nature [4,5,7]. It was first recorded in the classical masterpiece of Tibetan Medicine, Somaratsa [7] that has been used to treat traumatic injury, rheumatic arthritis (RA) and grasserie 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 chemicals, the researches are mainly focused on the involatile compounds. 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 are iridoid glycosides, which are. responsible for the analgesic effect [6,7], and are used as chemical markers (CMs) to evaluate the quality of Duyiwei [7]. The C_{13}-norisoprenoids are usually neglected, in which only 5*β*, 6*α*-dihydroxy-3*β*-(*β*-D-glucopyranosyloxy)-7-megastigmen-9-one was reported previously, to the best of our knowledge [8]. Meanwhile, fourteen organic acids including palmitic acid (PA) have been isolated and identified [6]. Up to now, there is only one paper reported the chemicals in essential oils (Es) extracted by steam distillation from PR, and another paper reported the lipophilic composition in the CH_2Cl_2 extracted part, seen in supplemental Table 1, to the best of our knowledge [9,10]. In terms of content, the main compounds are fatty acids (FAs), especially long-chain FAs (LCFAs) such as palmitic acid (PA), myristic acid (MA), oleic acid (OA) and linoleic acid (LA). Some identifications are debatable such as linoleic acid ethyl ester, 2-butyl-1-octanol, cyclohexenylacetic acid, *etc.*, considering the values of linear retention indices (LRIs) or the mass spectra [9,10]. Only three compounds including MA, PA and LA are detected in both studies [9,10]. Usually, there are hundreds of compounds in EOs from plants. The selection of chemical markers (CMs) from the EOs is important since these CMs can differentiate such EOs from others quickly and set up the corresponding chemotype [11,12].

Meanwhile, studies on the more in-depth biological effects of volatile oils from PR have been very limited in recent years [7]. Whereas, the petroleum ether extracted part has been reported to have the anti-tumor activities, which means the volatile compounds may have such activities [13]. By now, there is no test on the anti-oxidant activities (AOAs) of volatile chemicals extracted from PR. The oxidative stress has been linked to many diseases such as RA, cancer and diabetes [14,15]. Moreover, the supplemental FAs have important meaning for keeping the balance between oxidation and anti-oxidation in cells [16–23]. The effects of FAs on oxidant injury appear to be related to the degree of their unsaturation [17]. PA, a kind of saturated FAs (SFAs), can increase oxidative stress in cells in a concentration dependent manner [22,23], because it can induce over-expression of the pro-oxidant protein p66Shc [22] or react with cells to generate reactive oxygen species (ROS), reduce the content of NO and make cells more prone to oxidative stress [23]. However, stearic acid as another kind of SFAs is reported to protect pulmonary artery endothelial cell from oxidant injury [17]. Generally, polyunsaturated FAs (PUFAs) can reduce oxidant injury [18–21], but there also has contradicted results [17].

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 [7]. Based on the previous study [24], we now focus on the volatile chemicals and their AAs. Considering the complexity of EOs, it is necessary to do some separation for the further study [11]. As a result, crystals were isolated from the EOs of PR through cryoprecipitation.

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 (%, mL/g) of the light yellow Es with fresh and elegant smell is obtained from of PR with Voucher No. as 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 Es of PR

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

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

No.	Compounds	CAS#	Linear retention indices (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	0.1	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
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

doi:10.20944/preprints202310.1959.v1

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
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	MA (14:0)	544-63-8	1748, 2694	-	2685	3.69	5.36	5.67	2.51	4.1	4.85	2.89	5.1	5.6
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	0.5	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	0.1	0.24	nd	0.13	0.3	0.15	0.12	0.22

doi:10.20944/preprints202310.1959.v1

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 palmitate	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	0.7	nd	nd	0.32	0.19	0.21	0.42
53	Isophytol	505-32-8	1948, 2296	-	2290	0.31	0.37	0.9	nd	0.23	0.56	0.39	0.39	0.8
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	0.3	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	15.9	54.8	64.57	32.31	43.15	58.49	41.1
57	Ethyl palmitate	628-97-7	1993, 2251	-	2253	0.04	0.09	0.23	nd	0.1	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	1.9	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

doi:10.20944/preprints202310.1959.v1

66	LA (18:2, n-6)	60-33-3	2133, 3164	-	2884	7.9	1.01	8.62	5.6	0.9	1.27	4.71	0.56	nd
67	OA (18:1, n-9)	112-80-1	2141, 3173	-	2770	2.75	2.81	6.21	2.73	3.09	9.05	3.4	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	0.1	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	0.1	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	0.3	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	0.1	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	0.4
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	1.4	1.38	1.7
	Total (81)					98.47	98.22	94.75	99.5	98.42	96.67	97.29	98.06	96.45
	Hydrocarbon monoterpenes (HMs) (4)					3.48	3.27	0.77	1.37	0.20	0.53	0.91	0.10	0.31
	Alcohol monoterpenes (AMs) (11)					3.08	1.05	5.06	5.21	2.63	5.19	5.25	2.81	5.20
	Hydrocarbon sesquiterpenes (HSs) (1)					0.07	0.08	0.08	0.00	0.04	0.04	0.13	0.01	0.03
	Alcohol sesquiterpenes (ASs) (1)					0.02	0.05	0.14	0.00	0.09	0.18	0.07	0.08	0.10
	Alcohol diterpenes (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
FAs with odd carbons (5)	0.74	1.14	1.82	0	0.78	1.23	0.67	1.00	1.30
FAs with even carbons (11)	68.81	78.75	45.42	67.08	79.04	54.97	58.01	74.52	51.17
LCFAs (9)	68.48	78.37	44.27	65.63	77.61	51.61	56.69	73.28	48.74
Medium-chain FAs (MCFAs) (5)	1.07	1.40	2.57	1.44	2.03	4.21	1.87	2.04	3.35
Short-chain FAs (SCFAs) (2)	0	0.12	0.4	0	0.18	0.38	0.12	0.19	0.38
SFAs (12)	56.36	73.90	26.20	58.75	74.91	44.35	49.59	70.08	51.05
Monounsaturated FAs (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
Phthalates (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
Total oxygenated compounds (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
C_{13}-Norisoprenoids (4)	0.79	0.05	0.64	0.76	0.08	0.18	1.50	0.15	0.26
Unknowns (4)	2.28	2.62	7.92	4.82	3.00	3.69	2.39	3.16	7.58

Note: The data of content are gotten from the Gas Chromatography-Mass Spectrometer (GC-MS) using a free FA phase (FFAP) column. The numbers 8, 9 and 10 after E, C and RC refer to the corresponding Voucher No. of PR, respectively; 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 $LRIs^{c}$ are gotten by DB-5 and FFAP 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.

doi:10.20944/preprints202310.1959.v1

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 October 2023 doi:10.20944/preprints202310.1959.v1

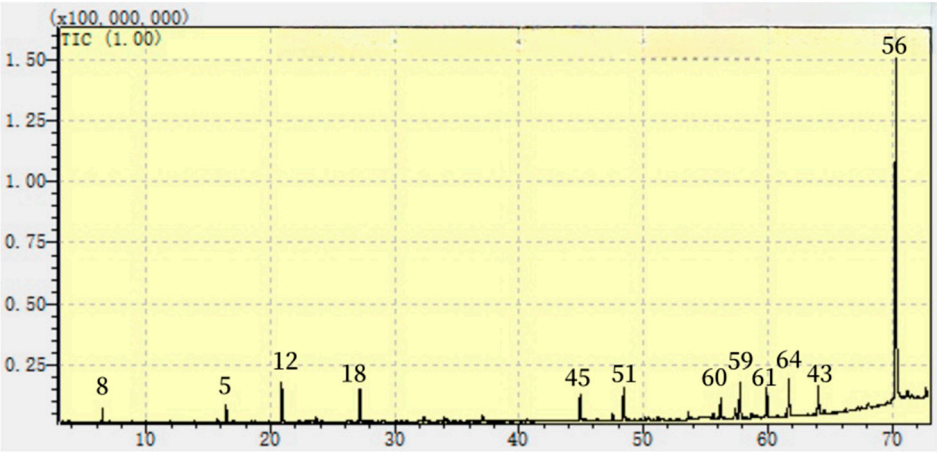


Figure 1. Total ion chromatogram (TIC) of E10. The peak corresponds to the compound with the same No. listed in Table 1.

Four compounds, which characteristic ion peaks can be seen in Table 2, can not be elucidated by their mass spectra and LRIs values [25].

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

Only eight compounds including hexanal, 1-octen-3-ol, limonene, linalool, *α*-terpineol, hexahydrofarnesyl acetone, methyl palmitate and PA are detected by MS using DB-5 due to the low concentrations 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 Es 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, their has the possibility that these compounds were introduced from such Es which are abundant in limonene and *α*-terpineol [11]. In such scenario, the quantitation results are based on the data gotten from MS using 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 compounds are first reported from the EOs of PR.

The Es, Cs and RCs are mainly consisted of FAs, especially LCFAs. PA is the most outstanding one, which is in line with the reported results [9,10]. Then, MA, OA and LA 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 the esters of FAs, the major compounds are methyl palmitate and methyl linolenate [10]. Among the aldehydes & ketones, hexahydrofarnesyl acetone is prominent. Tricosane and pentacosane are two highlight *n*-alkanes. In terms of AMs, linalool and *α*-terpineol are prominent. Phytol as a major compound is the only one of ADs.

C_{13}-norisoprenoids including dehydro-ar-ionene, *trans*-*β*-damascenone, *trans*-geranylacetone and *trans*-*β*-ionone are first reported from PR to the best of our knowledge. Its content is relatively less, and the content in the EOs is relatively higher compared with that in the corresponding crystals or EOs removed crystals.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 October 2023 doi:10.20944/preprints202310.1959.v1

2.3. Principal Component Analysis (PCA)

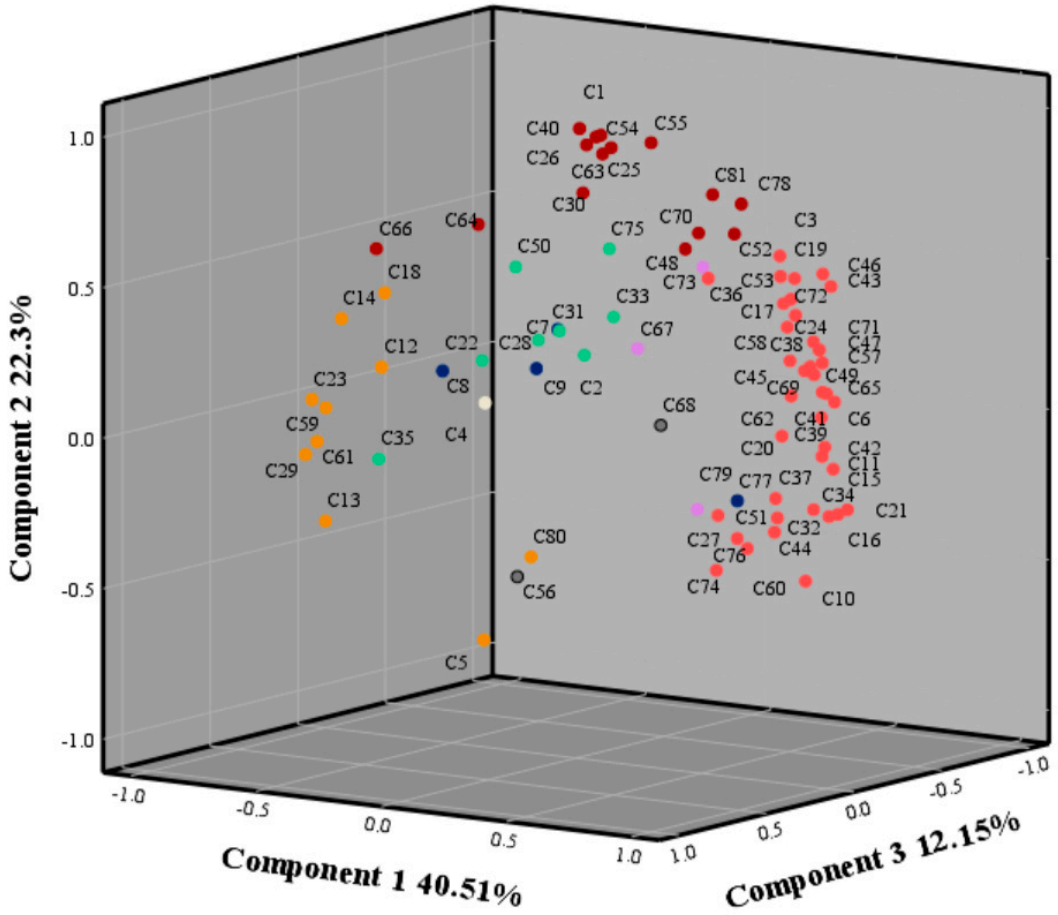


Figure 2. The PCA result. The number after "C" corresponds to the same No. compound listed in Table 1. Except for C56 "PA" and C68 "stearic acid", the other 79 compounds can be divided into seven components denoted with seven different color such as red, brown, orange yellow, green, dark blue, pinkish purple and white corresponding to components 1, 2, 3, 4, 5, 6 and 7, respectively.

A total of thirty-eight compounds such as C43 "MA", C45 "hexahydrofarnesyl acetone" and C51 "methyl palmitate" belongs to component 1; fifteen compounds such as C66 "LA" belong to component 2; ten compounds some such C29 "*trans*-*β*-damascenone" belong to component 3; eight compounds for instance C28 "dehydro-ar-ionene", C33 "*trans*-geranylacetone" and C35 "*trans*-*β*-ionone" belong to component 4; four compounds including C7 "p-cymene", C8 "limonene", C9 "*γ*-terpinene" and C77 "octacosane" belong to component 5; three compounds including C67 "OA", C73 "pentacosane" and C79 "nonacosane" belong to component 6; only C4 "β-Pinene" belongs to component 7.

2.4. AOAs of Es, Cs, RCs, four CMs and two references

For 1,1-Diphenyl-2-picrylhydrazy1 radical (DPPH) and (2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assays, only the IC_{50} (inhibitory concentration of 50%) of vitamin C (Vc) was detected, the IC_{50} of other samples were deduced through the results (Supplemental Table S2).

In DPPH assay (Figure 3**),** the RC10 presents the highest radical scavenging activity (RSA) as 14.7% in 110 μg·mL^{-1} among the nine samples including E8, E9, E10, RC8, RC9, RC10, C8, C9 and C10. PA shows a certain AOA at 100 μmol·L^{-1}, but its RSA values are negative with the increase of concentration. The RSA values (Supplemental Table S2) of OA and LA in the high concentration

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 October 2023 doi:10.20944/preprints202310.1959.v1

group are positive correlation to the concentration, but the initial value of OA is negative. At 1200 mmol·L^{-1}, the RSA value of OA is 6.39%, while that of LA is 35 %. Vc shows the strongest IC_{50}.

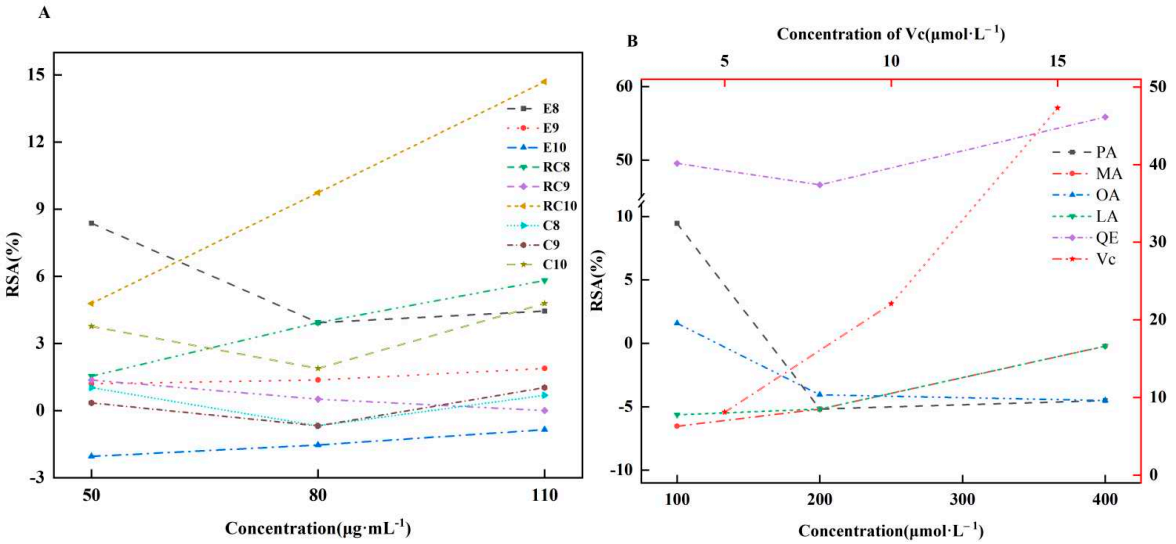


Figure 3. A: RSA of Es and their separated parts. B: RSA of CMs and references. QE: quercetin.

ABTS assay (Figure 4) demonstrates that the RSA values of RCs are higher than those of the corresponding Es or Cs in most cases, which implies that the Cs should contain chemicals showing antagonistic AOAs. The IC_{50} of Vc is higher than that in DPPH method, whereas the IC_{50} of QE is lower than that in DPPH assay. It is worth noting that most of the samples show better RSA values compared with those detected by the DPPH assay, which should be due to the higher reactivities of ABTS radical cations [26].

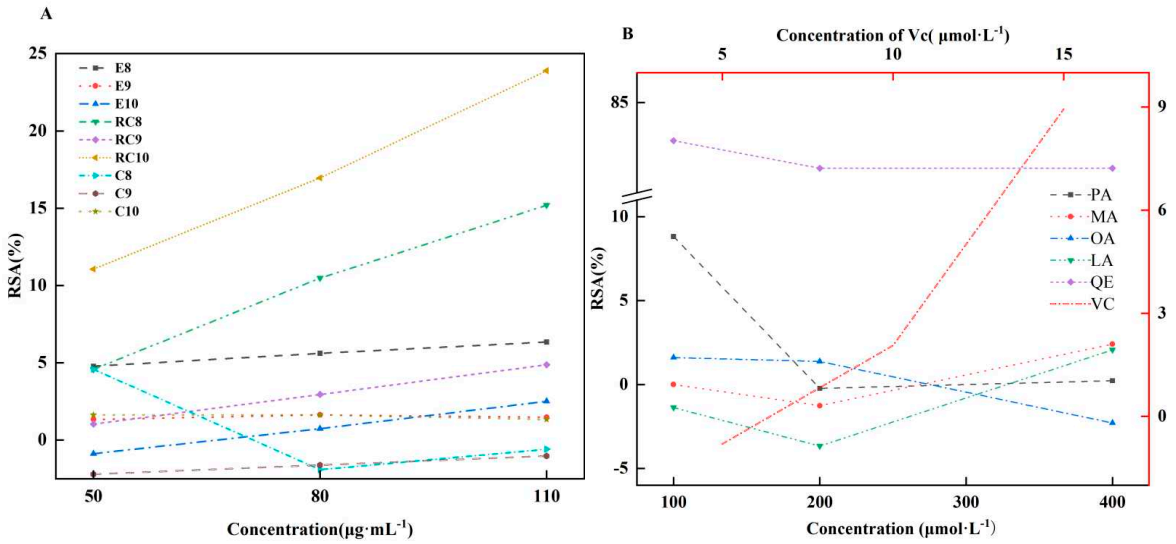


Figure 4. A: RSA of Es and their separated parts. B: RSA of CMs and references.

In the ferric reducing/antioxidant power (FRAP) assay, the AOAs are expressed as the concentration of Fe^{2+} (mmol·L^{-1}). The FRAP values of OA and LA in 300 mmol·L^{-1} are significantly higher than that of Vc at 15 μmol·L^{-1}, and similar to that of QE at 200 and 100 μmol·L^{-1}, respectively. However, the FRAP values of OA and LA gradually decrease when the concentration increase to 600 and 1200 mmol·L^{-1}. It is worth noting that the FRAP values of nine samples including E8, E9, E10, RC8, RC9, RC10, C8, C9 and C10 are close to that of Vc at 10 μmol·L^{-1}.

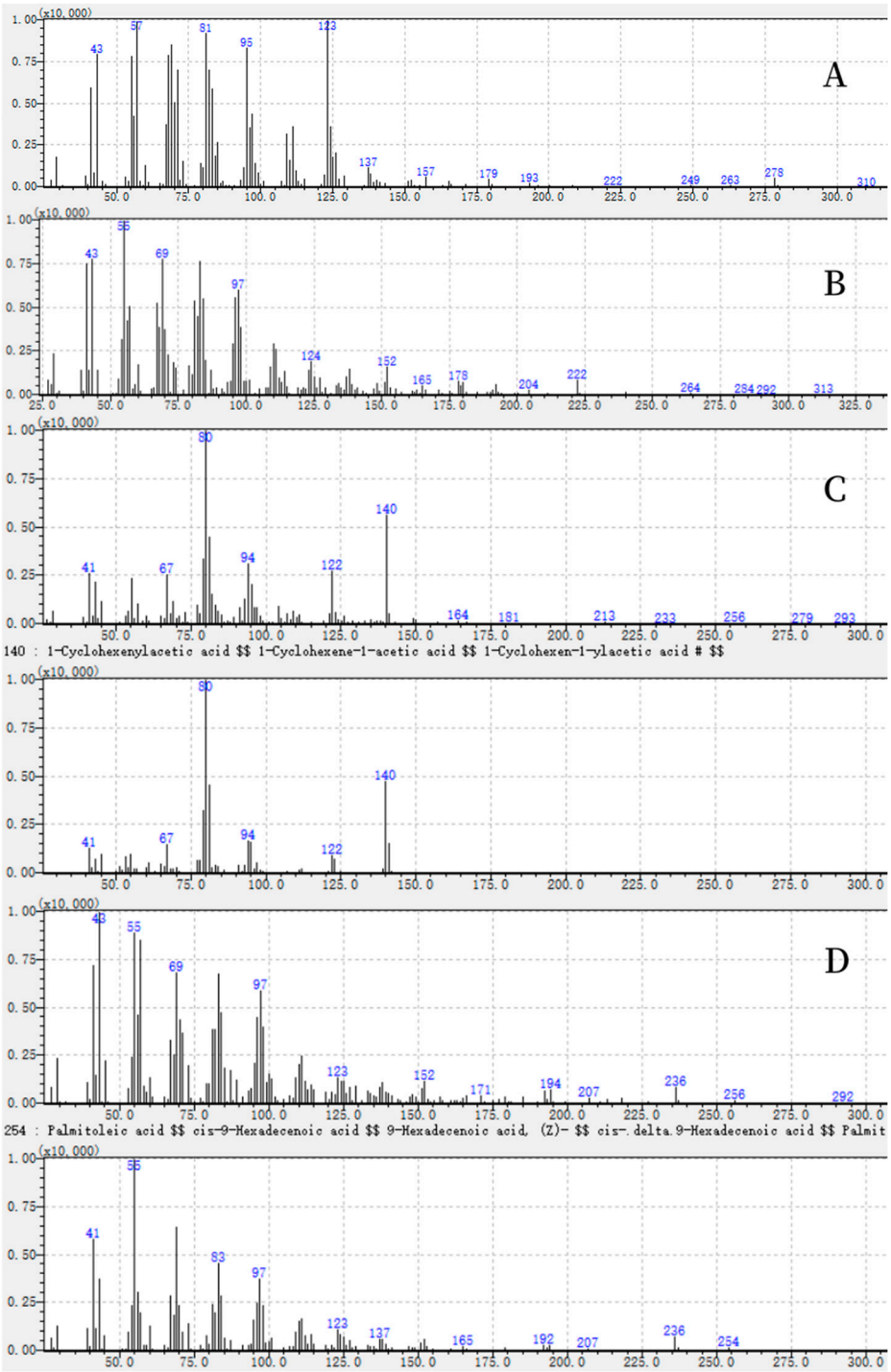
The OA and LA show some pro-oxidation activities (POAs) in lower concentration group, whereas demonstrate well AOAs in higher concentration group.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 October 2023 doi:10.20944/preprints202310.1959.v1

3. Discussion

The mass spectra of compounds 66, 67 and 68 are highly similar with those of LA, OA 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. Consequently, the chemicals with LRIs[d] higher than 2984 such as LA, OA 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 LA, OA and stearic acid, respectively, which are also reported previously [9,10].

Unknown-1 should be an analogue of phytol acetate according to its characteristic ion peaks (Table 2, Figure 5A) and LRI value.



 doi:10.20944/preprints202310.1959.v1

Figure 5. A: The mass spectrum of unknown-1 from RC8; B: The mass spectrum of unknown-2 from RC9; **C:** The mass spectra of unknown-3 from RC10 and the corresponding match of 1-cyclohexenylacetic acid from NIST 14 library; **D:** The mass spectra of unknown-4 from C10 and the corresponding match of palmitoleic acid from NIST 14 library.

Unknown-2 should be an unsaturated LCFA or the corresponding ester based on its characteristic ion peaks (Table 2, Figure 5B) and LRI value.

The most suitable match for unknown-3 is 1-cyclohexenylacetic acid with a molecular wight (M_w) of 140 (Figure 5C). 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-3 should be a derivative of 1-cyclohexenylacetic acid. Cyclohexenylacetic acid is reported as a compound in the CH_2Cl_2 extract of PA, which should be corresponding to unknown-3 in this study [10].

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

The Es are mainly composed of LCFAs, which is in agreement with the previous reports [9,10]. The Cs and RCs are also mainly composed of LCFAs. The Cs have relatively higher content of PA, while the RCs have relatively lower content of PA, compared with that of Es. The chemicals, which have high boiling points (BPs) such as LCFAs and their ester, lead to the lower extraction rate compared with that of the Es extracted from other plants, for example *Citrus* L. [11,12]. As for HMs, only *α*-pinene was reported in flower and leaf of PR previously [10]. In terms of AMs, only linalool was reported in leaf of PR previously [10]. The 1-octene-3-ol is reported to have a typical mushroom flavor [27].

According to the percentages, seven compounds including PA, MA, LA, OA, methyl palmitate, hexahydrofarnesyl acetone and phytol can be chosen as the CMs in these Es. Compared with previous studies [9,10], methyl palmitate, hexahydrofarnesyl acetone and phytol are three new CMs.

The C_{13}-norisoprenoids are important to the flavor of Es althoutgh their content is relatively less [28]. Among them, *trans*-*β*-damascenone is one of the most important natural aroma compounds known and is one of the backbones of the international perfume industry [29], which has a characteristic sweet, fruity and warm flavor [30]. Moreover, its odour thresholds are very low at 0.002 $\mu g \cdot L^{-1}$ in water and 5 $\mu g \cdot L^{-1}$ in 25% alcohol [28,30], only trace quantities are necessary for odor [31]. This compound should do an improtant contribution to the special odour of Es extracted from PR.

trans-*β*-Damascenone is primarily degraded from precursors when heated in the acidic environment [28,32]. It was probably produced from the progenitors during the hydro-distillation in this study [33]. Many other C_{13}-norisoprenoids volatile compounds have been elucidated to be generated in the same way [28,33]. The 5*β*, 6*α*-dihydroxy-3*β*-(*β*-D-glucopyranosyloxy)-7-megastigmen-9-one [8] as a probable progenitor can generate these four kinds of C_{13}-norisoprenoids (Figure 6).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 October 2023 doi:10.20944/preprints202310.1959.v1

