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Essential Oil Constituents from the Leaves of *Anoectochilus setaceus, Codonopsis javanica* and *Aristolochia kwangsiensis* from Vietnam

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Abstract: There are very few reports on the phytochemistry of *Anoectochilus setaceus* Blume, *Codonopsis javanica* (Blume) Hook. f. and *Aristolochia kwangsiensis* Chun & F.C.How ex S.Yun Liang species in the literature. Here we present essential oil compositions of the three endemic plants from Vietnam. The analysis of the chemical constituents of the hydrodistilled essential oils was achieved by using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The essential oils of *A. setaceus, C. javanica* and *A. kwangsiensis* afforded very low oil yields: 0.12%, 0.31% and 0.10% (v/w), respectively, calculated on a dry weight basis. The result indicated that the major components of the leaf oil of *A. setaceus* consist mainly of α-cadinol (17.1%), (*E,E*)-farnesol (14.0%) and terpinen-4-ol (11.0%) while β-pinene (20.8%) and α-pinene (15.4%) were the main compounds identified in *C. javanica.* However, the significant compounds of *A. kwangsiensis* were sabinene (34.8%), β-caryophyllene (8.8%) and terpinen-4-ol (8.6%). To the best of our knowledge this is the first report on the essential oil compositions of these species.

Keywords: *Anoectochilus setaceus; Codonopsis javanica; Aristolochia kwangsiensis*; essential oil; terpenoids.

1. Plant Source

The present study report the chemical constituents identified in the essential oils of from the leaves of *Anoectochilus setaceus* Blume, *Codonopsis javanica* (Blume) