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Chemical composition and antioxidant activity of petroleum ether fraction of *Rosmarinus officinalis*

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ABSTRACT

The presented study examines the chemical composition and antioxidant activity of the petroleum ether fraction of *Rosmarinus officinalis* (PEF-RO), which was obtained via 75 % ethanol extraction followed by petroleum ether extraction. The obtained fractions were analyzed by gas chromatography-mass spectrometry (GC-MS). The *in vitro* antioxidant activity of PEF-RO was investigated using various assays, including 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate (ABTS) free radical scavenging, and ferric reducing antioxidant power (FRAP) method. A total of 82 chemical components were successfully identified, totaling 10.06 % of PEF-RO content. The identified components consisted of 24 hydrocarbons, 14 ketones, 16 alcohols, 4 phenols, 14 esters, and 10 other compounds. Notably, verbenone (2.4377 %), vitamin A (0.6854 %), *trans*-geraniol (0.5998 %), linolenic acid (0.5713 %), and 1,8-eucalyptol (0.5323 %) were the most abundant compounds, and there are many trace components in PEF-RO. PEF-RO's IC_{50} values of DPPH and ABTS free radical scavenging were determined as 0.36 mg/mL and 0.19 mg/mL, respectively. FRAP-method was employed to measure the total antioxidant energy of PEF-RO, which displayed good antioxidant activity. The obtained data provides the foundation for the comprehensive development and utilization of *Rosmarinus officinalis*.

1. Introduction

Rosmarinus officinalis L. is a small, perennial, green shrub from the Lamiaceae family [1]. It is originally native to the Mediterranean coast and is now widely grown in southern Europe. The earliest record of rosemary in China dates back to the Cao Wei period and was documented in the famous Chinese herbal medicine book 'Herbal Supplements' [2]. Today, *Rosmarinus officinalis* is extensively cultivated in Yunnan, Guizhou, Xinjiang, Henan, Jiangxi, and other regions of China. *Rosmarinus officinalis* is a versatile plant, in which its stems and elongated green leaves are the most commonly utilized parts [3]. During the growing season, the leaves emit a strong and

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refreshing aroma known to relieve depression [4–6]. Due to its medicinal and culinary properties, *Rosmarinus officinalis* is widely used in the food and cosmetics industries [7]. *Rosmarinus officinalis* and its extracts have been found to possess antioxidant properties [8,9] and have shown effectiveness in combatting bacterial infections [10,11], inflammation [12–14] neurological disorders [15,16], tumors [17,18], and cardiovascular diseases [19]. These functions are attributed to the presence of various active components such as terpenoids, polyphenols, flavonoids, and organic acids. Rosemary essential oil, obtained through steam distillation or hydrodistillation, contains various components such as 1,8-cineole, a-pinene, camphor, camphene, and b-pinene. These components exhibit notable antioxidant effects [20,21]. The antioxidant properties of *Rosmarinus officinalis* essential oil have been extensively studied due to its diverse uses [22,23]. Synthetic commercially available antioxidants exhibit potential toxic effects on the human body upon long-term usage, whereas rosemary's volatile components, as a natural extract, are non-toxic, harmless, highly efficient, and possess high-temperature resistance. Additionally, they have greater effectiveness than artificial antioxidants such as butyl hydroxyanisole, butylated hydroxytoluene, and *tert*-butyl hydroquinone [24,25]. Hence, the research and development of natural antioxidants can benefit from the application of the aforementioned volatile components. Currently, *Rosmarinus officinalis*'s essential oils are mainly obtained by steam distillation, which has a low yield and requires high extraction equipment.

The presented study focused on the petroleum ether fraction of *Rosmarinus officinalis* (PEF-RO), as well as its chemical composition and antioxidant activity. PEF-RO was easily obtained at a low cost, exhibits high active ingredient content, and retains the volatile components of *Rosmarinus officinalis*. GC-MS was used to identify and analyze the chemical components of PEF-RO and examined the characteristic components. The *in vitro* antioxidant capacity of PEF-RO was determined through various methods, and 14 main components were identified as PEF-RO markers. Additionally, the study compared the antioxidant capacity of these components to understand their contribution to PEF-RO's antioxidant activity. Overall, this study provides a theoretical basis for the development and utilization of *Rosmarinus officinalis*.

