

Article

Comparative Analysis of Chemical Composition, Anti-Inflammatory Activity and Antitumor Activity in Essential Oils from *Siegesbeckia orientalis*, *S. glabrescens* and *S. pubescens* with an ITS Sequence Analysis

Xiaoxu Gao, Jiangchun Wei, Lina Hong, Sanpeng Fan, Gaosheng Hu *  and Jingming Jia *

School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China; 18842643805@163.com (X.G.); jiangchun_w@126.com (J.W.); honglina777@163.com (L.H.); threehedgedhod@163.com (S.F.)

* Correspondence: hugsh_2011@163.com (G.H.); jiajingming@163.com (J.J.);
Tel.: +86-24-2398-6501 (J.J. & G.H.); Fax: +86-24-2398-6216 (J.J. & G.H.)

Received: 2 August 2018; Accepted: 21 August 2018; Published: 30 August 2018



Abstract: Herba *Siegesbeckiae* (HS), derived from the aerial parts of three plants, *Siegesbeckia orientalis* (SO), *S. glabrescens* (SG), and *S. pubescens* (SP), has been used for the treatment of inflammatory diseases in China for centuries. In the present study, hydrodistillation was applied to extract essential oils from dried SO, SG, and SP aerial parts, and chemical composition analysis by gas chromatography–mass spectrometry (GC-MS) led to the identification of a total of 148 compounds (56 in SO, 62 in SG, and 59 in SP). The main components in the essential oils of SO, SG, and SP differed significantly. In vitro anti-inflammatory activity assays showed that SP essential oils (IC₅₀, 0.97 µg/mL) significantly reduced the ability of lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages to release NO, and the SO essential oil (IC₅₀, 14.99 µg/mL) was better than the others at inhibiting the LPS-induced release of cytokine IL-6. Furthermore, the essential oils exhibited antitumor activities (IC₅₀, 37.72–123.16 µg/mL) against Hep3B (liver) and Hela (cervical) cells. Linear regression analysis showed that, caryophyllene oxide peak area percentages showed remarkably high negative correlation coefficients with IC₅₀ values of Hep3B and Hela cytotoxicity, which suggested the contribution of this compound on the cancer cell cytotoxicity of three essential oils. Finally, the ITS1-5.8S-ITS2 region was amplified and sequenced in order to generate genomic reference sequences for each plant. These can be used to identify the origins of the plants, and will assist other research studies related to these three plants.

Keywords: *Siegesbeckia orientalis*; *S. glabrescens*; *S. pubescens*; essential oil composition; GC-MS; anti-inflammatory; antitumor; ITS1-5.8S-ITS2

1. Introduction

Plants produce a wide variety of volatile compounds, including terpenes, alcohols, aldehydes, esters, ethers, ketones, phenols, and oxides, that play important roles in defense, pollinator attraction, signal transduction, and so on. These volatile compounds are also important resources for the pharmaceutical, food, beverages, perfume, and cosmetics industries, and are thus economically significant [1–3]. The mixture of plant volatile compounds, which is also known as essential oil, can be extracted using hydrodistillation, steam distillation, supercritical fluid extraction, Soxhlet's extraction, and ultrasound-assisted extraction [4,5].

Herba Siegesbeckiae (HS), a medicinal material used for the treatment of rheumatic arthritis, malaria, and snake bites in China since centuries ago [6], is derived from the aerial parts of three annual medicinal plants, *Siegesbeckia orientalis* (SO), *S. glabrescens* (SG), and *S. pubescens* (SP), which belong to the genus *Siegesbeckia* and the family Compositae. The main activities reported for HS extract are anti-inflammatory [7], antiallergic [8], antithrombotic [9], and immunosuppressive [10,11], but other activities have also been identified [12,13]. Recently, it was reported that SP essential oil significantly inhibited the proliferation of hepatocellular carcinoma cells (HepG2, Hep3B, Huh7, SMMC-7721). The 50% proliferation inhibition concentration of the SP essential oils was 42.0–95.2 $\mu\text{g}/\text{mL}$ [14]. In the past few decades, through systematic chemical studies, three main categories of compound have been identified in HS: diterpenes, sesquiterpenes, and flavonoids [15–18]. Pharmacological studies suggested that diterpenoids are the main antirheumatic constituents of HS [19,20]. A series of ent-kaurane and ent-pimarane diterpenoids from HS has been reported [17,18,21–23]. However, to the best of our knowledge, a comparative study related to the chemical composition, anti-inflammatory activity, and antitumor activity of essential oils from the three individual plant species has not been reported.

In this study, the hydrodistillation method was used to extract essential oils from dried SO, SG, and SP aerial parts, and chemical composition analysis was carried out using gas chromatography–mass spectrometry (GC-MS). Subsequently, the bioactivities of the oils, including anti-inflammatory and antitumor activities, were evaluated *in vitro*. Furthermore, in order to provide a reference molecular marker for the plant origin, the ITS1-5.8S-ITS2 genomic regions of the three plants were amplified and sequenced. Our results revealed remarkable differences in the essential oil composition and bioactivities, and in the characteristic ITS sequence, which will facilitate the discrimination of the origins of the three plants and their subsequent utilization.

2. Results and Discussion

2.1. Analysis of Essential Oils

The yield of essential oils from the dried aerial tissue of the three plants was 0.06% (SO), 0.08% (SG), and 0.13% (SP) (*w/w*). Representative GC-MS chromatograms of the three essential oils are shown in Figure 1. A total of 56 (SO), 62 (SG), and 59 (SP) constituents were identified, representing 98.72% (SO), 93.89% (SG), and 91.35% (SP) of the total peak area. Across the three plants, 148 compounds were identified in total. The retention time, compound name, molecular formula, literature retention indices (*RI*), experimental retention indices relative to C_8 – C_{20} *n*-alkanes (*RI*), and peak area percentages are listed in Table 1.

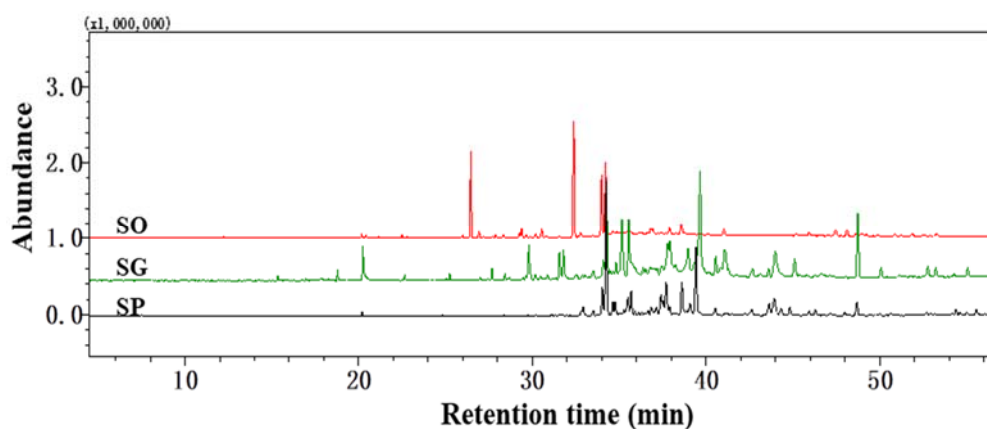


Figure 1. Representative GC-MS chromatograms of SO (red), SG (green), and SP (black) essential oils.

Table 1. Chemical composition (peak area percentage) of the essential oils of SO, SG, and SP.

No.	Rt (min)	Compound Name	Molecular Formula	RL	RI	SO	SG	SP
1	6.14	(3E)-Hexenol	C ₆ H ₁₂ O	855	868		0.05	
2	6.59	Hexanol	C ₆ H ₁₄ O	868	860		0.04	
3	6.95	Dibutyl oxide	C ₈ H ₁₈ O	878	882			0.09
4	7.49	Butyl acrylate	C ₇ H ₁₂ O ₂	893	884			0.11
5	7.96	Butyl propionate	C ₇ H ₁₄ O ₂	906	894			0.02
6	11.01	Nopinene	C ₁₀ H ₁₆	992	978			0.03
7	11.28	Butyl butyrate	C ₈ H ₁₆ O ₂	1000	984			0.07
8	12.24	α-Cymene	C ₁₀ H ₁₄	1025	1027	0.21		
9	12.41	Clearene	C ₁₀ H ₁₆	1029	1018			0.07
10	13.57	6-Ethyl-2-methyloctane	C ₁₁ H ₂₄	1059	1046			0.03
11	13.61	2,4-Dimethyldecane	C ₁₂ H ₂₆	1060	1086		0.03	
12	15.35	Linalool	C ₁₀ H ₁₈ O	1105	1082		0.33	
13	15.58	Dehydrocineole	C ₁₀ H ₁₆ O	1111	1041		0.03	
14	16.45	4-Acetyl-1-methylcyclohexene	C ₉ H ₁₄ O	1133	1118	0.12		
15	16.96	Camphor	C ₁₀ H ₁₆ O	1147	1121		0.07	
16	17.20	Mentha-1,5-diene-8-ol	C ₁₀ H ₁₆ O	1153	1125		0.04	
17	17.39	Neryl oxide	C ₁₀ H ₁₆ O	1158	1135	0.11		
18	17.78	2-Camphanol	C ₁₀ H ₁₈ O	1168	1165			0.04
19	17.83	Borneol	C ₁₀ H ₁₈ O	1169	1165		0.16	
20	17.90	β-Phellandren-8-ol	C ₁₀ H ₁₆ O	1171	1178		0.13	
21	18.20	4-terpineol	C₁₀H₁₈O	1179	1174	0.01	0.07	
22	18.55	p-Cymen-8-ol	C ₁₀ H ₁₄ O	1188	1197	0.07		
23	18.73	(-)-α-Terpineol	C ₁₀ H ₁₈ O	1192	1196	0.08		
24	18.78	α-terpineol	C₁₀H₁₈O	1194	1194		0.71	0.04
25	18.83	2-Decanone	C ₁₀ H ₂₀ O	1195	1181	0.09		
26	19.16	2-Decanol	C ₁₀ H ₂₂ O	1204	1218	0.11		
27	20.18	Nerol	C₁₀H₁₈O	1233	1228	0.56	2.85	0.48
28	20.42	O-Methylthymol	C ₁₁ H ₁₆ O	1239	1231	0.42		
29	20.97	2-Hexanoylfuran	C ₁₀ H ₁₄ O ₂	1255	1265			0.04
30	21.16	Geraniol	C₁₀H₁₈O	1260	1256	0.27		0.07
31	21.72	2,4-Dimethylphenethyl alcohol	C ₁₀ H ₁₄ O	1276	1292	0.01		
32	21.76	Dihydro-4-(3-methyl-2-methylenbutyl)-2(3H)-furanone	C ₁₀ H ₁₆ O	1277	1286		0.02	
33	21.98	4-Methyl-dodecane	C ₁₃ H ₂₈	1284	1269			0.03
34	22.02	4,6-Dimethyl-dodecane	C ₁₃ H ₃₀	1285	1285		0.04	
35	22.49	2-Undecanone	C ₁₁ H ₂₂ O	1298	1295	0.58		
36	22.65	Thymol	C ₁₀ H ₁₄ O	1302	1293		0.33	
37	22.77	2-Undecanol	C ₁₁ H ₂₄ O	1306	1297	0.23		
38	23.33	(E,E)-2,4-Decadienal	C ₁₀ H ₁₆ O	1322	1321		0.03	
39	24.71	Hexahydro-pseudoionone	C ₁₃ H ₂₆ O	1361	1341	0.05		
40	24.85	Eugenol	C₁₀H₁₂O₂	1365	1359		0.03	0.11
41	25.05	Nerol acetate	C ₁₂ H ₂₀ O ₂	1370	1352		0.13	
42	25.24	α-Cubebene	C ₁₅ H ₂₄	1376	1355		0.42	
43	25.98	2-Dodecanone	C ₁₂ H ₂₄ O	1397	1396	0.45		
44	26.48	4,8,8-Trimethyl-2-methylene-4-vinylbicyclo[5.2.0]nonane	C ₁₅ H ₂₄	1409	1407			0.10
45	26.93	cis-Caryophyllene	C ₁₅ H ₂₄	1419	1425	1.30		
46	27.00	Caryophyllene	C₁₅H₂₄	1420	1418	14.13	0.21	
47	27.19	1,4-Dimethoxy-2-tert-butylbenzene	C₁₂H₁₈O₂	1425	1406	0.21	0.02	
48	27.67	trans-α-Bergamotene	C ₁₅ H ₂₄	1436	1430		0.89	
49	27.72	Aromandendrene	C ₁₅ H ₂₄	1437	1416	0.23		
50	27.88	γ-Elemene	C ₁₅ H ₂₄	1440	1431	0.62		
51	28.34	cis-α-Bisabolene	C ₁₅ H ₂₄	1451	1478	0.56		
52	28.37	α-Farnesene	C ₁₅ H ₂₄	1451	1458			0.12
53	28.56	(E)-β-Farnesene	C ₁₅ H ₂₄	1456	1440		0.13	
54	29.26	Acoradiene	C ₁₅ H ₂₄	1471	1474	0.61		
55	29.39	γ-Muurolene	C ₁₅ H ₂₄	1474	1483	1.42	0.28	
56	29.45	Guaia-1(10),11-diene	C ₁₅ H ₂₄	1476	1490		0.21	
57	29.57	1,5-Cadinadiene	C ₁₅ H ₂₄	1479	1460			0.11
58	29.64	Curcumene	C ₁₅ H ₂₂	1480	1524	0.51		
59	29.8	cis-β-Farnesene	C ₁₅ H ₂₄	1484	1458		3.96	
60	30.15	γ-Curcumene	C ₁₅ H ₂₄	1492	1486		0.53	

Table 1. Cont.

