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CHEMICAL COMPOSITION AND ANTIOXIDANT EFFECT OF ESSENTIAL OILS OF THREE MEDICINAL SPECIES OF SELAGINELLA P. BEAUV.

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Abstract

The volatile oils and multiple antioxidant effects of three medicinal species of *Selaginella* namely, *S. sinensis*, *S. labordei* and *S. tamariscina* were compared. Gas chromatography-mass spectrometry (GC-MS) facilitated the rapid analysis of the volatile constituents. As a result, a total of 55 compounds were detected. Among them, 8 compounds were recognized as the common components. The DPPH-scavenging, ferric ion reducing antioxidant power (FRAP) and metal ion chelation abilities of these 3 species were evaluated *in vitro*. The results showed that *S. tamariscina* had the best FRAP abilities, and *S. sinensis* possessed the strongest DPPH-scavenging and metal chelation effects. This study revealed the constituent and activity differences of 3 medicinal species of *Selaginella*, which could provide reference for their medicinal application.

Introduction

Selaginellae Herba, which is derived from a perennial herb, is a traditional Chinese medicine with a long history of thousand years (Li *et al.* 2017). Clinically, it is often applied to treat pneumonia, nephritis and jaundice (Wang *et al.* 2015, 2016). There are about 20 species of medicinal plants of the genus *Selaginella*, and they are abundant in plant resources. Currently, *S. tamariscina* (P. Beauv.) Spring (ST) Spring and *S. pulvinata* (SP) Maxim are included in the Chinese pharmacopoeia as statutory medicinal materials (Li *et al.* 2018). In fact, many other species are widely used in rural areas of China, such as *S. sinensis* (Desv.) Spring (SS) and *S. labordei* Hieron. ex H. Christ (SL) (Wang *et al.* 2015). So far, the differences in chemical composition and activity between the civil and statutory materials have not been fully elucidated.

There are different types of volatile components in natural plants, including organic acids, monoterpenes, sesquiterpenes, aliphatic compounds, ketone etc. (Wang *et al.* 2013, 2017). Different *in vitro* assays characterize most of the essential oils and phenolic compounds as antioxidants (Rafaela *et al.* 2010). Indeed, several medicinal plants have been reported as sources of safe natural antioxidants (Alvin *et al.* 2014). In living systems antioxidants play a major role in the prevention of cancer, arteriosclerosis, malaria, rheumatoid arthritis, neurodegenerative diseases and aging processes by protecting the organism against oxidative damage (Karlson *et al.* 2010, Trouw *et al.* 2010, Qadir *et al.* 2017). Mechanism of the activity can include suppressing reactive oxygen species formation, scavenging free-radical production, chelating trace elements and upregulating or protecting antioxidant defences (Ran *et al.* 2016).

Currently, there is lack of information concerning the bioactive properties of the volatile oils in ST, SS and SL. Recently, Jung *et al.* (2006) characterized chemical structures of ST essential oils, yet no information is available on antioxidant properties of this species. In this context, the aim of this study was to study the chemical composition of essential oils in ST, SS and SL, and to

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evaluate their antioxidant activity, which was would be helpful to layout a foundation for comprehensive exploitation and utilization of three *Selaginella* species.

Materials and Methods

Selaginella sinensis, *S. labordei* and *S. tamariscina* were purchased from Meizhou Market, Guangdong Province. Anhydrous ethanol and hydrochloric acid were obtained from Chongqing Chuandong Chemical Group Co., Ltd. Ferrous sulfate, ferric chloride, ferrozine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) were purchased from Tianjin Damao Chemical Reagent Co., Ltd. Vitamin C and EDTA was purchased from Sinopharm Group Co., Ltd.

The steam distillation method was used to extract volatile oils. The plant materials were crushed into powder. 100 g of sample was weighed and placed in a 5000 ml round-bottomed flask. After that, 3000 ml distilled water was added. The sample was soaked at room temperature for 2 hrs and then extracted under microboiling condition for 6 hrs. The distillate was extracted by 30 ml hexane for 3 times. The extracts were combined and dried with anhydrous sodium sulfate. After vacuum distillation, a colorless or yellowish oily liquid was obtained and stored in a refrigerator for use.