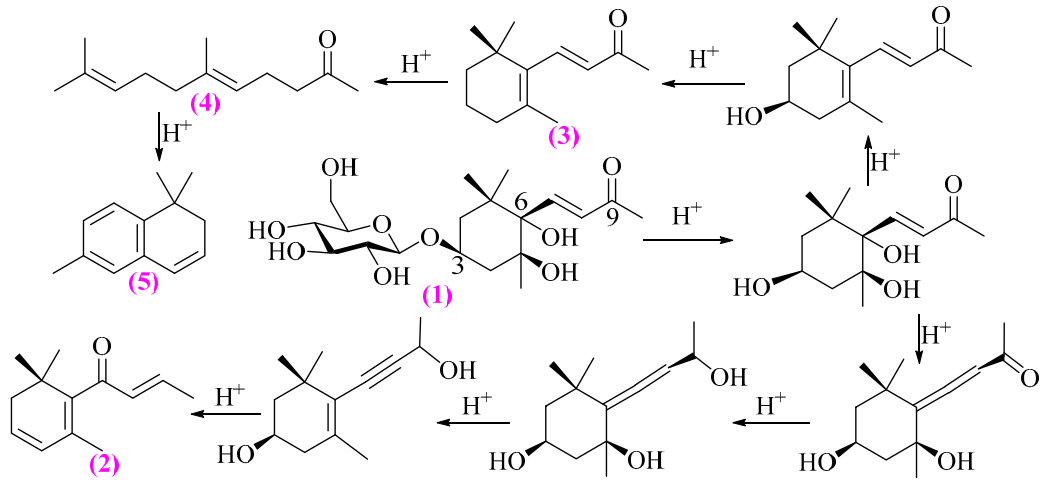


Figure 6. Hypothetical transformation from 5*β*, 6*α*-dihydroxy-3*β*-(*β*-D-glucopyranosyloxy)-7-megastigmen-9-one **(1)** to *trans*-*β*-damascenone **(2)**, *trans*-*β*-ionone **(3)**, *trans*-geranylacetone **(4)** and dehydro-ar-ionene **(5)**.

The Es extracted from PR present some similarities with the Es extracted from *M. sylvestris* [34], *Cirsium japonicum* var. *ussurience* Kitamura, *Ixeris dentate* and *I. stolonifera* [35,36], because they are all represented with the high BPs compounds such as PA and hexahydrofarnesyl acetone as the major components.

As for the assays to detect AOAs, 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 ROS of an antioxidant, climate and temperature [37]. It is hardly to get the same result under the "equal condition".

In DPPH and ABTS methods, the RCs usually show better AOAs than those of the corresponding Es and Cs, which should be related to the higher content of PA in the Cs. Sometimes, the Cs even present POAs. As a member of SFAs, PA can cause oxidative stress, inflammation, insulin resistance and impair endothelial function in a concentration dependent manner [18–23,38,39]. For example, PA in high concentration (400 μM) can not only increase oxidative stress injury, but also induce the overexpression of $P66^{Shc}$ protein, whereas in low concentration (200 μM) only slightly increased the expression of $P66^{Shc}$ protein [22], which can promote oxidation and apoptosis by regulating the production of ROS [39]. In this study, the RSA values of PA also present in a concentration dependent manner. As for the AOAs of MA, the previous studies are contradicted. On the one hand, MA can potently stimulate human polymorphonuclear leukocytes, which play an important role in host defense against microbial infections, to produce ROS. Excessive intake of MA can lead to detrimental consequences by uncontrolled production of ROS [40]. On the other hand, MA can protect the testes against oxidative stress caused by hyperglycemia [41]. MA shows weak AOAs or some POAs in this study [42]. Under which kinds of circumstances can MA present AOAs or POAs needs to be studied further.

OA can prevent the production of ROS in the endoplasmic reticulum and endoplasmic reticulum stress-induced inflammatory response, change membrane lipid peroxidation [43,44]. OA and LA can produce AOAs by reducing pro-inflammatory signals [45]. Here, LA shows better AOAs compared with that of OA in higher concentration group, which should be related to the number and conjugations of double bonds [46,47]. Usually, the more double bonds and conjugations in PUFAs will result in the better AOAs [18–21,46,47] 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 [16]. On the other hand, the more double bonds and conjugations also mean the PUFAs are easily to react with oxygen and generate ROS [48,49]. As a result, LA can be used as a free radical product to evaluate the AOAs of compounds [48]. Linolenic acid (l8:3, n-6) and eicosatrienoic acid

 doi:10.20944/preprints202310.1959.v1

(20:3, n-3) can enhance oxidant injury [17]. Excessive intake of MUFAs and PUFAs can also lead to oxidative stress and inflammation *in vivo* models [50]. The relationship between the degree of unsaturation and susceptibility to oxidant injury remains unclearly [16].

In addition, the synergistic AOAs of four CMs were not detected. The results of the *in vitro* study demonstrated that SFAs induced significant cellular lipotoxic damage, whereas the combination of MUFAs/PUFAs with SFAs markedly enhanced the impaired cell viability [51]. The RSA values of RC10 are significantly higher than those of the other eight samples of Es, Cs and RCs, and its other biological activities deserve further investigation.

4. Materials and Methods

4.1. Plant Materials, Reagents and Chemicals

The information of three populations of the aboveground portion of PR, named L8, L9 and L10, which were corresponding to the same No. samples in previous research [24], were presented in Table 3. The collected populations were authenticated by Professor Yi Zhang (Chengdu university of traditional Chinese medicine (CUTCM), Chengdu, China) and internal transcribed spacer 2 DNA barcodes in previous study [24]. 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 3. The origins of PR and GenBank accession numbers of ITS2 sequences [24].

Voucher No.	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 of high-performance liquid chromatography grade, 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. 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 weighed powders 315 g of L8, L9 and L10 were 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 Es 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 Es was removed by anhydrous Na_2SO_4.

The Es of L8, L9 and L10 were stored at 4, -4 and -80 °C, respectively, to evaluate crystallization. Cs were obtained at 4 or -4 °C, respectively. At -80 °C, the RCs were all being solid state. As a result, there were three samples as E, C and RC 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.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 October 2023 doi:10.20944/preprints202310.1959.v1

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).

The E8, E9, E10, C8, C9, C10, RC8, RC9 and RC10 were diluted by MeOH to the concentration such as 50, 80 and 110 μg·mL^{-1}, respectively. The PA, MA, OA, LA and QE were diluted by MeOH to the concentration such as 100, 200 and 400 μmol·L^{-1}, respectively. Moreover, the OA and LA were diluted by MeOH to the concentration such as 300, 600 and 1200 mmol·L^{-1}. The Vc was diluted in MeOH to the concentration such as 5, 10 and 15 μmol·L^{-1}, respectively. The above samples were detected by DPPH, ABTS and FRAP assays.