No.	Rt (min)	Compound Name	Molecular Formula	RL	RI	SO	SG	SP
61	30.18	β -Funebrene	C ₁₅ H ₂₄	1492	1479			0.15
62	30.19	Elemol	C ₁₅ H ₂₆ O	1492	1512	0.72		
63	30.47	β -Himachalene	C ₁₅ H ₂₄	1499	1518		0.19	
64	30.54	α -Bisabolene	C ₁₅ H ₂₄	1500	1518	1.83		
65	30.73	Cuparene	C ₁₅ H ₂₂	1505	1526		0.04	
66	30.79	β -Bisabolene	C ₁₅ H ₂₄	1506	1500	0.31		
67	31.12	Valencen	C ₁₅ H ₂₄	1513	1504			0.29
68	31.52	Cadina-1(10),4-diene	C ₁₅ H ₂₄	1523	1526			0.17
69	31.52	Methyl dodecanoate	C ₁₃ H ₂₆ O ₂	1523	1535	0.31		
70	31.56	β -Sesquiphellandrene	C ₁₅ H ₂₄	1523	1525		2.74	
71	31.78	2-penten-1-ol	C ₁₅ H ₂₄ O	1528	1504		3.14	
72	32.37	trans- α -Bisabolene	C ₁₅ H ₂₄	1542	1540	24.41		
73	32.55	trans-Farnesene epoxide	C ₁₅ H ₂₄ O	1546	1540		0.65	
74	32.76	(Z)- α -Bisabolene epoxide	C ₁₅ H ₂₄ O	1551	1531	1.00		
75	32.89	6,10-Dimethyl-3-(1-methylethylidene)cyclodecene	C ₁₅ H ₂₆	1553	1564		0.19	
76	32.93	γ -Costol	C ₁₅ H ₂₄ O	1554	1542			0.94
77	33.04	Sesquirosefuran	C ₁₅ H ₂₂ O	1557	1577		0.15	
78	33.09	Ledol	C ₁₅ H ₂₆ O	1558	1566	0.16		
79	33.26	6-epi-shyobunol	C ₁₅ H ₂₆ O	1562	1555		0.09	
80	33.46	Globulol	C ₁₅ H ₂₆ O	1566	1569	0.22		
81	33.52	1,5-Epoxyalvial-4-ene	C₁₅H₂₄O	1568	1560		1.07	0.99
82	33.98	Spathulenol	C₁₅H₂₄O	1578	1577	12.46	0.87	3.44
83	34.21	Caryophyllene oxide	C₁₅H₂₄O	1583	1582	16.88	7.18	21.89
84	34.50	1,2-Dihydroneolidol	C₁₅H₂₈O	1590	1574		0.27	
85	34.63	6-(1-methylethyl)-1-naphthalenol	C ₁₅ H ₂₄ O	1593	1584			1.51
86	34.64	(-)-Globulol	C ₁₅ H ₂₆ O	1593	1590	0.51		
87	34.81	Mintketone	C₁₅H₂₄O	1597	1579		1.00	1.53
88	35.13	Rosifoliol	C ₁₅ H ₂₆ O	1603	1598	0.33		
89	35.15	Cedrol	C ₁₅ H ₂₆ O	1603	1583		8.35	
90	35.27	Copaborneol	C ₁₅ H ₂₆ O	1606	-			0.46
91	35.45	Humulene epoxide	C₁₅H₂₄O	1609	1592	0.25	4.75	2.62
92	35.68	Octahydro-2,2,7a-trimethyl-4-methylene-1,3a-ethano-3aH-inden-5-ol	C ₁₅ H ₂₄ O	1613	1599			3.33
93	35.90	Allohimachalol	C ₁₅ H ₂₆ O	1617	-			0.48
94	36.12	Z-3-Hexadecen-7-yne	C ₁₆ H ₂₈	1621	1637			0.38
95	36.30	Aromadendrane-4,10-diol	C ₁₅ H ₂₆ O ₂	1624	1619			0.26
96	36.37	Agarospinol	C ₁₅ H ₂₆ O	1626	1608		0.89	
97	36.45	Bisabolol	C ₁₅ H ₂₆ O	1627	1625			0.20
98	36.63	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5-ol	C₁₅H₂₄O	1630	1647	0.23		0.67
99	36.78	α -acorenol	C ₁₅ H ₂₆ O	1633	1635		1.47	
100	36.81	11,11-Dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol	C₁₅H₂₄O	1633	1651	0.41		1.20
101	36.92	Isospathulenol	C ₁₅ H ₂₄ O	1636	-	0.34		
102	37.18	α -Cadinol	C ₁₅ H ₂₆ O	1640	1637		0.28	1.35
103	37.40	Cedrenol	C ₁₅ H ₂₄ O	1644	1636			1.99
104	37.44	γ -Gurjunenepoxide-(2)	C ₁₅ H ₂₄ O	1645	1628		0.83	
105	37.68	Sesquibenihol	C₁₅H₂₄O	1649	1653	0.14	0.30	
106	37.70	(-)-Spathulenol	C ₁₅ H ₂₄ O	1650	1636			5.14
107	37.88	Himbaccol	C ₁₅ H ₂₆ O	1653	1631	1.60		
108	37.89	(1S,4R,5S)-1-Methyl-4-(prop-1-en-2-yl)spiro[4.5]dec-7-ene-8-carbaldehyde	C ₁₅ H ₂₂ O	1653	1642			0.80
109	37.90	Dehydroneolidol	C ₁₅ H ₂₄ O	1653	1632		6.28	
110	38.57	trans-Longipinocarveol	C₁₅H₂₄O	1665	1659	2.84		5.87

Table 1. Cont.

