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Identification of antioxidant ingredients by GC-MS from the essential oil of Purple *Eleutherococcus simonii* leaves

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Abstract

The main objective of the current study was to investigate the components and antioxidant activity of essential oil extracted from Purple Eleutherococcus leaves (identified as *Eleutherococcus simonii*) (PEEO) by hydrodistillation (HD) and solid-phase microextraction (SPME). Fourteen chemical components were identified in PEEO using gas chromatography-mass spectrometry (GC-MS), accounting for 41.21% of the total essential oil extracted by SPME, including isopropyl toluene, 4-methylene-1-(1-methylethyl) cyclohexene (3.24%), fumaric-red myrrh alcohol (2.94%), farnesol acetate (2.92%), etc. Forty-three compounds were identified by HD, accounting for 73.28% of the total essential oil extracted by HD, including α-Farnesene (3.4%), Hexadecanoic acid, methyl ester (1.28%), (α-Bisabolol (0.87%), etc. The antioxidant activity of PEEO was characterized for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, ABTS method and FRAP method, and the IC_{50} values were 1.125 mg/mL, 0.945 mg/mL and 0.862 mg/mL, respectively. The IC_{50} values for DPPH, ABTS and FRAP of essential oil were similar to those of ascorbic acid (V_C; 0.853, 0.945 and 0.037 mg/mL). The results showed that the essential oil of PEEO has a high radical scavenging activity.

Keywords: *Eleutherococcus simonii* leaves; essential oil; GC-MS; hydrodistillation; solid phase microextraction; antioxidant activity.

Practical Application: As a vegetable with high antioxidant properties, *Eleutherococcus simonii* has a good application prospect in the field of health care products or cosmetics.

1 Introduction

There are 37 species of Eleutherococcus plants found all over the world, mainly distributed in China, Japan and Korea, also found in other countries such as India, Bhutan, Mongolia, Nepal, Philippines, northeast Russia, Thailand and Vietnam. Most plants of them have medicinal value, such as tonic, anti-rheumatism, anti-stress, anti-fatigue, anti-tumor and other effects, which are clinically used to treat rheumatism numbness, waist and knee weakness and other diseases (Ni & Liu, 2006). Due to the special climate of the Puer city in Yunnan province, a kind of Eleutherococcus specie (*Eleutherococcus simonii*) showed purple characteristics, named Purple Eleutherococcus by locals. The tender leaves are purple, which is caused by plateau environment. Purple Eleutherococcus have an aromatic smell and rich of anthocyanins with great potential as local vegetables and tea. In our previous study, Purple Eleutherococcus was identified as *Eleutherococcus simonii* by ITS2 marker and morphology. In recent years, researchers have found that the leaves of Eleutherococcus plants contain diterpenoids, lignans, phytosterol, triterpenoids, flavonoids, phenols, coumarins, and fatty acids (Liu et al., 2002; Yook et al., 2002). Pharmacological studies reported that Eleutherococcus plants have anti-tumor, anti-inflammatory, hypoglycemic, anti-aging, liver protective and suppressive effects (Liu et al., 2017; Lu et al., 2018b). At present, *Eleutherococcus simonii* has been applied clinically (Zhang et al., 2011). However, its chemical composition and biological activity has not yet been carried out a lot. Therefore, the aim of this study was to investigate the composition and biological activity of the essential oil from Purple *Eleutherococcus simonii* leaves collected from the Puer city in Yunnan province and define the chemodiversity of the plant and the consequent potential value of this natural resource.

According to current reports, the essential oil of the Eleutherococcus plants is mainly extracted from leaves (Zheng et al., 2012), stems (Yu et al., 2005) and roots bark (Hu et al., 2009b, 2012). The main essential oil components of leaves include 1, 5, 8-menthtriene, ditert-butyl-p-cresol butyl isobutyl phthalate and β-linalool (Hu et al., 2009a; Muselli et al., 1999). There are caryophyllene oxide, palmitic acid and γ-elemene,etc in the root of Eleutherococcus plants (Song et al., 2015). In addition, studies compared the constituents of the essential oils from the root bark of 8 species of Acanthopanax, and identified 24 common components, mainly terpenoids. Its reported activities include

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antimicrobial activity (Hu et al., 2009a) and anti-inflammatory (Hu et al., 2014), antioxidant and cytotoxic activity (Hu et al., 2012).