Gas chromatography-mass spectrometry (HP6890/5975C GC-MS, Agilent corporation, USA) was applied for the analysis of chemical components in 3 *Selaginella* species. Elastic quartz capillary column (30 m × 0.25 mm × 0. 25 μm) were used to separate volatile oils.

The program of column temperature is: 0 - 2 min, 54 - 54°C; 2 - 11 min, 54 - 126°C; 11 - 36 min, 126 - 226°C; 36 - 46 min, 226 - 306°C; 47 - 54 min, 306 - 306°C. The parameters for GC-MS were set as follows: vaporization chamber temperature, 250°C; carrier gas, He (99.999%); pre-column pressure, 7.65 psi; carrier gas flow, 1.0 ml/min; injection volume, 1 μl; shunt ratio, 20:1; solvent delay time, 4.0 min; ion source, EI; ion source temperature, 230°C; quadrupole temperature, 150°C; electron energy; 70 eV; emission current, 34.6 μA; multiplier voltage, 1482 V; interface temperature, 280°C; mass range, 29 - 500 amu. The volatile oils were identified by Nist database and their retention indices (RI). Then, Kovat's RI values calculated according to the alkane series (C_7 - C_{30}) (Beijing Ruizhi Hanxing Technology Co., Ltd.), which had the same GC - MS analysis program as that applied to the sample.

The method for DPPH detection is based on previous works (Bandoniene *et al*. 2002). Four mg DPPH were accurately weighed and placed in a 100 ml brown volumetric flask. Ethanol was added to dissolve DPPH. 0.04 mg/ml DPPH solution was stored at low temperature for use. The samples were diluted to gradient concentrations with ethanol. The 2 ml sample was mixed evenly with the 2 ml DPPH solution. The mixture was put in a dark place at room temperature for a 30 min reaction. The absorbance of the reaction solution was measured at 517 nm. Ethanol and vitamin C were used as the blank control and the positive control, respectively. The DPPH-scavenging rate was calculated as follows: DPPH-scavenging rate (%) = [(Ac-As)/Ac] × 100%, where Ac is the absorbance of the blank control and As is the absorbance of the sample solution.

FRAP was operated according to the reported method (Arcari *et al*. 2013). Briefly, 27.8 mg $FeSO_4.7H_2O$ was weighed and placed in a 100 ml volumetric flask. Distilled water was added to dissolve the powder, and then ferrous solutions with gradient concentrations were prepared. FRAP solution was prepared by mixing 25 ml sodium acetate buffer (pH = 3.6), 2.5 ml TPTZ solution and 2.5 ml $FeCl_3$ solution (10 : 1 : 1). After that, 0.1 ml ferrous solution, 3 ml FRAP solution and 0.3 ml distilled water were mixed and reacted for 8 min. The absorbance of the reaction solution was detected at 593 nm. The standard curve of $FeSO_4$ solution was plotted. For the sample test,

0.1 ml of ferrous solution was replaced by the same volume of plant extract. Distilled water was used as a blank control. Vitamin C was the positive control.

The metal ion-chelating ability of plant extracts was evaluated by detecting the complex of Fe^{2+} with ferrozine. 50 mg philozine was accurately weighed and placed in a 100 ml volumetric flask. Ethanol was added to dissolve philozine. The solution was diluted to 0.2 mg/ml for storage. Then, 1 ml sample solution, 2.8 ml distilled water, 50 µl 2 mmol/l $FeCl_3$ and 150 µl 0.1 mmol/L ferrozine were mixed for 10 min. The absorbance of the reaction solution was detected at 562 nm. EDTA was used as a positive control. The chelating rate was calculated according to the following formula. Chelating rate (%) = (1-As/Ac) × 100%, where As is the absorbance of the sample solution and Ac is the absorbance of the blank control.

Results and Discussion

GC-MS was used to analyze the chemical constituents from 3 *Selaginella* species, namely, *S. sinensis*, *S. labordei* and *S. tamariscina*. The total ion chromatograms are shown in Fig. 1. By comparing the MS database (the matching degree is more than 80%) and the data in the literature, 22, 33 and 32 components were characterized from above 3 plants, respectively.

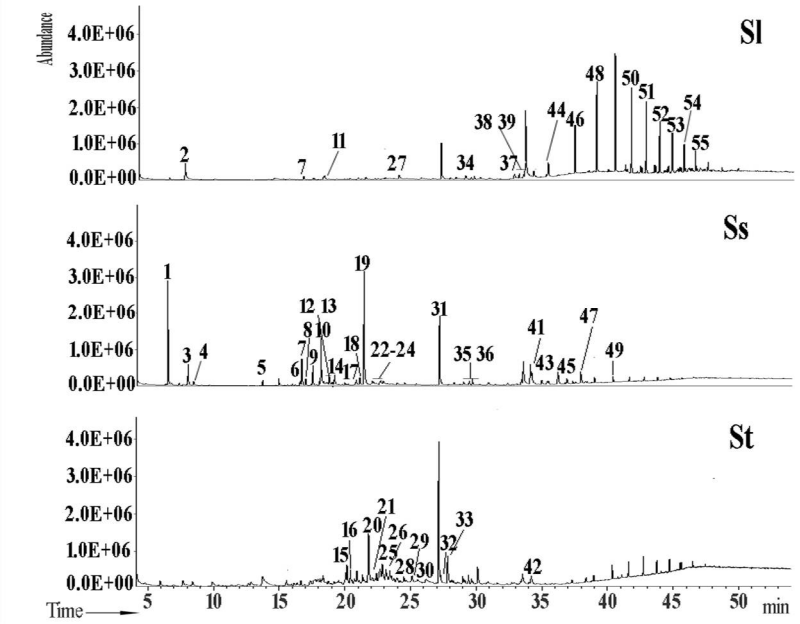

Fig. 1. Analysis diagram of the volatile oil of three *Selaginella* species by GC-MS.

The detailed information of compounds including retention time, molecular formula and relative content was summarized in Table 1. It could be seen that the 55 compounds are mainly sesquiterpenes (11), monoterpenes (3), ketones (7) and hydrocarbons (15). Eight common components were found in 3 different *Selaginella* species, including n-docosane, 3-methyltricosane, 4-methyltricosane, n-tetracosane, n-pentacosane, phytol, n-heptadecane, fitone. The distribution of different kinds of volatile components was presented in Fig. 2. The details of each species of *Selaginella* are as follows.

Table 1. Essential oil component of three *Selaginella* species.

No	RI	Rt/ min	English name	Formula	Family	Volatile oil compounds content/% ds content from three habitats/%		
						SL	SS	ST
1	917	6.47	alpha-pinene	$C_{10}H_{16}$	Monoterpenes		11.28 ± 0.02	
2	996	7.64	2-pentylfuran	$C_9H_{14}O$	Furan compounds	3.77 ± 0.01		1.56 ± 0.03
3	1005	8.01	3-carene	$C_{10}H_{16}$	Monoterpenes		2.83 ± 0.04	
4	1030	8.39	dl-limonene	$C_{10}H_{16}$	"		0.85 ± 0.02	
5	1289	13.74	cis-anethol	$C_{10}H_{12}O$	Ethers		0.91 ± 0.03	5.82 ± 0.02
6	1367	16.37	terpinyl acetate	$C_{12}H_{20}O_2$	Esters		0.74 ± 0.02	
7	1410	16.67	(+)-longifolene	$C_{15}H_{24}$	Sesquiterpene	0.55 ± 0.01	4.22 ± 0.03	
8	1428	16.96	cedrene	$C_{15}H_{24}$	"		1.16 ± 0.02	
9	1439	17.51	(-)-thujopsen	$C_{15}H_{24}$	"		2.86 ± 0.04	
10	1442	18.12	geranylacetone	$C_{13}H_{22}O$	Ketones	0.51 ± 0.01	8.19 ± 0.25	
11	1458	18.26	irisone	$C_{13}H_{20}O$	"	0.78 ± 0.05		
12	1464	18.34	alpha-caryophyllene	$C_{15}H_{22}$	Aliphatic hydrocarbon		0.74 ± 0.02	2.77 ± 0.11
13	1484	18.61	α-curcumene	$C_{15}H_{24}$	Sesquiterpene		1.71 ± 0.32	
14	1491	19.16	acetovanillone	$C_9H_{10}O_3$	Ketones		1.95 ±	
15	1517	20.01	β-selinene	$C_{15}H_{24}$	Sesquiterpene			2.51 ± 0.25
16	1525	20.17	farnesol	$C_{15}H_{26}O$	Higher alcohols			4.13 ±
17	1534	20.43	selinene	$C_{15}H_{24}$	Sesquiterpene		1.04 ± 0.02	2.26 ± 0.3
18	1539	20.92	elemicin	$C_{12}H_{16}O_3$	Ethers		1.17 ± 0.15	
19	1543	21.39	α-muurolene	$C_{15}H_{24}$	Sesquiterpene		19.27 ± 0.68	2.55 ± 0.12
20	1549	21.94	cadinene	$C_{15}H_{24}$	"			1.87 ± 0.23
21	1571	20.43	nerolidol	$C_{15}H_{26}O$	Higher alcohols			10.21 ± 0.86
22	1586	22.52	copaene	$C_{15}H_{24}$	Sesquiterpene		0.42 ± 0.01	
23	1605	22.6	cedarwood oil	$C_{15}H_{26}O$	Higher alcohols		0.95 ± 0.06	0.95 ± 0.12
24	1631	22.87	(-)-alpha-copaene	$C_{15}H_{24}$	Sesquiterpene		0.91 ± 0.02	4.98 ± 0.65
25	1645	23.12	beta-eudesmol	$C_{15}H_{26}O$	Higher alcohols			1.67 ± 0.31
26	1650	23.43	beta-eudesmol	$C_{15}H_{26}O$	"			1.18 ± 0.05
27	1665	23.92	(e,e)-farnesol	$C_{15}H_{26}O$	"	0.99 ± 0.05		

(Contd.)

28	1687	24.48	y-muurolene	$C_{15}H_{24}$	Sesquiterpene			0.88 ± 0.25
29	1700	24.93	1-heptadecene	$C_{17}H_{34}$	Aliphatic hydrocarbon			0.62 ± 0.21
30	1726	25.13	methyl hexadecanoate	$C_{17}H_{34}O_2$	Esters			1.71 ± 0.34
31	1738	27.15	fitone	$C_{18}H_{36}O$	Ketones	5.48 ± 0.25	10.64 ± 0.56	20.08 ± 0.15
32	1745	27.29	1-bromooctadecane	$C_{18}H_{37}Br$	halohydrocarbon			1.83 ± 0.21
33	1763	27.83	myristic acid	$C_{14}H_{28}O_2$	Fatty acid			5.31 ± 0.33
34	1834	28.99	n-heptadecane	$C_{17}H_{36}$	Aliphatic hydrocarbon	0.94 ± 0.25	0.48 ± 0.06	1.12 ± 0.05
35	1852	29.41	2-heptadecanone	$C_{17}H_{34}O$	Ketones		0.64 ± 0.21	1.31 ± 0.52
36	1877	30.11	diisobutyl phthalate	$C_{16}H_{22}O_4$ C16H22O4 C16H22O4	Esters		1.03 ± 0.31	4.21 ± 0.02
37	1939	32.75	margaric acid(p)	$C_{17}H_{34}O_2$	Fatty acid	1.17 ± 0.15		
38	1954	33.08	oleic acid	$C_{18}H_{34}O_2$	"	0.77 ± 0.31		
39	1967	33.42	2-methylheptadecane	$C_{20}H_{42}$	Aliphatic hydrocarbon	0.67 ± 0.05		
40	1973	33.53	phytol	$C_{20}H_{40}O$	Higher alcohols	14.13 ± 0.15	5.86 ± 0.69	3.09 ± 0.58
41	1989	34.17	dehydroepiandrosterone	$C_{19}H_{28}O_2$	Ketones		3.29 ± 0.65	
42	1995	34.2	octadecane	$C_{18}H_{38}$	Aliphatic hydrocarbon			2.53 ± 0.21
43	2038	34.2	n-nonadecane	$C_{19}H_{40}$	"	1.17 ± 0.11	2.84 ± 0.35	
44	2065	35.27	stearic acid	$C_{18}H_{36}O_2$	Fatty acid	2.64 ± 0.35	3.64 ± 0.25	
45	2129	36.9	n-eicosane	$C_{20}H_{42}$	Aliphatic hydrocarbon		1.47 ± 0.25	
46	2147	37.34	nonadecanoic acid	$C_{19}H_{38}O_2$	Fatty acid	5.93 ± 0.25		
47	2153	37.92	totarol	$C_{20}H_{30}O$	Phenols		2.64 ± 0.68	
48	2192	38.96	n-docosane	$C_{22}H_{46}$	Aliphatic hydrocarbon	10.01 ± 0.15	0.59 ± 0.06	0.73 ± 0.05
49	2219	40.41	3-methyltricosane	$C_{24}H_{50}$	"	12.22 ± 0.89	0.61 ± 0.311	1.76 ± 0.24
50	2230	41.61	2-methyltricosane	$C_{24}H_{50}$	"	7.99 ± 0.21	0.62 ± 0.1	1.61 ± 0.25
51	2235	42.74	n-tetracosane	$C_{24}H_{50}$	"	6.81 ± 0.05	0.47 ± 0.36	2.02 ± 0.21
52	2246	43.76	n-pentacosane	$C_{25}H_{52}$	"	4.77 ± 0.35	0.42 ± 0.06	1.28 ± 0.58
53	2267	44.72	8- hexylnonadecane	$C_{25}H_{52}$	"	3.09 ± 0.25		1.31 ± 0.56
54	2289	45.5	n-hexacosane	$C_{26}H_{54}$	"	1.87 ± 0.25		0.81 ± 0.06
55	2312	46.5	n-heptacosane	$C_{27}H_{56}$	"	1.09 ± 0.21		0.62 ± 0.04

In SS, α-muurolene (19.27%), alpha-pinene (11.28%), fitone (10.64%), geranyl acetone (8.19%), phytol (5.86%) and longifolene (4.22%) were the three components with the highest content. In SL, the most abundant ingredients include phytol (14.13%), 3-methyltricosane (12.22%), n-docosane (10.01%), n-tetracosane (6.81%) and fitone (5.28%). In ST, fitone (20.08%) remains the most abundant ingredient followed by nerolidol (10.21%), anethole (5.82%), myristic acid (5.31%), ylangene (4.98%), linalool (4.13%) and phytol (3.09%). These results suggest that some *Selaginella* species have common principal components, implying that they may have similar biological activity.

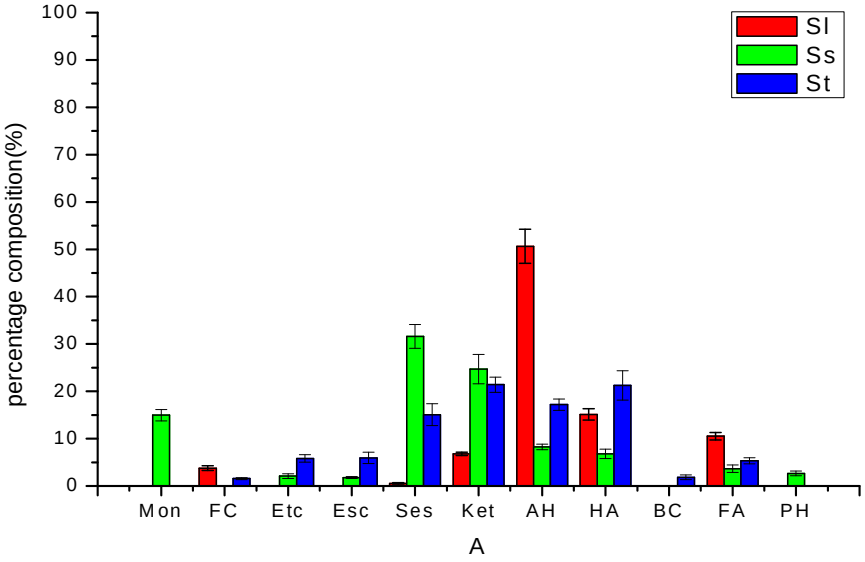

Fig. 2. Distribution of chemical classes of volatile components extracted from three species *Selaginella*. Chemical class code: Mon: monoterpenes; FC: Furan compounds; Etc: Ether compounds; Esc: Ester compound: Ses: Sesquiterpenes; Ket: Ketone; AH: Aliphatic hydrocarbon; HA: Higher alcohols; BC: Bromine compound; FA: Fatty acid; PH: Phenolic.

DPPH, FRAP and metal ion-chelating ability were applied to evaluate the antioxidant activity of 3 *Selaginella* species. As shown in Fig. 3, the plant extracts could exert antioxidant effect in a concentration-dependent manner. Their IC_{50} values were compared as shown in Fig. 3. The DPPH-scavenging activities of different extracts were ranked as vitamin C (the positive control) > SS > ST > SL. It is noteworthy that SS has the strongest scavenging effect on DPPH among the three plants, of which the IC_{50} value (3.85 μg/ml) is higher than that of vitamin C (2.45 μg/ml). SL possesses the weakest activity, and its IC_{50} value is 5.67 μg/ml.

The order of FRAP activity was vitamin C (the positive control) > ST > SS > SL. Interestingly, the order of FRAP activity of the three plants was opposite to that of DPPH-scavenging activity. Among them, ST possesses the highest FRAP value of 9136 μmol/g, while SL has the weakest activity of 5764 μmol/g. The metal ion-chelating abilities were ranked as EDTA (the positive control) > SS > ST > SL. The activities of the *Selaginella* species were all weaker than that of EDTA. Among the 3 plants, SS is the most effective with the EC_{50} of 3.58 μg/ml, and SL is the weakest with EC_{50} of 4.81 μg/ml. In addition, the results were in line with the DPPH free radicals scavenging data mentioned above. For the above three activities, the activity of SS is noticeable, because its DPPH-scavenging activity and metal ion-chelating ability are the strongest.

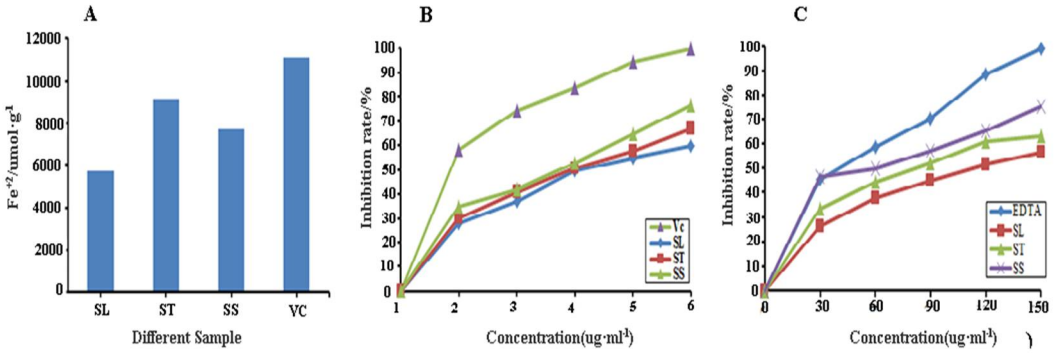

Fig. 3. Antioxidant activity of three *Selaginella* sp. (**A**) ferric ion reducing antioxidant power; (**B**) DPPH radical scavenging ability and (**C**) ferric chelation power.

The chemical composition and antioxidant potential of three medicinal plants of *Selaginella* were compared. A total of 55 compounds were characterized by GC-MS, of which 8 were recognized as the common components. Three antioxidant activities of the plant extracts were evaluated *in vitro*. *S. sinensis* possessed the strongest DPPH-scavenging activity and metal ion-chelating ability. *S. tamariscina* had the best FARP ability. Compared to *S. tamariscina*, which is listed in Chinese pharmacopoeia, *S. sinensis* may have better activity in some aspects. This study would be helpful to expand the sources of medicinal *Selaginella*.

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Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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