No.	Rt (min)	Compound Name	Molecular Formula	RL	RI	SO	SG	SP
111	38.79	Isovalencenol	C ₁₅ H ₂₄ O	1669	-	0.28		
112	38.87	3-Isopropyl-6,7-dimethyltricyclo[4.4.0.0(2,8)]decane-9,10-diol	C ₁₅ H ₂₆ O ₂	1671	1687			0.23
113	38.96	3,7,11-Trimethyl-dodeca-2,4,6,10-tetraenal	C ₁₅ H ₂₂ O	1672	1664		3.26	
114	39.07	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	C ₁₅ H ₂₄ O	1674	1690			2.02
115	39.36	1-Isopropyl-4,8-dimethylspiro[4.5]dec-8-en-7-ol	C ₁₅ H ₂₆ O	1680	1660		0.28	
116	39.41	Germacra-4(15),5E,10(14)-trien-1β-ol	C ₁₅ H ₂₄ O	1680	-			14.10
117	39.64	4-(1,5-Dimethylhex-4-enyl)cyclohex-2-enone	C ₁₄ H ₂₂ O	1685	1661		12.90	
118	40.53	(-)-Isolongifolol, acetate	C ₁₇ H ₂₈ O ₂	1701	1719			1.19
119	40.55	m-Camphorene	C ₂₀ H ₃₂	1701	-		1.79	
120	41.03	Pentadecanal	C₁₅H₃₀O	1710	1701	1.65		0.19
121	41.05	Cuparenal	C ₁₅ H ₂₀ O	1710	-		3.46	
122	41.21	α-Costol	C ₁₅ H ₂₄ O	1713	-			0.19
123	42.23	Platambin-1,6-dione	C ₁₅ H ₂₂ O ₂	1731	1740			0.38
124	42.62	Epicedrol	C ₁₅ H ₂₆ O	1738	1743			0.81
125	43.62	Ylangenol	C ₁₅ H ₂₄ O	1757	-			2.33
126	43.98	Dehydroaussurea lactone	C₁₅H₂₀O₂	1763	1755		5.49	4.85
127	44.32	Valerenic acid	C ₁₅ H ₂₂ O ₂	1769	1759			1.53
128	45.09	β-Acoradienol	C ₁₅ H ₂₄ O	1783	-		2.42	
129	45.18	4a,5-Dimethyl-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalen-1-ol	C ₁₅ H ₂₄ O	1785	-	0.58		
130	45.92	Aristol-1(10)-en-9-ol	C ₁₅ H ₂₄ O	1798	1788	0.93		
131	46.27	Naphthalenemethanol	C ₁₅ H ₂₄ O	1805	-			1.16
132	46.64	(1-Methyldecyl)cyclohexane	C ₁₇ H ₃₄	1811	1790		0.35	
133	47.45	β-Copaen-4α-ol	C ₁₅ H ₂₄ O	1826	1820	1.96		
134	48.04	Sativene epoxide	C ₁₅ H ₂₄ O ₃	1837	1812		0.13	
135	48.10	(+)-Cycloisolongifol-5-ol	C ₁₅ H ₂₄ O	1838	-	1.80		
136	48.62	2,15-Hexadecanedione	C ₁₆ H ₃₀ O	1848	1864	0.66		
137	48.73	Phytone	C₁₈H₃₆O	1850	1844		7.83	2.74
138	48.94	6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-ol	C ₁₅ H ₂₄ O	1854	1868	1.00		
139	49.24	15-Hydroxy-α-muurolole	C ₁₅ H ₂₄ O	1859	-	0.35		
140	50.06	Diisobutyl phthalate	C₁₆H₂₂O₄	1875	1888		1.09	0.29
141	50.62	1-Hexadecanol	C ₁₆ H ₃₄ O	1885	1870			0.49
142	50.87	11-Hexadecyn-1-ol	C ₁₆ H ₃₀ O	1890	1872	0.44		
143	51.21	Z,Z,Z-4,6,9-Nonadecatriene	C ₁₉ H ₃₄	1896	1934	0.32		
144	51.87	Geranyl-α-terpinene	C ₂₀ H ₃₂	1908	1962	0.84		
145	52.75	Farnesyl acetone	C₁₈H₃₀O	1924	1909		1.17	0.59
146	53.16	Methyl isoheptadecanoate	C ₁₈ H ₃₆ O ₂	1932	1909			0.19
147	54.25	Isophytol	C₂₀H₄₀O	1952	1939		0.26	0.36
148	55.04	Butyl isobutyl phthalate	C₁₆H₂₂O₄	1967	1973		0.99	0.49
Sum						98.72	93.89	91.35

RI: Experimental retention indices relative to C₈-C₂₀n-alkanes; RL: Literature retention indices. Compounds shared in common in two or three essential oils are in bold.

As shown in Figure 1 and Table 1, the composition of the three essential oils differed significantly. The essential oil of SO was dominated by hydrocarbon sesquiterpenes (45.93%), oxygenated sesquiterpenes (44.99%), and oxygenated monoterpenes (1.53%), among which trans-α-bisabolene (24.41%), caryophyllene oxide (16.88%), caryophyllene (14.13%), and spathulenolspathulenol (12.46%) were found to be the main components. The SG essential oil mainly contained oxygenated sesquiterpenes (65.51%), hydrocarbon sesquiterpenes (9.79%), oxygenated diterpenes (9.26%), and oxygenated monoterpenes (4.90%), among which 4-(1,5-dimethylhex-4-enyl)cyclohex-2-enone (12.90%), cedrol (8.35%), phytone (7.83%), caryophyllene

oxide (7.18%), dehydronerolidol (6.28%), and dehydrosaussurea lactone (5.49%) were the main components. The essential oil of SP was dominated by oxygenated sesquiterpenes (83.46%), oxygenated diterpenes (3.69%), and hydrocarbon sesquiterpenes (0.94%), with caryophyllene oxide (21.89%), germacra-4(15),5E,10(14)-trien-1 β -ol (14.10%), trans-longipinocarveol (5.87%), (–)-spathulenol (5.14%), and dehydrosaussurea lactone (4.85%) as the main components.

Only four chemical components were in common among all three species, namely nerol, spathulenol, caryophyllene oxide, and humulene epoxide. These accounted for 30.15%, 15.65%, and 28.43% of the total peak area in SO, SG, and SP essential oils, respectively (Figure 2). The essential oils of SO and SG shared 5 chemical components, as did the essential oils of SO and SP (Figure 2). SG and SP shared eleven identical components, as shown in Figure 2.

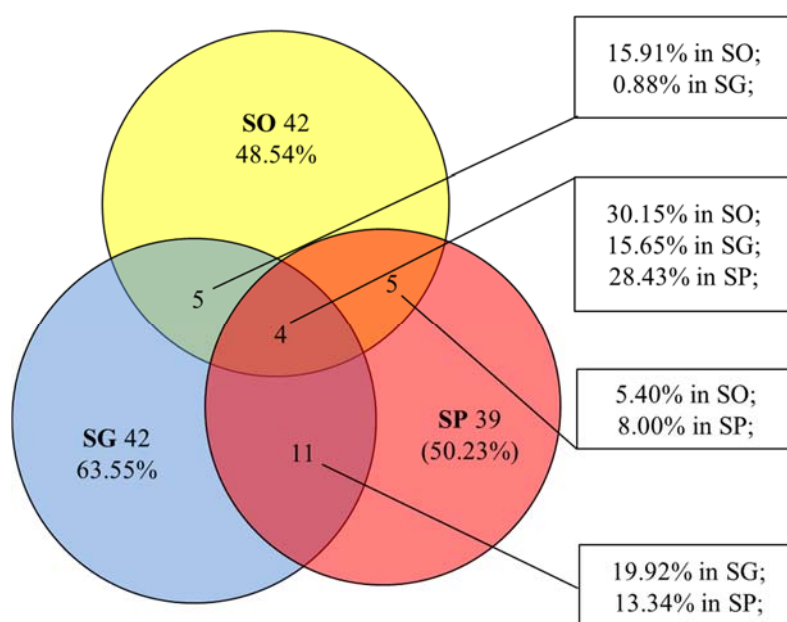


Figure 2. Venn diagram of essential oil compositions of SO, SG, and SP.

2.2. Anti-Inflammatory Activity

According to traditional use and recently published results, anti-inflammatory [7], antiallergic [8], antithrombotic [9], and immunosuppressive [10,11] effects are reported as the main activities of HS. Diterpenoids were the main antirheumatic constituents of HS [19], of which kirenol was the main chemical constituent. Pharmacological studies suggested that kirenol could inhibit paw swelling, boost up the proliferation of splenic lymphocyte of adjuvant arthritis rats, regulate T lymphocyte subpopulation ratio, and inhibit specific immune response of normal mice [20]. As we know, NO plays an important role in the inflammatory, immune responses, and in thrombotic pathogenesis processes. Inhibition of excess NO production has been used as an assay in the screening of anti-inflammatory, antithrombotic, and immunosuppressive drugs. Therefore, in this study, we treated the mouse macrophage cell line RAW264.7 with lipopolysaccharide (LPS) to induce an inflammatory response and we investigated the inhibitory effects of the three essential oils on NO production by the activated cells. Before the anti-inflammatory activity assay, the cytotoxicity of the three essential oils was evaluated by MTT assay. Our results suggested that the three essential oils had no obvious cytotoxic effects on the RAW264.7 cell line when the concentration was lower than 200 $\mu\text{g}/\text{mL}$ (shown in Figure S1).

As shown in Figure 3, the positive control minocycline showed the strongest NO inhibition activity with $\text{IC}_{50} = 0.37 \mu\text{g}/\text{mL}$, which is significantly lower than SG oil ($p < 0.05$), SO oil ($p < 0.01$), and SP oil ($p < 0.01$). Among the three essential oils, SG oil showed the lowest IC_{50} of $0.97 \mu\text{g}/\text{mL}$, which is significantly lower ($p < 0.01$) than SO oil ($\text{IC}_{50} = 2.83 \mu\text{g}/\text{mL}$) and SP oil ($\text{IC}_{50} = 13.48 \mu\text{g}/\text{mL}$).

Even though the SG oil showed weaker NO inhibition activity than minocycline, it still has the strongest NO inhibition effect of any crude essential oil as far as we know.

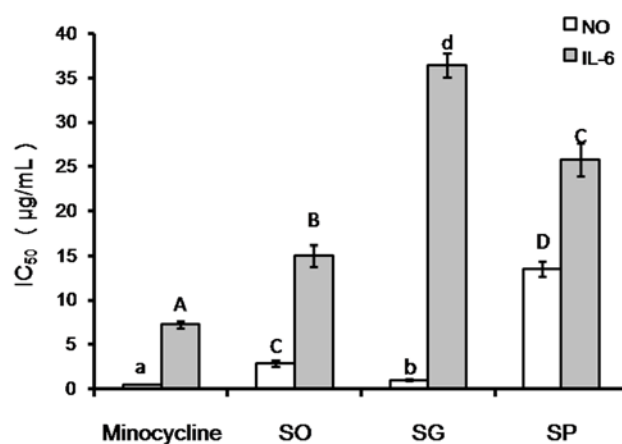


Figure 3. Anti-inflammatory effect of the SO, SG, and SP essential oils. A–D, $p < 0.01$; a,b,d, $p < 0.05$.

Inflammatory cells can release a variety of inflammatory cytokines which participate in the regulation of the body's innate immune response and directly kill target cells or mediate apoptosis, thus promoting the repair of damaged tissues. One of these cytokines is IL-6, a critical component of the inflammatory mediator network which plays an important role in the inflammatory response. The ability of the essential oils to inhibit the release of IL-6 was tested in LPS-treated RAW264.7 cells. As indicated in Figure 3, the ability of SO essential oil (IC_{50} , 14.99 µg/mL) to inhibit the release of cytokine IL-6 was significantly weaker ($p < 0.01$) than that of the positive control (minocycline) (IC_{50} , 7.21 µg/mL), but stronger ($p < 0.01$) than that of SP (IC_{50} , 25.76 µg/mL) and SG (IC_{50} , 36.41 µg/mL) essential oils.

NO and cytokine IL-6 are important indicators of the cellular inflammatory response. Our results demonstrated that the three essential oils showed significant anti-inflammatory activity. In summary, the three essential oils significantly inhibited the LPS-induced secretion of NO and cytokine IL-6 by the macrophage cell line RAW264.7. These results provide important clues for the identification of possible candidate compounds from the three essential oils, especially SG and SO essential oils.

2.3. Antitumor Activity

The *in vitro* antitumor activities of the essential oils were evaluated using two cancer cell lines, Hep3B (liver) and Hela (cervical). The cytotoxicity was expressed as IC_{50} values, as shown in Figure 4. The IC_{50} of the positive control 5-Fu (4.19 µg/mL in Hep3B, 2.08 µg/mL in Hela) was significantly lower than the three essential oils tested ($p < 0.01$). Among the three essential oils, SP exhibited the strongest cytotoxicity (38.10 µg/mL for Hep3B, 37.72 µg/mL for Hela). These results suggest that essential oils from the three plants might be used as a natural source of compounds for anticancer compound screening.

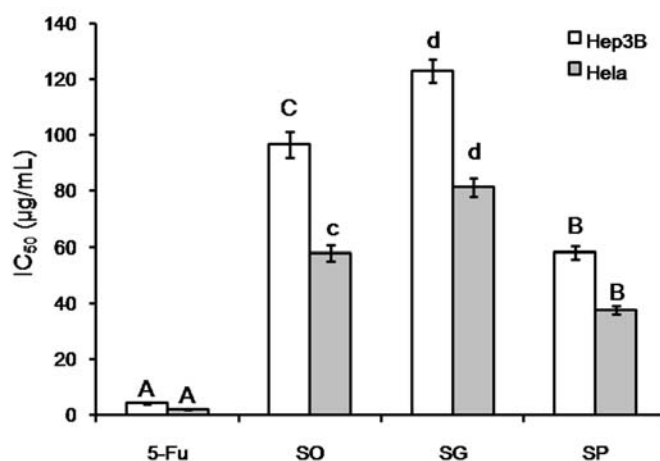


Figure 4. Cytotoxicity of the essential oils of SO, SG, and SP against Hep3B and Hela cell lines. A–C, $p < 0.01$; c,d, $p < 0.05$.

A recent study [24] showed that SO ethanol extract significantly inhibited the proliferation of RL 95-2 human endometrial cancer cells. Furthermore, SO ethanol extract was effective against A549 (lung cancer), HepG2 (hepatoma), MDA-MB-231 (breast cancer), and especially on LNCaP (prostate cancer) cell lines. At the same time, it was reported that caryophyllene oxide (IC₅₀ value = 14.90 µg/mL) and caryophyllene (IC₅₀ value = 33.20 µg/mL) were mainly responsible for most cytotoxic activity of SO ethanol extract against RL 95-2 cells. As mentioned previously in Section 2.1, there are only four common compounds in the three essential oils, and caryophyllene oxide is one of the four common components in the three essential oils.

In order to find out the possible relationships between common compounds and two kinds of activities, a linear regression analysis was carried out between percentages and IC₅₀ values. Our results showed that (Figure S2) only caryophyllene oxide peak area percentages showed remarkably strong negative correlation coefficients with IC₅₀ of NO inhibition ($R^2 = 0.959$), Hep3B ($R^2 = 0.918$), and Hela ($R^2 = 0.980$) cytotoxicity. These results suggested the possible important contribution of caryophyllene oxide on the anticancer activity and anti-inflammatory effects of Herba Siegesbeckiae essential oil.