Purple *Eleutherococcus simonii* is a local vegetable in Yunnan. It has a unique aroma and may have special essential oil components. Therefore, we need to verify its composition and pharmacological activity, and then we can expand the medicinal resources and avoid the waste of resources. It can also promote the comprehensive development and utilization of Acanthopanax. In this study, Purple *Eleutherococcus simonii* leaves' essential oil was extracted by HD and SPME method. The constituents were determined by GC-MS. The antioxidant activity was analyzed using three methods (DPPH, ABTS, FRAP) by radical scavenging activity assays.

2 Materials and methods

2.1 Plant materials and chemical reagents

Dry Purple *Eleutherococcus simonii* leaves were collected in Mojiang county, Puer city in Yunnan province, China. The antioxidant activity was determined by using DPPH reagent (Shanghai Maclean Biochemical Technology Co., Ltd., China), ABTS (Shanghai Maclean Biochemical Technology Co., Ltd., China), TPTZ Reagent (Yuanye Biotechnology Co., Ltd., China), Potassium persulfate (Tianjin Yongda Chemical Reagent Co., Ltd., China), and Ferrous sulfate heptahydrate (Pharmaceutical Group Chemical Reagent Co., Ltd., China). There are some other reagents such as ferric chloride hexahydrate (Wuxi Prospect Chemical Reagent Co., Ltd., China), anhydrous sodium acetate (Wenzhou Reagent Chemical Factory, China), ether, ethanol, anhydrous magnesium sulfate, concentrated hydrochloric acid, concentrated sulfuric acid and glacial acetic acid (Hangzhou Gaojing Fine Chemical Co., Ltd., China).

2.2 Instruments and equipment

The SPME was carried out on Manual solid-phase microextraction device (Shanghai Anpu Experimental Technology Co., Ltd., China), using 65 μm PDME/DVB extraction fiber head (Shanghai Anpu Experimental Technology Co., Ltd., China), Magnetic stirrer (Heidolph Company, Germany). The GC-MS was carried out on 6890/5973 GC-MS combined instrument (Agilent Company, USA). The Hydrodistillation was performed by using HD device (Nantong Tongzhou Shentong Heater Factory, China) and heating jacket (Nantong Tongzhou Shentong Heater Factory, China). The absorbance was measured by UV-5200 visible spectrophotometer (Shanghai Yuanxi Instrument Co., Ltd., China). The thermostatic water bath was used on HWS28 type electric heating constant temperature water bath (Shanghai Yiheng Scientific Instrument Co., Ltd., China).

2.3 Extraction of essential oil

Solid-phase microextraction (SPME)

The shredded sample (1 g) was placed in a 15 mL special sampling bottle, inserted into a manual sampler with 65 micron PDME/DVB fiber head, extracted at a magnetic stirring speed of 480 r/min at 65 lower headspace for 1 h, and then immediately inserted into the chromatograph sample inlet (temperature 250) for desorption for 5 min.

Hydrodistillation (HD)

The medicinal PEEO (200 g) was mixed with appropriate amount of distilled water and shaken and sealed for 1 hour. The mixture was heating for 5 hours on the essential oil tester with the reflux condenser. The essential oil was extracted by 10 mL of ether and combined and dried by anhydrous sodium sulfate after collection. The essential oil was stored in the sample bottle and put in the refrigerator after filtration and evaporation.

2.4 Gas chromatography (GC) conditions

The column was a DB-5MS quartz elastic capillary column (30 m × 0.25 mm × 0.25 μm). Carrier gas was He. The flow rate was 1 mL/min. Inlet temperature was 250 °C. Column initial temperature was 50 °C (for 1 min). Raise to 210 °C at a heating rate of 5 °C/min (for 10 min). No split injection.

2.5 Mass spectrometry (MS) conditions

The ion source temperature was 230 °C. The quadrupole temperature was 150 °C. The interface temperature was 250 °C. The transmission line temperature was 280 °C. The mass range was 30-350 amu. The electron multiplier voltage was 1765V. Ionization mode: EI, energy 70 eV.