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

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, $t_n \le t_x \le t_{(n+1)}$) by the equation proposed by Van Den Dool and Kratz [52]. The t_{30} was deduced from the TIC.

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

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. PCA

The PCA for the 81 compounds was run by Solutionsstatistical Package for the Social Sciences Statistics 26 of International Business Machine.

4.7. AOAs

Nine samples of Es and their separated parts including E8, E9, E10, C8, C9, C10, RC8, RC9, RC10, four CMs including PA, MA, OA, LA and two references such as Vc and QE were tested the AOAs.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 October 2023 doi:10.20944/preprints202310.1959.v1

All the subjects were diluted in MeOH. Due to the limited amount of volatile oils, only three low concentrations of 50, 80 and 110 μg·mL^{-1} were set up for the nine samples of Es and their separated parts. For OA and LA, higher concentration group of 300, 600 and 1200 mmol·L^{-1} was founded. Their AOAs were tested by DPPH, ABTS and FRAP assays, respectively.

4.7.1. DPPH assay

A slight improvement was made according to the literature method [53]. The sample 100 μL at different concentration 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.

4.7.2. ABTS assay

A slightly modification was made based on the previously method [54]. 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.

4.7.3. FRAP assay

A slight modification was made based on the literature method [53]. 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.

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·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 AOAs.

5. Conclusions

A total of 44 chemicals are first reported from the Es of PR. As for content, seven compounds including PA, MA, LA, OA, methyl palmitate, hexahydrofarnesyl acetone and phytol can be chosen as the CMs. In terms of flavour, *trans*-*β*-damascenone can be selected as a CM. In sum, these eight compouds are selected as the CMs in Es of PR. The percentage of PA, the most abundance compound, is higher in Cs but lower in RCs compared with that in Es. Usually, the RCs demonstrate stronger AOAs and the Cs show weaker AOAs compared with those of Es, which should be related to the different content of PA in these samples. This study advance the knowledge in the Es of PR and can give some hints for the utilization of such Es which are abundant in LCFAs.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, Z.P. and J.W.; methodology, C.X. and J.W.; software, C.X., X.Y., A.H. and J.W.; validation, Z.P., C.X., X.Y., Y.S., A.H. and J.W.; investigation, C.X. and J.W.; resources, Z.P.; data

curation, C.X., X.Y. and J.W.; writing—original draft preparation, C.X., X.Y. and J.W.; writing—review and editing, Z.P., C.X., X.Y., Y.S., A.H. and J.W.; supervision, Z.P. and J.W.; project administration, Z.P. and J.W.; funding acquisition, Z.P. and J.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (Grant No. 81973567), Chongqing Science and Technology Bureau (Grant No. cstc2020jcyj-msxmX0310), and Chongqing Municipal Health Commission (Grant No. 2020ZY023793; ZY201602104).

Acknowledgments: The authors thank the undergraduates, Hang Shi, Qin Duan, Meiying Luo, Shanshan Jiang, and Churui Xiao, for their contributions.

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. Zhang, F.; Wu, Z.J.; Sun, L.; Wang, J.; Tao, X.; Chen, W.S. Iridoid glucosides and a C_{13}-norisoprenoid from *Lamiophlomis rotata* and their effects on NF-kB activation. *Bioorg. Med. Chem. Lett.* 2012, 22, 4447-4452.
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. 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.
12. Ul Haq, A.; Wang, J. Identification of varieties and biomarkers analyses on essential oils from peels of *Citrus* L. collected in Pakistan. *Pak. J. Bot.* **2023**, *55*, 1407-1418.
13. 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.
14. Zhou, Z.; Li, T.; Du, R.; Liu, C.; Huang, S.; Han, L.; Zhang, P.; Wang, Y.; Jiang, M. *Lamiophlomis rotata* attenuates rheumatoid arthritis by regulating sphingolipid and steroid hormone metabolism. *Mol. Omics* **2023**, *19*, 72-83.
15. Aebisher, D.; Cichonski, J.; Szpyrka, E.; Masjonis, S.; Chrzanowski, G. Essential oils of seven Lamiaceae plants and their antioxidant capacity. *Molecules* **2021**, *26*, 3793.
16. 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.
17. 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.
18. 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.
19. 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.
20. 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.
21. 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.