2.4. ITS1-5.8S-ITS2 Sequence

Due to the similarity in morphological characteristics of dried and chopped commercial materials, it is difficult to distinguish the three plants from each other. Therefore, it is important to provide a reliable molecular marker for the identification of the plant origin and for comparison with other research studies. It has been accepted that genomic ribosomal DNA sequences, especially ITS1-5.8S-ITS2, can be used for the interspecific identification of plant origin. Therefore, the full ITS1-5.8S-ITS2 sequence was amplified from genomic DNA of the three plants and sequenced. The accession numbers in Genbank are MH701787 (SO), MH701847 (SG), and MH701848 (SP). Multiple alignment of the ITS1-5.8S-ITS2 sequence from the three plants was carried out using DNAMAN 9.0 software. As shown in Figure S3, across the total sequence of 730–732 bp, the three plants shared 99.27% similarity with each other. There are 16 single nucleotide polymorphism (SNP), which can be used for the development of specific Polymerase chain reaction (PCR) assays to distinguish the origins of samples of these three plants.

3. Conclusions

In this study, hydrodistillation was used to extract essential oils from the dried aerial parts of SO, SG, and SP, and their chemical compositions and bioactivities were analyzed and compared. A total of 56, 62, and 59 constituents were identified in SO, SG, and SP essential oils, respectively,

by GC-MS. There was a marked difference between the components of the three essential oils. The essential oils of SO, SG, and SP exhibited various levels of anti-inflammatory and antitumor activity in vitro. The essential oil of SP (IC_{50} , 0.97 $\mu\text{g}/\text{mL}$) significantly reduced NO release by LPS-induced RAW264.7 macrophages, and the essential oil of SO was the most effective at reducing the release of cytokine IL-6. The antitumor activities of the essential oil from SP were much better than SG and SO. Therefore, the essential oils of SO, SG, and SP were significantly different from each other in terms of phytochemical composition, but with similar bioactivities with various activity levels. Among four common compounds in three essential oils, caryophyllene oxide peak area percentages showed remarkably high negative correlation coefficients with IC_{50} values of Hep3B and Hela cytotoxicity, which suggested the contribution of this compound on the cancer cell cytotoxicity of three essential oils. Finally, in order to provide detailed information about the origin of the plants used in the present work, the complete ITS1-5.8S-ITS2 region was amplified from plant genomic DNA and sequenced. This sequence can be used by other researchers to confirm whether their plant specimens have the same origin as ours or not.

4. Materials and Methods

4.1. Materials

The flowering SP and SG plant materials were collected in October 2017 from Jinzhai, Anhui province, China. The flowering SO plant material was collected in April 2018 from Zhazhou, Hainan province, China. Voucher specimens were deposited at the Herbarium of Shenyang Pharmaceutical University (Shenyang, China). The voucher numbers were SYPU-P-201710-007 (SP), SYPU-P-201710-008 (SG), and SYPU-P-201804-025 (SO). They were identified by Associate Professor Jia Lingyun in Shenyang Pharmaceutical University (Shenyang, China). The fresh plants were air-dried and ground into a fine powder which was passed through a 20 mesh and stored in sealed plastic bags at $-20\text{ }^{\circ}\text{C}$ for future use. RAW264.7, a mouse macrophage cell line, was purchased from the Cell Bank of the Shanghai Institute of Cell Biology and Biochemistry, Chinese Academy of Sciences (Shanghai, China). The cancer cells lines Hep3B (liver) and Hela (cervical) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China).

4.2. Reagents

He (purity > 99.999%) and N_2 (purity > 99.999%) were supplied by Shenyang Qianzhen Chemical Gas (Shenyang, China). Hexane (HPLC grade) and anhydrous sodium sulfate (analytical grade) were purchased from Shandong Yuwang Chemical Group (Shandong, China). Plant Genomic DNA Extraction Kits, 2X Taq master premix, and GoldView dye were purchased from Kangweishiji Bio. (Beijing, China). Agarose G-10 powder was purchased from Biowest (Hongkong, China). Synthesis of primers ITS1 and ITS4 as well as Sanger sequencing were carried out at Genewiz Bio. Ltd. (Suzhou, China). DMSO and 1% penicillin-streptomycin antibiotics were purchased from Sigma (biology grade, Burlington, VT, USA). RPMI 1640, Eagle's Minimum Essential Medium, and 10% fetal bovine serum were purchased from Gibco (Waltham, MA, USA). Nitric Oxide Assay Kit was purchased from Beyotime Institute of Biotechnology (Jiangsu, China). ELISA kits were purchased from Shanghai Joyee Biotechnics Co., Ltd. (Shanghai, China). Minocycline was purchased from Dalian Meilun Biotech Co., Ltd. (Dalian, China). 5-Fluorouracil was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China).

4.3. DNA Extraction, ITS Amplification, Electrophoresis and Sequencing

Dried leaf tissues were ground into a fine powder. Then, DNA was extracted using a Plant Genomic DNA Extraction Kit following the manufacturer's instructions. Extracted DNA (1 μL) was used as the template for PCR amplification, with 25 μL 2X Taq master premix, 3 μL 10 μM ITS1 primer (5'-TCCGTAGGTGAACCTGCGG-3'), 3 μL 10 μM ITS4 primer (5'-TCCTCCGCTT ATTGATATGC-3'),

and 18 μL double-distilled H_2O . The amplification conditions were as follows: 94 $^\circ\text{C}$ for 5 min (1 cycle); 94 $^\circ\text{C}$ for 30 s, 55 $^\circ\text{C}$ for 30 s, and 72 $^\circ\text{C}$ for 40 s (30 cycles); 72 $^\circ\text{C}$ for 10 min (1 cycle). PCR product (5 μL) was applied to a 1% agarose gel containing GoldView dye and electrophoresis was carried out for 15 min at 150 V followed by observation under UV light in a Tenon gel imaging system. After confirmation of a strong, single amplified band of about 700 bp, the PCR product was sent for Sanger sequencing using ITS1 and ITS4 as sequencing primers. Sequences were confirmed by comparing them with the original sequencing chromatogram and were connected using Seqman software (Madison, MI, USA).

4.4. Essential Oil Extraction

100 g powder of dried aerial parts were added together with 1 L distilled water into a 2 L round-bottomed bottle, which was connected to a Clevenger-type apparatus with tap water for cooling. The hydrodistillation was continued for 4 h to extract the essential oils. The obtained essential oil was collected from the side arm and dried with anhydrous sodium sulphate, then sealed in a headspace bottle and stored at 4 $^\circ\text{C}$ until it was tested.