2.6 Determination of antioxidant activity of essential oil

DPPH method

The standard stock solution (absorption value of 0.78-0.82 at 517 nm) was obtained mixing 3.943 mg of DPPH reagent and absolute ethanol in 100 mL brown volumetric. The essential oil (1 g/mL) was subjected to gradient dilution (0.5, 1.0, 1.5, 2.0, 2.5 mg/mL), each of essential oil was mixed with DPPH (1:3) to 5 centrifuge tubes and placed in the dark for 30 min. The absorbance value was measured at 517 nm (Note: Ai). Repeat the above steps with mixing essential oil and absolute ethanol solution (Note: Aj). Same to mixing absolute ethanol solution and DPPH (Note: A_0). Perform 3 parallel tests. The DPPH radical scavenging rate (E_0) was calculated according to the formula. V_C was used as a positive control, and the calculation result was used to prepare a radical scavenging rate-sample mass concentration curve.

$$E_0 = \left(1 - \left(A_i - A_j\right) / A_0\right) \times 100\% \quad (1)$$

ABTS method

The ABTS solution was mixing ABTS reagent (20.3 mg), potassium persulfate powder (3.51 mg) and distilled water (10 mL). Place the mixture at room temperature, reacted in the dark for 12-16h. Mix the mixture with ethanol (1:40) and measure the absorbance value at 734 nm (preferably about 0.7). The essential oil (1 g/mL) was subjected to gradient dilution (0.5, 1.0, 1.5, 2.0, 2.5 mg/mL), each of 5 concentrations of essential oil (0.1 mL) was mixed with ABTS (4 mL) in 5 centrifuge tubes, and then place

them in the dark for 5 min. The absorbance value was measured at 734 nm (Note: A_i). Repeat the above operation with essential and ethanol solution (Note: A_j). Same to ethanol solution and ABTS (Note: A_0). Perform 3 parallel tests. The ABTS radical scavenging rate (E_0) was calculated according to the Formula 1. V_C was used as a positive control, and the calculation result was used to prepare a radical scavenging rate-sample mass concentration curve.

FRAP assay

Drawing of the $FeSO_4$ standard curve

The concentration of $FeSO_4$ standard solution is 800 μmol/L, then obtain gradient $FeSO_4$ dilution (400 μmol/L, 200 μmol/L, 100 μmol/L, 50 μmol/L and 25 μmol/L) and measure the absorbance value at 593 nm to prepare a standard curve.

Solution preparation

40 mmol/L hydrochloric acid solution: The concentrated hydrochloric acid (0.1 mL, 12 mol/L) was added to distilled water (30 mL) and stored in the dark.

0.3 mol/L sodium acetate buffer solution: Sodium acetate (5.1 g) was added to glacial acetic acid (20 mL). The mixture was diluted to 250 mL with distilled water and stored in the dark.

10 mmol/L TPTZ solution: TPTZ (31.233 mg) was added to appropriate amount of hydrochloric acid (40 mmol/L). The mixture was diluted to 250 mL with hydrochloric acid and stored in the refrigerator.

FRAP working solution: TPTZ solution (2.5 mL, 10 mmol/L), $FeCl_3{\cdot}6H_2O$ (2.5 mL, 20 mmol/L), acetate buffer (25 mL, 0.3 mol/L, pH 3.6) and FRAP working solution (2.7 mL, 37 °C) was added to essential oil (0.3 mL) in five centrifuge tubes, respectively. The absorbance was measured at 593 nm (Note: A_i). Perform 3 parallel tests. Replace FRAP working solution with ethanol solution and repeat the above operation and measure the absorbance value at 593 nm (Note: A_0). According to the measured absorbance value, the corresponding $FeSO_4$ concentration was determined on the standard curve, defined as FRAP value. The larger the value, the stronger the antioxidant activity. V_C was used as the positive control, and the calculation result was used to produce FRAP value-sample mass concentration curve.

The 50% inhibitory concentration (IC_{50}) can be determined by a regression model of the radical scavenging rate-sample concentration curve. The larger the value is, the weaker the antioxidant activity of the essential oil has.

2.7 Statistical analysis

The DPPH radical, ABTS and FRAP scavenging activities were analyzed using analysis of variance and Origin 8.0 (Origin Lab Corporation, USA). The IC_{50} value was calculated by using SPSS Statistics 19 software (IBM, USA). Each experiment was repeated three times, the mean value was calculated, and the experimental results are presented as the means ± SD (standard deviation).

3 Results and discussion

3.1 GC-MS results

The results are shown in Figure 1 and Figure 2, Table 1 and Table 2. Among them, the essential oil extracted by SPME method identified 14 kinds of compounds, accounting for 41.21% of the total essential oil extracted by this method, mainly including p-Cymene (4.28%) and cyclohexene, 4-methylene-1-(1-methylethyl) (3.24%), α-Bisabolol (2.94%), farnesol acetate (2.92%). The essential oil extracted by SPME method is mainly alkane (22.63%). The essential oil extracted by HD method identified kinds of compounds, which accounted for 73.28% of

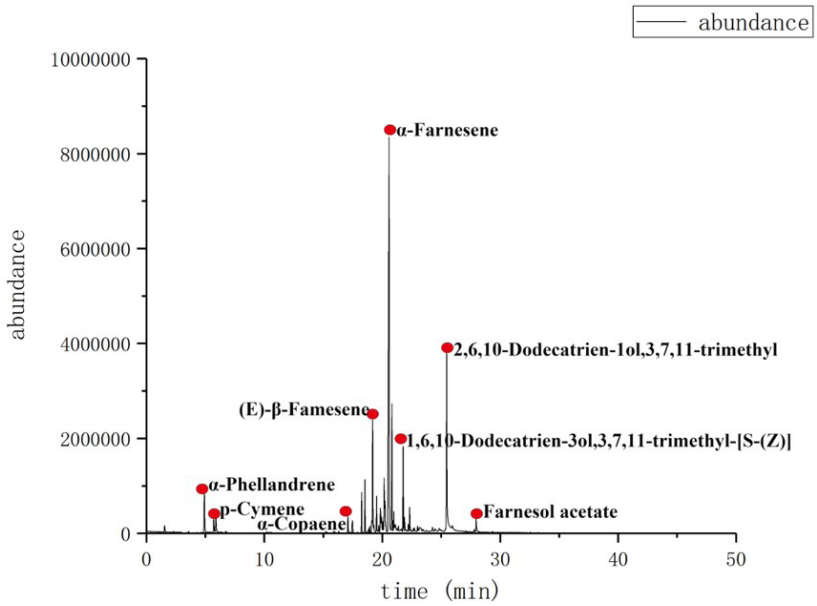

Figure 1. Total ionic flux of essential oil extracted by solid phase microextraction.

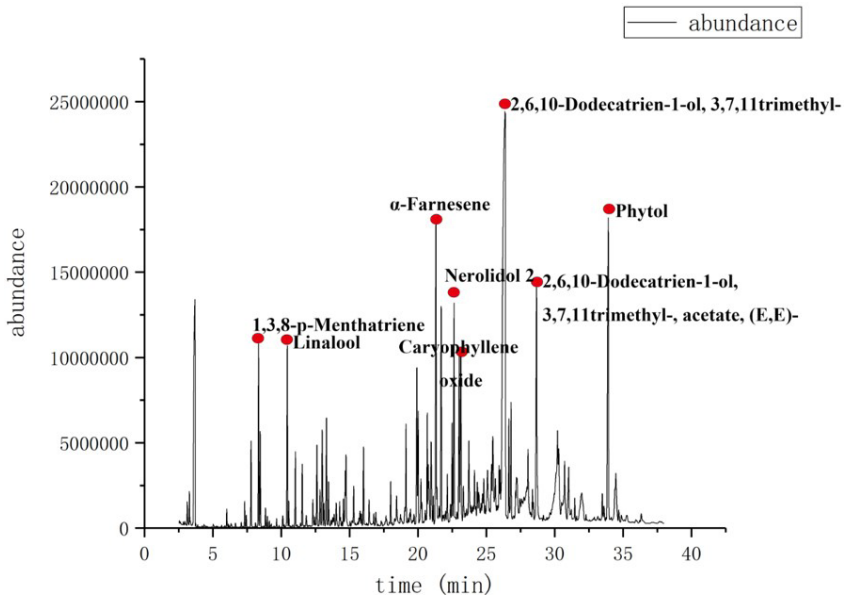

Figure 2. Total ionic flux of essential oil extracted by Steam distillation method.

Table 1. The constituents and relative contents of essential oil extracted by SPME.

No.	Retention Time	Compound Molecular	formula	Molecular weight	Relative content (%)
1	4.912	α-Phellandrene	C10H16	136	2.6
2	5.706	p-Cymene	C10H14	134	4.28
3	5.895	Cyclohexene,4-methylene-1-(1-methylethyl)	C10H16	136	3.24
4	17.085	α-Copaene	C15H24	204	2.78
5	18.246	Caryophyllene	C15H24	204	2.59
6	18.531	γ-Elemene	C15H24	204	3.11
7	19.187	(E)-β-Famesene	C15H24	204	2.58
8	19.704	γ-Muurelene	C15H24	204	2.89
9	20.573	α-Farnesene	C15H24	204	2.84
10	21.882	1,6,10-Dodecatrien-3ol,3,7,11-trimethyl-[S-(Z)]	C15H26O	222	2.81
11	22.326	Caryophyllene oxide	C15H24O	220	2.76
12	24.82	α-Bisabolol	C15H26O	222	2.94
13	25.465	2,6,10-Dodecatrien-1ol,3,7,11-trimethyl	C15H26O	222	2.87
14	27.951	Farnesol acetate	C17H28O2	264	2.92

the total essential oil extracted by this method, mainly including Caryophyllene (1.01%), (E)-β-Famesene (2.26%), α-Farnesene (3.40%), Caryophyllene oxide (1.85%) and 2,6,10-Dodecatrien-1ol,3,7,11-trimethyl (18.61%). The constituents of the essential oil by HD method were mainly alcohols (41.44%). α-Bisabolol is a sesquiterpene alcohol found in chamomile (Matricaria sp.) and other plants and has been widely used in dermatological and cosmetic formulations (Gomes-Carneiro et al., 2005). Previous studies showed that α-Bisabolol demonstrated the activities of major human drug-metabolizing enzymes (Ganzera et al., 2006), and antimutagenic (Gomes-Carneiro et al., 2005) and antiseptic (Isaac & Thiemer, 1975) properties. Other studies have found that α-Bisabolol have cytoprotective effects on ethanol-induced mucosal damage. The mechanism of gastric protection is multifactorial and may involve endogenous prostaglandins, nitric oxide release and activation of k-atp channels (Bezerra et al., 2009). Elemene is a sesquiterpene compound, there are a variety of isomers, including methyl-elemene and ramethyl-elemene, which are optical isomers. Both of them have inhibitory effects on tumors (Li et al., 2013; Shi et al., 2014). Studies have shown that elemene inhibits the activity of DNA methyltransferase in tumor cells by activating the erk1/2 and AMPK signaling pathways (Zhao et al., 2015). p-Cymene has been reported to have antibacterial activity (Bagamboula et al., 2004). 2,6,10-Dodecatrien-1ol,3,7,11-trimethyl is a bioactive flavoring and pharmaceutical intermediate compound and can be used for psychotropic drugs and skin protection of topical drugs.In addition, it has an inhibitory effect on cancer (Burke et al., 1997). According to the previous report, caryophyllene is a dicyclic sesquiterpene compound, the pharmacological action of caryophyllene mainly has the local anesthesia effect, the treatment colitis, the antitussive effect and so on (Liu et al., 2012). α-Farnesene is a sesquiterpene substance

Table 2. The constituents and relative contents of essential oil extracted by HD.

No.	Retention Time	Compound Molecular	formula	Molecular weight	Relative content (%)
1	7.792	α-Phellandrene	C10H16	136	0.73
2	8.343	1,3,8-p-Menthatriene	C10H14	134	1.61
3	8.457	1,7-Octadiene, 2-methyl-6-methylene-	C10H16	134	0.87
4	10.442	Linalool	C10H18O	154	1.74
5	11.035	2-Cyclohexen-1-ol, 1-methyl-4-(1methylethyl)-, cis-	C10H18O	154	0.68
6	11.537	Hexadecanoic acid, methyl ester	C15H26O	172	0.55
7	12.609	3-Cyclohexen-1-ol, 4-methyl-1-(1methylethyl)-, (R)-	C10H18O	172	0.79
8	12.833	Benzenemethanol, α,α,4trimethyl-	C10H14O	150	0.44
9	12.993	α-Terpineol	C10H18O	150	1.15
10	13.46	2-Cyclohexen-1-ol, 3-methyl-6-(1methylethyl)-, trans-	C10H18O	150	0.42
11	14.738	Geraniol	C10H18O	150	0.70
12	16.019	Thymol	C10H14O	150	0.78
13	18	α-Cubebene	C15H24	152	0.38
14	19.129	Caryophyllene	C15H24	152	1.01
15	19.916	5,9-Undecadien-2-one, 6,10-dimethyl-,E	C13H22O	158	1.61
16	20.019	cis-β-Farnesene	C15H24	158	1.34
17	20.677	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4methyl-	C15H22	160	0.99
18	20.749	trans-β-Ionone	C13H20O	156	0.51
19	20.962	cis-α-Bergamotene	C15H24	156	0.95
20	21.312	α-Farnesene	C15H24	156	3.40
21	21.699	(E)-β-Famesene	C15H24	156	2.26
22	22.148	α-Calacorene	C15H20	200	0.54
23	22.498	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1methylethylidene)-, (E,E)-	C15H24	200	0.97
24	22.638	Nerolidol 2	C15H26O	200	2.41
25	23.137	Caryophyllene oxide	C15H24O	216	1.86
26	23.718	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12oxabicyclo[9.1.0]dodeca-3,7-diene	C15H24O	216	1.00
27	24.133	Isospathulenol	C15H24O	216	0.52
28	24.342	(-)-Spathulenol	C15H24O	216	0.41
29	24.813	Selin-6-en-4α-ol	C15H26O	216	0.45
30	25.384	α-Bisabolol	C15H26O	216	0.87
31	25.474	6,10-Dodecadien-1-ol, 3,7,11-trimethyl-	C15H28O	240	1.43
32	25.657	(E)-3,7-Dimethylocta-2,6-dienyl ethyl carbonate	C13H22O3	226	0.68
33	26.387	2,6,10-Dodecatrien-1-ol, 3,7,11trimethyl-	C15H26O	226	18.62
34	26.65	2,6,10-Dodecatrienal, 3,7,11-trimethyl-,	C15H24O	226	0.94
35	26.809	Benzene, 1,1'-(1,2-cyclobutanediyl) bis-, cis-	C16H16	208	1.35
36	28.049	Farnesol isomer a	C15H26O	208	1.19
37	28.672	2,6,10-Dodecatrien-1-ol, 3,7,11trimethyl-, acetate, (E,E)-	C17H28O2	264	3.43
38	30.208	1,3-Butadiene, 1,4-diphenyl-, (E,E)-	C16H14	206	3.66
39	30.296	Hexadecanoic acid, methyl ester	C17H34O2	270	1.28
40	30.722	1-Hexadecen-3-ol, 3,5,11,15tetramethyl-	C20H40O	296	1.43
41	31.026	n-Hexadecanoic acid	C16H32O2	256	0.89
42	31.98	1,3-Butadiene, 1,4-diphenyl-, (E,E)-	C16H14	206	1.29
43	33.919	Phytol	C20H40O	296	5.17

with strong fruit aroma, which is related to the inductivity of insects, but its pharmacological effect has not been studied yet (Huelin & Coggiola, 1970). Hosam O. Elansary (2015) found that cyclohexene, 4-methylene-1-(1-methylethyl) had an antioxidant effect. Therefore, according to the active substances identified in PEEO, this essential oil may have analgesic activities, antibacterial and anti-cancer effects.