2. Materials and methods

2.1. Plant collection

In December 2020, a total of 5 kg of dry *Rosmarinus officinalis* L. leaves were purchased from the Juhuayuan professional market of traditional Chinese medicinal materials in Kunming. These leaves were picked in Yuxi City, Yunnan Province during the summer of 2020. The plant was identified as *Rosmarinus officinalis* L. by Professor Xu Junju of Yunnan Agricultural University. A voucher specimen (RO202012) was deposited at the laboratory of the College of Science of Yunnan Agricultural University.

2.2. PEF-RO extraction

After crushing 5 kg of dried rosemary leaves (150 mesh), they were cold-soaked and extracted three times with 8 equiv. amount of 75 % ethanol aqueous solution for 2 h each time. The resulting ethanol extract was filtered and ethanol recovery with rotary evaporator (temperature below 50 °C). Next, the extract was shaken and extracted with an equal volume of petroleum ether three times to obtain the petroleum ether extract of *Rosmarinus officinalis* (PEF-RO). This extract was then spin-dried and concentrated to obtain 800 g of PEF-RO (16 % yield), which was sealed and low temperature stored for further use (under 4 °C).

2.3. GC-MS analysis

7890B–7000C Gas Chromatography-Triple Quadrupole Mass Spectrometry (Agilent Technologies (China) Co., Ltd.) was used to analyze PEF-RO samples both qualitatively and quantitatively. GC conditions: Agilent VF-17 ms chromatographic column (50 m × 0.25 mm × 0.25 μm); carrier gas: helium, carrier gas flow rate: 1.0 mL/min; inlet temperature: 250 °C; injection volume 1 μL Split injection, split ratio 10:1; temperature program: initial temperature: 50 °C for 1 min, then increased from 50 °C to 200 °C at a rate of 5 °C/min, maintained for 1 min, increased from 200 °C at a rate of 10 °C/min to 280 °C, held for 10 min, and the total running time was 50 min. MS conditions: detector: mass spectrometer detector; solvent delay: 2 min; ionization voltage: 70 eV; ion source temperature: 230 °C; transfer line temperature: 280 °C; scanning ion range: 35–450 amu; mass spectrometry scanning method: SCAN. The peak area normalization method was used to determine the relative content of the components. The chemical structures of the components were identified by searching the standard mass spectrum library of NIST11 Chemworkstation and referring to relevant literature.

2.4. Determination of in vitro antioxidant activity of PEF-RO

2.4.1. Determination of DPPH free radical scavenging ability

2 mg of DPPH was accurately weighed and dissolved in 50 mL of absolute ethanol producing a 0.04 mg/mL DPPH solution, which was stored in the dark at 4 °C. A 1.0 mg/mL PEF-RO ethanol solution was prepared and diluted with absolute ethanol to create solutions with concentration gradients of 1.0, 0.8, 0.6, 0.4, 0.2, and 0.1 mg/mL 200 μL of each gradient sample solution and 3 mL of DPPH ethanol solution were accurately measured and fully mixed as the experimental group. After allowing the solution to stand in the dark at room temperature for 30 min, the absorbance (A_1) of the sample solutions with different concentrations was measured at 517 nm. At the same time, the absorbance (A_2) of 200 μL sample solution mixed with 3 mL of absolute ethanol and the absorbance (A_0) of 200 μL of absolute ethanol mixed with 3 mL of DPPH solution was also measured. Vitamin C (Vc) was used as the positive control and the parallel experiment was measured in triplicate, and the mean value was obtained. DPPH free radical scavenging ability was then

calculated using formula (1), and the IC_{50} value was determined.

$$\text{DPPH Clearance}/\% = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100\% \quad (1)$$