4.5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis was carried out on a Shimadzu (TQ-8040) series GC-MS system (n) equipped with an AOC-20i autosampler. The columns used an HP-5 capillary column (30 m \times 0.32 mm, i.d. 0.25 μm) with a stationary phase of 5% diphenyl/95% dimethylpolysiloxane. Helium was used as the carrier gas. The column temperature was maintained at 40 $^\circ\text{C}$ for 3 min and then programmed to increase as follows: rising from 40 $^\circ\text{C}$ to 130 $^\circ\text{C}$ at a rate of 4 $^\circ\text{C}/\text{min}$ and holding for 2 min; rising from 130 $^\circ\text{C}$ to 150 $^\circ\text{C}$ at a rate of 2 $^\circ\text{C}/\text{min}$ and holding for 3 min; rising from 150 $^\circ\text{C}$ to 180 $^\circ\text{C}$ at a rate of 2 $^\circ\text{C}/\text{min}$ and holding for 3 min; and finally rising from 180 $^\circ\text{C}$ to 210 $^\circ\text{C}$ at a rate of 5 $^\circ\text{C}/\text{min}$ and holding for 5 min. The ionization energy was 70 eV with a scan time of 0.3 s and a mass range of 45–500 AMU. The identification of the compounds was based on the comparison of their retention times and mass spectra with those from the NIST14 and NIST14s (National Institute of Standards and Technologies, Mass Spectra Libraries, Gaithersburg, MD, USA) [25].

4.6. Anti-Inflammatory Activities

4.6.1. Cell Line and Cell Culture

RAW264.7, a mouse macrophage cell line, was grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin in a 37 $^\circ\text{C}$ humidified incubator containing 5% CO_2 .

4.6.2. Cell Viability Assay

The MTT assay was used to evaluate the effect of essential oils on cell viability [26]. RAW264.7 cells were seeded in 96-well plates at a density of 5×10^4 cells/well. After overnight growth, cells were treated with different concentrations of essential oils for 1 h, then incubated in the presence or absence of LPS (100 ng/mL) for the next 24 h. Then, 20 μL of MTT solution (5 mg/mL) was added and the cells were cultured for a further 4 h. After that, the supernatant was carefully removed and then the resulting formed azan crystals were dissolved in 100 μL DMSO with horizontal shaking. The absorbance at 490 nm (ref. 570 nm) [24] was measured with a microplate reader.

4.6.3. Measurement of NO and IL-6 Release

RAW264.7 cells were seeded into 96-well plates at a density of 8×10^4 cells/mL and cultured overnight. After pretreatment with different concentrations of essential oils (0.2–200 $\mu\text{g}/\text{mL}$) or the positive control (minocycline) for 1 h, the cells were stimulated with LPS (100 ng/mL) for 24 h. The concentration of NO in the conditioned culture medium was examined with the Nitric Oxide

Assay Kit and the release of IL-6 in the supernatants was assayed using ELISA kits according to the manufacturer's instructions [27,28]. The concentrations were calculated from standard curves.

4.7. Antitumor Assay

4.7.1. Cell Lines and Cell Culture

The cancer cells lines Hep3B (liver) and Hela (cervical) were grown in media supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin antibiotics and maintained in a CO₂ incubator at 37 °C in 5% CO₂. The medium was changed every 2 days until the cells reached confluence, at which point they were subcultured.

4.7.2. Cytotoxicity Assay

The essential oils from HS were evaluated for their cytotoxic activities against cells using the MTT cell viability assay with 5-fluorouracil as the positive control. First, cellular suspensions (1×10^5 cells/mL) were cultured in 96-well plates and exposed to different concentrations of the essential oils (1–200 µg/mL) using 3 wells for each concentration. The plates were incubated at 37 °C in 5% CO₂ for 48 h [29]. Then, 200 µL MTT solution was added to each well and the plates were cultured for 4 h. The absorbance of each well was measured at 490 nm using a microplate reader. The data were expressed as the mean percentage of viable cells as compared to the respective control cultures treated with the solvent. The half maximal growth inhibitory concentration (IC₅₀ values) was calculated from the standard curves of the dose-dependent cytotoxicity of the essential oils.

4.8. Statistical Analysis

All activity assay experiments were repeated three times and the data were represented as mean ± SD. Significance analysis was carried out using the t-test in Microsoft Excel 2007. Letters above the columns in Figures 2 and 3 represent significance (A–D: $p < 0.01$; a,b,d: $p < 0.05$)

Supplementary Materials: The following are available online at <http://www.mdpi.com/1420-3049/23/9/2185/s1>, Figure S1: Effects of SO, SG and SP essential oil on cell viability of RAW264.7. Figure S2: Linear regression analysis of caryophyllene oxide peak area percentages and IC₅₀ of Hep3B and Hela cytotoxicity. Figure S3: Multiple alignment of the ITS1-5.8S-ITS2 sequence of SO, SG and SP.

Author Contributions: X.G., G.H., and J.J. conceived and designed the experiments; X.G. collected the plant material, extracted essential oil, and carried out statistical analysis of all data, and with the help of S.F., analyzed the components using GC-MS. The anti-inflammatory activity assay was carried out by J.W. and the antitumor activity was assayed by L.H. X.G. and G.H. conducted the ITS sequence amplification and wrote the manuscript.

Funding: This research was funded by Liaoning Provincial Engineering Center of Endangered and Daodi Medicinal Plant Resources Utilization in Shenyang Pharmaceutical University.

Acknowledgments: The authors also appreciate the authentication of the plant samples by Jia Lingyun, Shenyang Pharmaceutical University.

Conflicts of Interest: There is no conflict of interest.

References

1. Bajpaia, V.K.; Baekb, K.H. Biological efficacy and application of essential oils in foods—A review. *J. Essent. Oil Bear. Plants* **2016**, *19*, 1–19. [CrossRef]
2. Ghayempour, S.; Montazer, M. Micro/nanoencapsulation of essential oils and fragrances: Focus on perfumed, antimicrobial, mosquito-repellent and medical textiles. *J. Microencapsul.* **2016**, *33*, 497–510. [CrossRef] [PubMed]
3. Huang, X.W.; Feng, Y.C.; Huang, Y.; Li, H.L. Potential cosmetic application of essential oil extracted from *Litsea cubeba* fruits from China. *J. Essent. Oil Res.* **2013**, *25*, 112–119. [CrossRef]
4. Chang, K.M.; Choi, E.M.; Kim, G.H. Chemical constituents of *Chrysanthemum indicum* L. flower oil and effect on osteoblastic MC₃T₃-E₁ cells. *Food Sci. Biotechnol.* **2010**, *19*, 815–819. [CrossRef]