3.2 Determination of antioxidant activity

Scavenging effects of essential oil on DPPH free radicals

The antioxidant activity of the samples studied was expressed in IC_{50}. It was defined as the concentration of the antioxidant necessary to reduce or inhibit 50% of the DPPH radical in solution. The best activity against the DPPH radical

was obtained with the lowest IC_{50} value (Liu et al., 2012). The removal of DPPH free radicals can be measured by the reduction of absorbance at this wavelength. The higher the free radical scavenging rate of antioxidants has, the stronger the antioxidant activity is. As shown in Figure 3, the clearance rate of DPPH free radicals increased with the increase of sample concentration. The clearance rate reached 53.29% when the sample concentration was 2.5 mg/mL. There was a good dose-effect relationship between sample concentration (X) and DPPH free radical scavenging rate (Y), and its regression line equation is Y=0.1666X+0.0763 (R^2=0.9226), IC_{50}=1.125 mg/mL. The V_C regression linear equation is Y=0.3766X+0.1786 (R^2 = 0.8689), IC_{50}=0.853 mg/mL. Figure 3 show the scavenging activity of Vc for the DPPH radical was superior to that of PEEO at all concentrations, but PEEO showed a greater increase in DPPH scavenging with an increase in concentration. PEEO showed much stronger antioxidant activity against DPPH radicals compared with the activity of essential oil from *Amygdalus pedunculata* Pall leaves (IC_{50}=2.32 mg/mL) (Lu et al., 2018a).

Scavenging effects of essential oils on ABTS free radical

As shown in Figure 4, the clearance rate of ABTS free radicals increased with the increase of sample concentration. The clearance rate reached 59.54% when the sample concentration was 2.5 mg/mL. There is a good dose-effect relationship between sample concentration (X) and ABTS free radical scavenging rate (Y), and its regression line equation is Y=0.2433X+0.0225 (R^2=0.9553), IC_{50}=1.963 mg/mL. The V_C regression linear equation is Y=0.3971X+0.1247 (R^2 = 0.8852), IC_{50}=0.945 mg/mL. Figure 4 show the scavenging activity of Vc for the DPPH radical was superior to that of PEEO at all concentrations, but PEEO showed a greater increase in DPPH scavenging with an increase in concentration. And PEEO showed strong antioxidant activity against DPPH radicals compared with the activity of essential oil from *Hyptis suaveolens* (Nantitanon et al., 2007).

FRAP method

The standard curve of ferrous sulfate is shown in Figure 5, and the regression line equation is Y=0.0028X+0.1317 (R^2=0.991). The ability of essential oil to reduce Fe^{2+} is shown in Figure 6. There

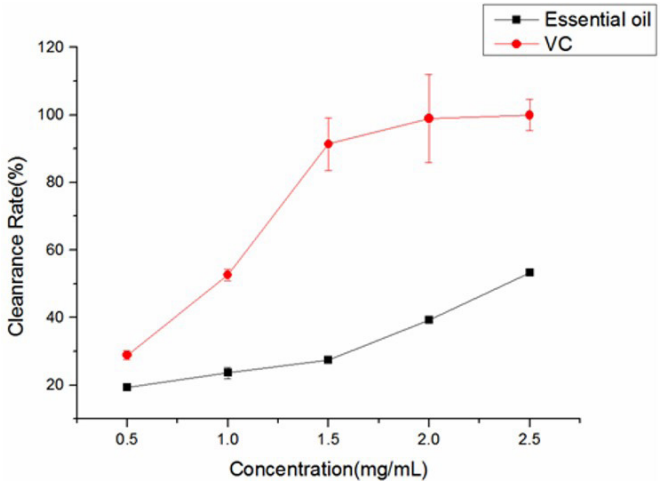

Figure 3. Scavenging effects of essential oil on DPPH free radicals.

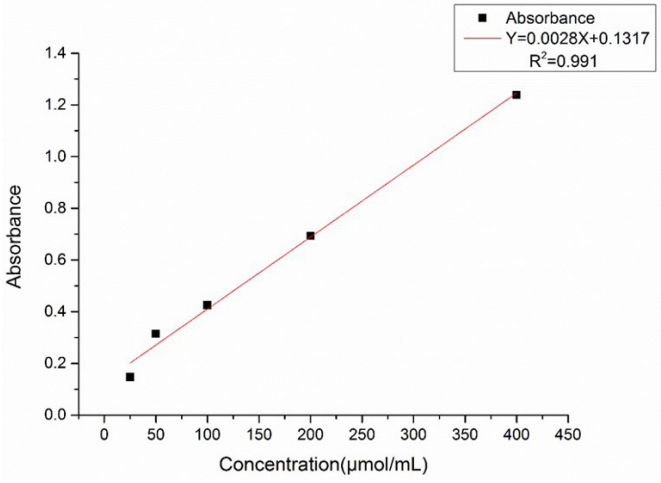

Figure 5. The standard curve of ferrous sulfate.