2.4.2. Determination of ABTS free radical cation scavenging capacity

30 mg of ABTS free radical powder was weighed and dissolved in 8 mL of pure water. 10 mg of $K_2S_2O_8$ was dissolved in 15 mL of pure water to obtain $K_2S_2O_8$ solution. The above two solutions were mixed in equal amounts at a volume ratio of 1:1 to prepare $ABTS^{\cdot+}$ working solution. The solution was stored at 4 °C for 10–12 h before use. The working solution was diluted with absolute ethanol until the absorbance was within the range of 0.7 ± 0.02 Abs. 1.0 mg/mL PEF-RO ethanol solution was prepared and diluted with absolute ethanol generating solutions with concentration gradients of 1.0, 0.8, 0.6, 0.4, 0.2, and 0.1 mg/mL 200 μL of each gradient sample solution and 3 mL of $ABTS^{+}$ working solution for the experimental group were allowed to react in the dark for 30 min. The absorbance (A_1) of the sample solutions with different concentrations was measured at 734 nm. The absorbance (A_2) after mixing water and ethanol was measured, and the absorbance (A_0) after mixing 200 μL absolute ethanol with 3 mL $ABTS^{\cdot+}$ working solution. The parallel experiment was conducted in triplicate with Vc as the positive control, and the mean value was used to calculate the clearance rate using formula (2). Finally, IC_{50} value was calculated.

$$\text{ABTS Clearance}/\% = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100\% \quad (2)$$

2.4.3. Determination of FRAR

Preparation of FRAP working solution involved the mixing of 300 mmol/L sodium acetate buffer solution (pH = 3.6), 10 mmol/L tripyridine triazine (TPTZ) solution (with 40 mmol/L HCl as solvent), and 20 mmol/L $FeCl_3$ solution in a volume ratio of 10:1:1. The resulting mixture was incubated in a water bath at 37 °C for 30 min to obtain FRAP working solution. It was important to prepare FRAP fresh every time before use. A standard curve was prepared by accurately weighing 27.8 mg of $FeSO_4{\cdot}7H_2O$ standard substance and dissolved in pure water to obtain a 100 mmol/L mother liquor. The mother liquor was diluted to 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mmol/L. Next, 200 μL of $FeSO_4{\cdot}7H_2O$ standard solution of various concentrations and 3 mL of FRAP working solution were mixed and the absorbance was measured at 593 nm. The standard concentration was taken as the abscissa, and the corresponding absorbance value was employed to create $FeSO_4{\cdot}7H_2O$ standard curve.

In this experiment, 1.0 mg/mL PEF-RO sample was prepared and diluted with absolute ethanol to create solutions of varying concentrations ranging from 1.0 to 0.1 mg/mL 200 μL of each gradient sample solution and 3 mL of FRAP working solution were mixed for the reaction. The absorbance was measured at 593 nm and the sample concentration was obtained using the standard curve. The concentration of the standard substance $FeSO_4$ solution was used to express the total antioxidant capacity of PEF-RO.

2.4.4. Determination of free radical scavenging ability of PEF-RO markers

14 marker components in PEF-RO were selected, and their DPPH clearance rate and ABTS clearance rate were measured, respectively. Verbenone, vitamin A, *trans*-geraniol, linolenic acid, 1,8-eucalyptol, *trans*-borneol, α-terpineol, linalool, palmitic acid ethyl ester, squalene, camphor, phytantriol, β-caryophyllene, and ethyl linoleate standard products were purchased from Sichuan Victory Biological Technology Co., Ltd., with a purity greater than 95 %. 14 marker components were dissolved in absolute ethanol individually to prepare at a concentration of 10 mg/mL each as standard solutions. For the experimental group, 200 μL of each standard solution and 2 mL of DPPH ethanol solution were mixed well and allowed to stand in a dark place at room temperature for 30 min. The absorbance (A_1) of the sample solutions was measured at 517 nm, as well as the absorbance (A_2) of the standard product solution and the absorbance of 200 μL of absolute ethanol mixed with 2 mL of DPPH solution (A_0). The same procedure was employed for $ABTS^{+}$ working solution, where 200 μL of each standard product solution and 2 mL of $ABTS^{+}$ working solution were mixed and allowed to stand in the dark for 30 min. The absorbance (A_1) of each standard product solution was measured at 734 nm, as well as the absorbance (A_2) of the standard product solution and the absorbance of 200 μL of absolute ethanol mixed with 2 mL of $ABTS^{\cdot+}$ working solution (A_0). The scavenging ability of DPPH and ABTS free radicals was calculated using formulas (1) and (2) respectively. Parallel experiments were measured in triplicate, and the mean value was determined.

2.5. Data analysis

The obtained results were presented as mean ± Standard Error of the Mean (SEM). The number of individuals and repeats tested in each series were provided along with the corresponding results. All statistical analyses were performed using Graph Pad Prism 7 software program.