22. 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.
23. 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.
24. 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.
25. Adams, R.P. *Identification of essential oil components by gas chromatography/mass spectrometry*, ed. 4.1. Allured publishing; Illinois, America, 2017; p. 1-804.
26. 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.
27. 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.
28. Carlin, S.; Mattivi, F.; Durantini, V.; Dalledonne, S.; Arapitsas, P. Flint glass bottles cause white wine aroma identity degradation. *PNAS* **2022**, *119*, e2121940119.
29. Pickenhagen, W. In *Flavor Chemistry-Thirty Years of Progress*; Teranishi, R., Wick, E.L., Hornstein, I., Eds. Kluwer Academic/Plenum Publishers: New York, U.S.A, 1999: p. 75-87.
30. Kaneshima, T.; Nojima, S.; Mori, S.; Myoda, T.; Nakahara, K.; Matsuo Y. Isolation and identification of progenitors, glycoconjugates of β-damascenone precursors, in sweet potato (*Ipomoea batatas*). *Flavour Fragr. J.* **2023**, *38*, 152-162.
31. Roberts, D.D.; Mordehai, A.P.; Acree, T.E. Detection and partial characterization of eight β-damascenone precursors in apples (*Malus domestica* Borkh. Cv empire). J. Agric. Food Chem. **1994**, *42*, 345-349.
32. Roberts, D.D.; Roberts, A.P.; Acree, T.E. Detection and partial characterization of eight β-Damascenone precursors in Apples (*Malus domestica* Borkh. Cv. Empire). *J. Agric. Food Chem.* **1994**, *42*, 345-349.
33. Suzuki, M.; Matsumoto, S.; Mizoguchi, M.; Hirata, S.; Takagi, K.; Hashimoto, I.; Yamano, Y.; Ito, M.; Fleischmann, P.; Winterhalter, P.; Morita, T.; Watanabe1, N. Identification of (3*S*, 9*R*)- and (3*S*, 9*S*)-Megastigma-6,7-dien-3,5,9-triol 9-*O*-β-D-glucopyranosides as Damascenone progenitors in the flowers of *Rosa damascena* Mill. *Biosci. Biotechnol. Biochem.* **2002**, *66*, 2692-2697.
34. 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.
35. 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.
36. 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.
37. Munteanu, I.G.; Apetrei, C. Analytical methods used in determining antioxidant activity: A review. *Int. J. Mol. Sci.* **2021**, *22*, 3380.
38. Fratantonio, D.; Speciale, A.; Ferrari, D.; Cristani, M.; Saija, A.; Cimino, F. Palmitate-induced endothelial dysfunction is attenuated by cyanidin-3-*o*-glucoside through modulation of Nrf2/Bach1 and NF-κB pathways. *Toxicol Lett* **2015**, *239*, 152–160.
39. Giorgio, M.; Migliaccio, E.; Orsini, F.; Paolucci, D.; Moroni, M.; Contursi, C.; Pelliccia, G.; Luzi, L.; Minucci, S.; Marcaccio, M.; Pinton, P.; Rizzuto, R.; Bernardi, P.; Paolucci, F.; Pelicci, P.G. Electron transfer between cytochrome c and $p66^{shc}$ generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell* **2005**, *122*, 221–233.
40. Tada, M.; Ichiishi, E.; Saito, R.; Emoto, N.; Niwano, Y.; Kohno, M. Myristic acid, a side chain of phorbol myristate acetate (PMA), can activate human polymorphonuclear leukocytes to produce oxygen radicals more potently than PMA. *J. Clin. Biochem. Nutr.* **2009**, *45*, 309–314.
41. Khalil, A.S.M.; Giribabu, N.; Yelumalai, S.; Shahzad, H.; Kilari, E.K.; Salleh, N. Myristic acid defends against testicular oxidative stress, inflammation, apoptosis: Restoration of spermatogenesis, steroidogenesis in diabetic rats. *Life Sci.* **2021**, *278*, 119605.
42. Fidrianny, I.; Suhendy, H.; Insanu, M. Correlation of phytochemical content with antioxidant potential of various sweet potato (*Ipomoea Batatas*) in West Java, Indonesia. *Asian Pac J Trop Biomed* **2018**, *8*, 25-30.
43. Sun, Y.; Wang, J.; Guo, X.; Zhu, N.; Niu, L.; Ding, X.; Xie, Z.; Chen, X.; Yang, F. Oleic acid and eicosapentaenoic acid reverse palmitic acid-induced insulin resistance in human Hepg2 cells via the reactive oxygen species/*Jun* pathway. *Genom. Proteom. Bioinf.* **2021**, *19*, 754–771.
44. Abenavoli, L.; Milanović, M.; Milić, N.; Luzza, F.; Giuffrè, A.M. Olive oil antioxidants and non-alcoholic fatty liver disease. *Expert Rev. Gastroenterol. Hepatol.* **2019**, *13*, 739-749.

45. Pauls, S.D.; Rodway, LA.; Winter, T.; Taylor, C.G.; Zahradka, P.; Aukema, H.M. Anti-inflammatory effects of α-linolenic acid in M1-like macrophages are associated with enhanced production of oxylipins from α-linolenic and linoleic acid. *J. Nutr. Biochem.* **2018**, *57*, 121–129.
46. Wu, S.H.; Sun, G.R.; Wang, Y.Y.; Tan, T.T.; Zhang, Y.; Deng, H.M.; Zhang, T.L.; Du, F.G. Chemical composition analysis and antioxidant activity of essential oil from *Magnolia sieboldii* Leaves. *Mol. Plant Breed.* **2023**, *21*, 1–12.
47. Lee, S.H.; Min, D.B. Effects, quenching mechanisms, and kinetics of carotenoids in chlorophyll-sensitized photooxidation of soybean oil. *J. Agri. Food Chem.* **1990**, *38*, 1630–1634.
48. Sila, A.; Sayari, N.; Balti, R.; Martinez-Alvarez, O.; Nedjar-Arroume, N.; Moncef, N.; Bougatef, A. Biochemical and antioxidant properties of peptidic fraction of carotenoproteins generated from shrimp by-products by enzymatic hydrolysis. *Food Chem.* **2014**, *148*, 445–452.
49. Burton, G.W.; Ingold, K.U. β-Carotene: An unusual type of antioxidant. *Science* **1984**, *224*, 569–573.
50. Grujić-Milanović, J.D.; Miloradović, Z.Z.; Mihailović-Stanojević, N.D.; Banjac, V.V.; Vidosavljević, S.; Ivanov, M.S.; Karanović, D.J.; Vajić, U.-J.V.; Jovović, D.M. Excesive consumption of unsaturated fatty acids leads to oxidative and inflammatory instability in wistar rats. *Biomed. Pharmaco.* **2021**, *139*, 111691.
51. Liu, W.; Zhu, M.; Gong, M.; Zheng, W.; Zeng, X.; Zheng, Q.; Li, X.; Fu, F.; Chen, Y.; Cheng, J.; Rao, Z.; Lu, Y.; Chen, Y.; Comparison of the effects of monounsaturated fatty acids and polyunsaturated fatty acids on liver lipid disorders in obese mice. *Nutrients* **2023**, *15*, 3200.
52. 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.
53. Zengin, G.; Sarikurkcu, C.; Uyar, P.; 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.
54. 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.