5. Wang, L.H.; Chen, Y.S.; Song, Y.T.; Chen, Y.; Liu, X.S. GC-MS of volatile components of *Schisandra chinensis* obtained by supercritical fluid and conventional extraction. *J. Sep. Sci.* **2008**, *31*, 3238–3245. [[CrossRef](#)] [[PubMed](#)]
6. State Pharmacopoeia Commission of the PRC. *Pharmacopoeia of the People's Republic of China*, 1st ed.; People's Medical Publishing House: Beijing, China, 2015; p. 368.
7. Wang, J.P.; Zhou, Y.M.; Ye, Y.J.; Shang, X.M.; Cai, Y.L.; Xiong, C.M.; Wu, Y.X.; Xu, H.X. Topical anti-inflammatory and analgesic activity of kirenol isolated from *Siegesbeckia orientalis*. *J. Ethnopharmacol.* **2011**, *137*, 1089–1094. [[CrossRef](#)] [[PubMed](#)]
8. Kim, H.M.; Kim, C.Y.; Kwon, M.H.; Shin, T.Y.; Lee, E.J. Suppression of anaphylactic reaction in mice by *Siegesbeckia pubescens*. *Arch. Pharm. Res.* **1997**, *20*, 122–127. [[CrossRef](#)] [[PubMed](#)]
9. Wang, J.P.; Xu, H.X.; Wu, Y.X.; Ye, Y.J.; Ruan, J.L.; Xiong, C.M.; Cai, Y.L. Ent-16 β ,17-dihydroxy-kauran-19-oic acid, a kaurane diterpene acid from *Siegesbeckia pubescens*, presents antiplatelet and antithrombotic effects in rats. *Phytomedicine* **2011**, *18*, 873–878. [[CrossRef](#)] [[PubMed](#)]
10. Sun, H.X.; Wang, H. Immunosuppressive activity of the ethanol extract of *Siegesbeckia orientalis* on the immune responses to ovalbumin in mice. *Chem. Biodivers.* **2006**, *3*, 754–761. [[CrossRef](#)] [[PubMed](#)]
11. Hwang, W.J.; Park, E.J.; Jang, C.H.; Han, S.W.; Oh, G.J.; Kim, N.S.; Kim, H.M. Inhibitory effect of immunoglobulin E production by jin-deuk-chal (*Siegesbeckia orientalis*). *Immunopharmacol. Immunotoxicol.* **2001**, *23*, 555–563. [[CrossRef](#)] [[PubMed](#)]
12. Dong, X.Y.; Chen, M.; Jing, W.; Huang, D.X.; Shen, S.M.; Li, H.T. Studies on antifertility constituents of *Siegesbeckia glabrescens*. *Acta Pharmacol. Sin.* **1989**, *24*, 833–836.
13. Su, J.D.; Osawa, T.; Namiki, M. Screening for antioxidative activity of crude drugs. *Agric. Biol. Chem.* **1986**, *50*, 199–203.
14. Lv, D.; Guo, K.W.; Xu, C.; Huang, M.; Zheng, S.J.; Ma, X.H.; Pan, L.H.; Wang, Q.; Yang, X.Z. Essential oil from *Siegesbeckia pubescens* induces apoptosis through the mitochondrial pathway in human HepG2 cells. *J. Huazhong Univ. Sci. Technol. Med. Sci.* **2017**, *37*, 87–92. [[CrossRef](#)] [[PubMed](#)]
15. Wu, Q.; Li, H.; Lee, S.Y.; Lee, H.J.; Ryu, J.H. New cytotoxic Sesquiterpenoids from *Siegesbeckia glabrescens*. *Molecules* **2015**, *20*, 2850–2856. [[CrossRef](#)] [[PubMed](#)]
16. Zeng, L.F.; Xu, J.W.; Xu, L.; Zhu, L.L.; Liu, H. Separation and identification of flavonoids from *Siegesbeckia glabrescens*. *Chin. J. Exp. Trad. Med. Form.* **2017**, *23*, 74–77.
17. Liu, K.; Roder, E. Diterpenes from *Siegesbeckia glabrescens*. *Planta Med.* **1991**, *57*, 395–396. [[CrossRef](#)] [[PubMed](#)]
18. Wang, J.B.; Duan, H.Q.; Wang, Y.; Pan, B.; Gao, C.; Gai, C.Y.; Wu, Q.; Fu, H.Z. ent-Strobane and ent-Pimarane Diterpenoids from *Siegesbeckia pubescens*. *J. Nat. Prod.* **2017**, *80*, 19–29. [[CrossRef](#)] [[PubMed](#)]
19. Hu, H.H.; Tang, L.X.; Li, X.M. Experimental research of effect of crude and processed *Herba siegesbeckiae* on anti-inflammation and anti-rheumatism. *J. Chin. Mater. Med.* **2004**, *29*, 542–545.
20. Xin, H.L.; Bi, J.; Liu, M.; Lin, W.H.; Qian, R.Q. Experimental study on anti-inflammatory and immuno-regulating effect of kirenol. *Chin. Tradit. Herb. Drugs* **2005**, *36*, 866–869.
21. Wang, F.; Cheng, X.L.; Li, Y.J.; Shi, S.; Liu, J.K. ent-Pimarane diterpenoids from *Siegesbeckia orientalis* and structure revision of a related compound. *J. Nat. Prod.* **2009**, *72*, 2005–2008. [[CrossRef](#)] [[PubMed](#)]
22. Xiong, J.; Ma, Y.B.; Xu, Y.L. Diterpenoids from *Siegesbeckia pubescens*. *Phytochemistry* **1992**, *31*, 917–921. [[CrossRef](#)]
23. Xiang, Y.; Zhang, H.; Fan, C.Q.; Yue, J.M. Novel diterpenoids and diterpenoid glycosides from *Siegesbeckia orientalis*. *J. Nat. Prod.* **2004**, *67*, 1517–1521. [[CrossRef](#)] [[PubMed](#)]
24. Chang, C.C.; Hsu, H.F.; Huang, K.H.; Wu, J.W.; Kuo, S.M.; Ling, X.H.; Houng, J.Y. Anti-proliferative effects of *Siegesbeckia orientalis* ethanol extract on human endometrial RL-95 cancer cells. *Molecules* **2014**, *19*, 19980–19994. [[CrossRef](#)] [[PubMed](#)]
25. NIST: National Institute of Standards and Technologies, Mass Spectra Libraries. Available online: <http://www.sisweb.com/software/nist-gc-library.htm> (accessed on 15 July 2018).
26. Kwon, O.K.; Lee, M.Y.; Yuk, J.E.; Oh, S.R.; Chin, Y.W.; Lee, H.K.; Ahn, K.S. Anti-inflammatory effects of methanol extracts of the root of *Lilium lancifolium* on LPS-stimulated raw264.7 cells. *J. Ethnopharmacol.* **2010**, *130*, 28–34. [[CrossRef](#)] [[PubMed](#)]
27. Kim, J.M.; Jung, H.A.; Choi, J.S.; Lee, N.G. Identification of anti-inflammatory target genes of *Rhizoma coptidis* extract in lipopolysaccharide-stimulated RAW264.7 murine macrophage-like cells. *J. Ethnopharmacol.* **2010**, *130*, 354–362. [[CrossRef](#)] [[PubMed](#)]

28. Yan, T.; Yu, X.Y.; Sun, X.D.; Meng, D.L.; Jia, J.M. A new steroidal saponin, furotrilliumoside from *Trillium tschonoskii* inhibits lipopolysaccharide-induced inflammation in Raw264.7 cells by targeting PI3K/Akt, MARK and Nrf2/HO-1 pathways. *Fitoterapia* **2016**, *115*, 37–45. [[CrossRef](#)] [[PubMed](#)]
29. He, Z.F.; Zhang, H.; Miao, H.W.; Li, Z.J.; Zhou, J.J.; Yan, X.Y.; Cai, X.J. 1-o-acetylbritannilactone (ABL) inhibits angiogenesis and lung cancer cell growth through regulating VEGF-Src-FAK signaling. *Biochem. Biophys. Res. Commun.* **2015**, *464*, 422–427.

Sample Availability: Samples of the compounds are not available from the authors.



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).