3. Results and discussion

3.1. Chemical composition analysis of PEF-RO

The chemical composition of PEF-RO was analyzed by the developed GC-MS conditions. The total ion flow chromatogram of PEF-RO was obtained (Fig. 1).

The obtained from GC-MS data was analyzed and compared using the NIST11 mass spectral library. The identified 82 chemical components were confirmed and their relative contents were calculated using the peak area normalization method after manual analysis of the standard mass spectrum (Table 1). Our analysis revealed 82 components, consisting of 10.06 % of the total PEF-RO. The identified components included 11 hydrocarbons, 32 terpenoids, 14 esters, 10 ketones, 6 alcohols, 3 phenols, and 6 other compounds (Fig. 2.).

Table 1 shows that verbenone had the highest content in PEF-RO, accounting for 2.4377 % of the total. Other compounds with relatively high content included vitamin A, *trans*-geraniol, linolenic acid, and 1,8-Eucalyptol, with relative contents of 0.6854 %, 0.5998 %, 0.5713 %, and 0.5323 %, respectively. According to reports, the chemical composition of *Rosmarinus officinalis* varies depending on several factors, including the plant parts used for extraction, genotype, harvest time, and experimental conditions such as the solvent, extraction time, temperature, and technical procedures [26]. Additionally, various components with high content in *Rosmarinus officinalis* essential oil, such as eucalyptol, *trans*-borneol, verbenone, and vamphor [20,21], were detected in PEF-RO prepared in this experiment. It was shown that PEF-RO could effectively extract the volatile components of *Rosmarinus officinalis*. However, the fat-soluble components of *Rosmarinus officinalis* that could be extracted with ethanol and petroleum ether are obviously more than the types of chemical components in *Rosmarinus officinalis* essential oil.

3.2. Results of the determination of the antioxidant activity of PEF-RO

DPPH free radical possesses a single electron, and in an ethanol solution, it appeared dark purple with a strong absorption band at 517 nm. It is known that antioxidants present in the solution react and consume DPPH free radicals, generating a color change from dark purple to light yellow [27]. The extent of the color change indicates the strength of the antioxidant capacity. Fig. 3 demonstrates that the scavenging rate of PEF-RO on DPPH radicals was directly proportional to the PEF-RO concentration. IC_{50} value of PEF-RO was determined as 0.3606 mg/mL, while Vc was 0.0212 mg/mL. The obtained results highlighted that PEF-RO had a high DPPH free radical scavenging ability.

A positive correlation was observed between ABTS free radical scavenging rate and PEF-RO concentration (Fig. 4.). IC_{50} value of PEF-RO was 0.1914 mg/mL, while that of Vc was 0.0890 mg/mL. Hence, PEF-RO had a high ability to clean ABTS free radicals.

According to the results presented in Fig. 5, $FeSO_4{\cdot}7H_2O$ solution exhibited a strong linear relationship between its concentration and absorbance, within the range of 0.1–1.0 mmol/L.

The regression equation for this relationship was $y = 0.5014x+0.2206$, with a high correlation coefficient of $R^2 = 0.9982$. The total antioxidant capacity of PEF-RO was expressed as the concentration of the standard substance $FeSO_4$ solution (Table 2).

3.3. PEF-RO markers of free radical scavenging ability

Under identical concentration conditions, verbenone, vitanmin A, tr*ans*-geraniol, linolenic acid, *α*-terpineol, and *β*-caryophyllene demonstrated superior scavenging ability of DPPH. Conversely, 1,8-eucalyptop, *trans*-borneol, linalool, palmitic acid ethyl ester, squalene, camphor, phytantriol, and ethyl linoleate exhibited weak or no scavenging effect of DPPH (Fig. 6). Verbenone, linolenic acid and *β*-caryophyllene show superior scavenging ability of ABTS free radicals (Fig. 7).