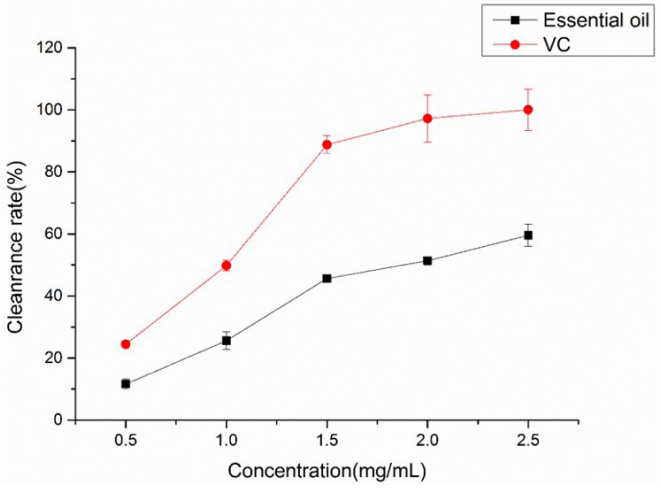

Figure 4. Scavenging effects of essential oils on ABTS free radicals.

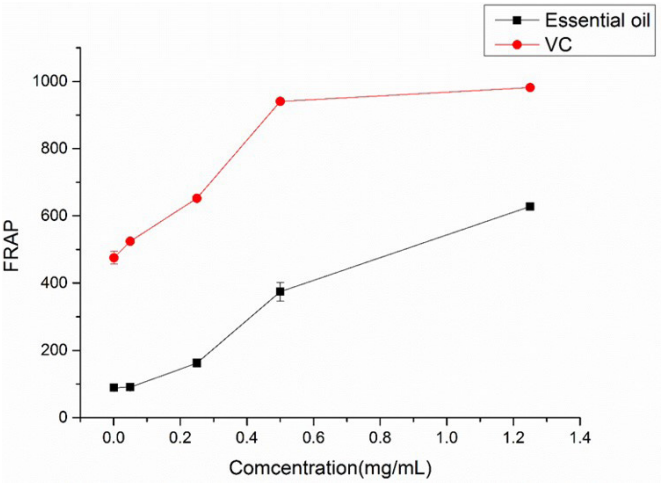

Figure 6. The reducing power of essential oil to Fe^{2+}.

is a good dose-effect relationship between sample concentration (X) and FRAP value of essential oil (Y), and its regression line equation is $Y= -132.7X^2+619.26X+64.609$ ($R^2=0.981$), $IC_{50}=0.862$ mg/mL. The V_C regression linear equation is $Y= -615.43X^2+1192.5X+456.71$ ($R^2=0.9716$), $IC_{50}=0.037$ mg/mL. The capacities of the reducing power of the essential oils were quite a bit lower than the reference antioxidant. When the concentration of essential oil was 1.25 mg/mL, its FRAP value reached 627.96, and PEEO showed a greater increase compared to Vc control. Compared with the experimental results of Abbdellaoui et al. (2019), PEEO had the better antioxidant activity.

4 Conclusion

In this experiment, we used HD and SPME two methods to extract the essential oil of Purple Eleutherococcus leaves. The former mainly extracts chemical components like alkanes and alcohols with high boiling point and high volatility, while the latter mainly extracts chemical components with low boiling point (alkanes, alcohols, esters and others). And the relative contents of essential oil extracted by HD method is higher than that by SPME method. The main chemical components of essential oil can be extracted completely by combining the two extraction methods. The essential oil has similar antioxidant activity measured by the method of DPPH, ABTS and FRAP under different concentration gradients. The antioxidant activities of essential oil were not much different from that of V_C control. Although the overall activity of essential oil was lower than that of V_C control group, it showed very good antioxidant activity. It can be concluded that PEEO is a potential source of natural antioxidants. It has a good application prospect in the field of traditional Chinese medicine health care products and cosmetics.

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