The antioxidant mechanism of natural ingredients is primarily attributed to their polyphenols, vitamins, alkaloids, saponins, polysaccharides, polypeptides, terpenes, and other antioxidant components. This mechanism involves three main aspects: direct scavenging of free radicals, inhibition of free radical formation, and activation of the antioxidant system [28,29]. This experiment shows that PEF-RO antioxidant activity exhibits excellent antioxidant properties. We conducted the antioxidant activity experiments on the higher contents components in PEF-RO and their scavenging rates for DPPH and ABTS were measured. The obtained results revealed that verbenone, vitamin A, *trans*-geraniol, linolenic acid, α-terpineol, and β-caryophyllene exhibited superior DPPH and ABTS free radical scavenging abilities. As previously reported, the high antioxidant activity can be attributed to the significant presence of terpenoids and olefins, as well as the effective capture of free radicals by the carbon-carbon double bonds [30]. These components

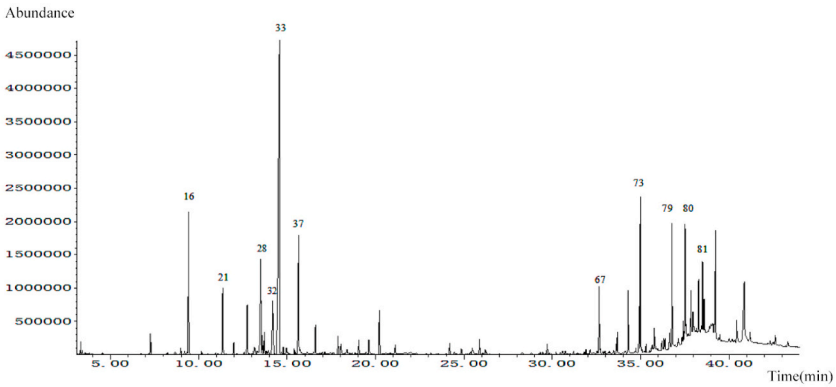

Fig. 1. The total ions chromatogram of PEF-RO.

Table 1
Composition analysis and relative content of PEF-RO.

NO.	Time (min)	Compound	relative content (%)	Category
1	3.32	2,2-diethoxypropane	0.03332	ethers
2	3.41	3-methylheptane	0.01457	hydrocarbons
3	3.57	*cis*-1,3-dimethylcyclohexane	0.00581	hydrocarbons
4	3.73	*cis*-1-ethyl-2-methylcyclopentane	0.00622	hydrocarbons
5	3.82	octane	0.00510	hydrocarbons
6	3.89	*trans*-1,2-dimethylcyclohexane	0.00187	hydrocarbons
7	3.99	*cis*-1,4-dimethylcyclohexane	0.00231	hydrocarbons
8	4.55	4-hydroxy-4-methyl-2-pentanone	0.00780	ketones
9	6.73	1R-α-pinene	0.00433	terpenes
10	7.16	(+)-campene	0.00148	terpenes
11	7.26	4-methylene-1-isopropyl-dicyclo[3.1.0]hex-2-ene	0.07462	terpenes
12	8.64	E,E−2,6-dimethyl-1,3,5,7-octatetraene	0.01768	terpenes
13	8.98	α-terpinene	0.02630	terpenes
14	9.19	*m*-thymein [1-Methyl-3-(1-methylethyl)-benzene]	0.01384	aromatic hydrocarbons
15	9.33	D-limonene	0.01054	terpenes
16	9.42	1,8-eucalyptol	0.53233	terpenes
17	10.15	γ-terpinene	0.01399	terpenes
18	10.50	*cis*-linardol epoxide	0.00367	terpenes
19	10.94	α- terpinolene	0.00861	terpenes
20	11.08	dehydro-p-thyme	0.00465	aromatic hydrocarbons
21	11.34	linalool	0.27585	terpenes
22	11.98	chrysanthemone	0.05167	ketones
23	12.57	*trans*-rosin celerythol	0.01211	terpenes
24	12.75	camphor	0.21821	terpenes
25	13.12	pinecone I	0.03189	terpenes
26	13.17	(+/−)-2(10)-pinec-3-one	0.02689	terpenes
27	13.29	2,2,4-trimethyl-3-cyclopentene-1-ethanol	0.01950	alcohols
28	13.49	*trans*-borneol	0.50808	terpenes
29	13.58	pincampone II	0.07321	terpenes
30	13.70	(−)-isoterpene pinol	0.09520	terpenes
31	13.93	α,α,4-trimethylbenzyl alcohol	0.02439	alcohols
32	14.17	α-terpineol	0.37909	terpenes
33	14.55	verbenone	2.43777	terpenes
34	14.96	citronellol	0.03887	terpenes
35	15.40	(−)-campene	0.02263	terpenes
36	15.47	(S)-2-methyl-5-(1-methylvinyl)-2-cyclohexen-1-one	0.00994	ketones
37	15.65	*trans*-geraniol	0.59978	terpenes
38	16.47	*cis*-chrysanthemenylformate	0.00868	esters
39	16.59	(1S-endo)-1,7,7-trimethyl-norbornan-2-yl acetate	0.12411	esters
40	16.81	carvacrol	0.01189	terpenes
41	17.01	4-isopropylbenzyl alcohol	0.01382	alcohols
42	18.04	3-methyl-6-(1-methylethylidene)-2-cyclohexen-1-one	0.06348	ketones
43	18.38	eugenol	0.05259	phenols
44	18.54	1,3,3-trimethyl-2-oxedicyclo[2.2.2]octan-6-yl acetate	0.00596	esters
45	18.86	7-(1-methylethylidene)dicyclo[4.1.0]heptane	0.01166	hydrocarbons
46	19.04	geranyl acetate	0.06356	esters
47	19.62	methyleugenol	0.07610	phenols
48	20.21	β-caryophyllene	0.18760	terpenes
49	20.84	geranyl acetone	0.00772	ketones
50	21.12	lymetholene	0.04307	terpenes
51	22.30	2,4-di-tert-butyl-phenol	0.01151	phenols
52	22.86	dihydrokiwi lactone	0.00446	ketones
53	23.12	dihydro-5,5-dimethyl-4-(3-oxobutyl)-2(3H)-furanone	0.00861	ketones
54	24.19	(−)-caryophyllene (or syringene) oxide	0.05098	terpenes
55	24.46	2-(4a,8-dimethyl-2,3,4,4a,5,6-hexahydronaphthal-2-yl)propan-1-ol	0.01117	alcohols
56	24.84	1,5,5,8-tetramethyl-12-oxadicyclo[9.1.0]dodecan-3,7-diene	0.02859	ethers
57	25.46	10,10-dimethyl-2,6-dimethylenedicyclo[7.2.0]undecane-5β-ol	0.06429	alcohols
58	25.87	1-methyl-6-methylenedicyclo[3.2.0]heptane	0.06843	hydrocarbons
59	28.27	6-hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	0.01725	ketones
60	28.81	ethyl tetradecanoate	0.00857	esters
61	29.71	neophytodiene	0.04421	terpenes
62	29.84	3,7,11,15-tetramethyl-2-hexadecene	0.00881	hydrocarbons
63	30.45	phenyl methyl 2-hydroxybenzoate	0.01427	esters
64	30.73	4,4,8-trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	0.02137	alcohols
65	31.20	farnesylacetone	0.01365	ketones

(*continued on next page*)

Table 1 (*continued*)

NO.	Time (min)	Compound	relative content (%)	Category
66	31.49	methyl hexadecanoate	0.00487	esters
67	32.65	palmitic acid ethyl ester	0.26321	esters
68	33.62	1,2,3,4,4a,9,10,10a-Octahydro-1,1,4a-trimethyl-7-isopropyl-phenanthrenene	0.03600	aromatic hydrocarbons
69	34.28	phytantriol	0.21486	terpenes
70	34.66	1,3-cyclooctadiene	0.01076	hydrocarbons
71	34.72	ethyl 3-hydroxyhexadecanoate	0.02707	esters
72	34.91	ethyl linoleate	0.14738	esters
73	34.99	linolenic acid	0.57134	organic acids
74	35.06	ethyl oleate	0.01858	esters
75	35.30	ethyl stearate	0.03007	esters
76	35.52	phytoacetate	0.00900	esters
77	36.21	6,10-dihydro-8-(4-methoxyphenyl)-10,10-dimethyl-6-oxo-pyridinyl [1,2-a]indole-7-nitrile	0.07790	heterocycles
78	36.32	1R,4S,7S,11R-2,2,4,8-tetramethyltricyclo[5.3.1.0(4,11)]undecy-8-ene	0.06193	hydrocarbons
79	36.76	ferruginal	0.51956	terpenes
80	37.50	vitamin A	0.68544	terpenes
81	38.59	13-isopropylmohansonene-12-ol-20-aldehyde	0.49316	aldehydes
82	41.19	squalene	0.22986	terpenes

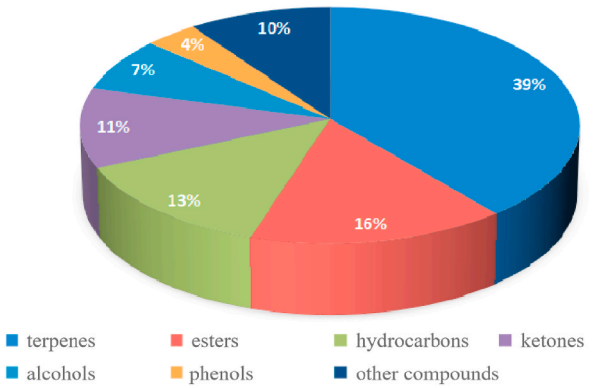

Fig. 2. Proportion of PEF-RO content classification.

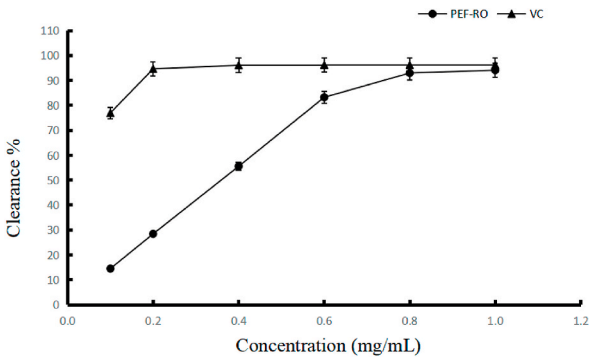

Fig. 3. DPPH radical scavenging of PEF-RO.

create a powerful synergy [31], enhancing the beneficial effects of the extract. However, the reported study highlighted that the antioxidant activity of PEF-RO is not solely dependent on its main components and may be influenced by minor ones. There are many trace components in PEF-RO. Therefore, it is vital to consider the synergistic, antagonistic, and additive effects when evaluating the antioxidant capacity of PEF-RO based on its constituents. Furthermore, different extraction methods and processes can influence the types and composition of chemical components in PEF-RO. In order to provide a theoretical basis for the development and utilization of PEF-RO as a natural antioxidant, further research should be conducted to study the influence of the extraction process on the types and

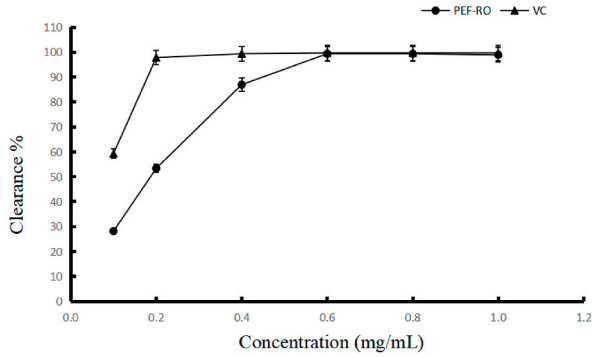

Fig. 4. Free radical scavenging of ABTS of PEF-RO.

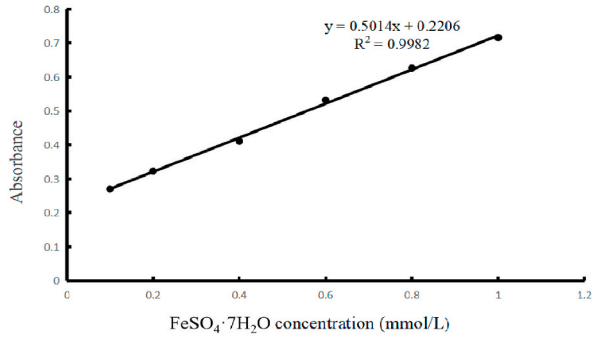

Fig. 5. $FeSO_4{\cdot}7H_2O$ standard curve.

Table 2
Antioxidant capacity of PEF-RO.

PEF-RO sample concentration (mg/mL)	Total antioxidant capacity (mmol Fe^{2+}/L)
0.1	0.2142 ± 0.0020
0.2	0.4010 ± 0.0016
0.4	0.7740 ± 0.0016
0.6	1.0618 ± 0.0035
0.8	1.4348 ± 0.0072
1.0	1.7452 ± 0.0180

contents of chemical components in PEF-RO and the interactions between compounds.

4. Conclusions

The presented work describes a method for the preparation of PEF-RO. GC-MS was used to detect and analyze the components of PEF-RO. A total of 82 volatile components were identified, totaling 10.06 % of PEF-RO. The identified components included 11 hydrocarbons, 32 terpenoids, 13 esters, 9 ketones, 6 alcohols, 3 phenols, and 8 other compounds. The diverse range of components identified had significant research value. Additionally, it was found that PEF-RO had excellent antioxidant properties, with the presence of high levels of verbenone, *trans*-geraniol, linolenic acid, α-terpineol, and β-caryophyllene, which displayed good scavenging ability of DPPH and ABTS free radicals. However, the concentration of antioxidant activity was higher in PEF-RO. Overall, PEF-RO exhibited great potential as a natural antioxidant and warrants further development.

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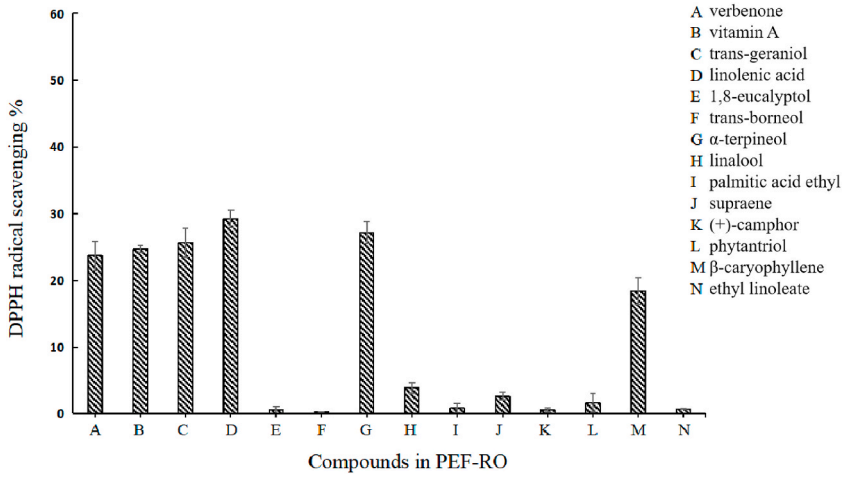

Fig. 6. Comparison of the free radical scavenging capacity of PEF-RO marker DPPH.

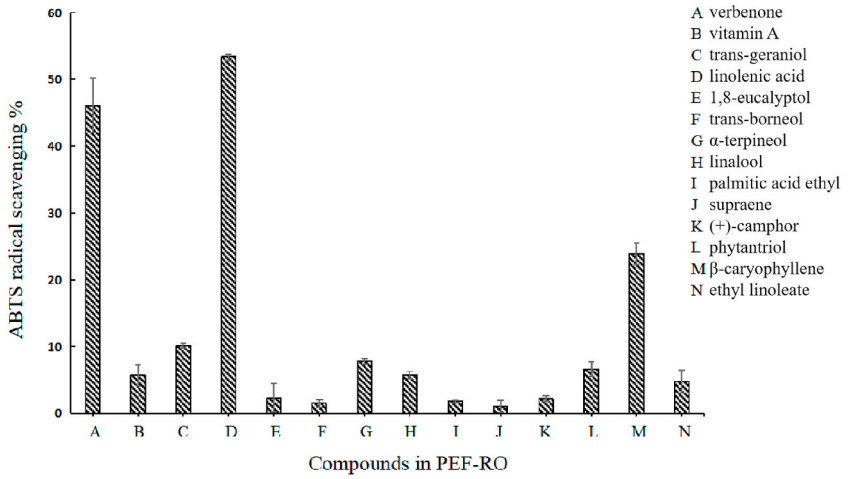

Fig. 7. Comparison of the free radical scavenging capacity of PEF-RO marker ABTS.

Data availability statement

Data included in article.

CRediT authorship contribution statement

Xiaojing Shen: Writing – original draft, Investigation, Conceptualization. **Ming Zhou:** Software, Methodology, Data curation. **Xingfan Zhu:** Software, Methodology, Data curation. **Jiaojiao Zhang:** Project administration, Software. **Junju Xu:** Supervision. **Weiwei Jiang:** Writing – review & editing, Writing – original draft, Resources, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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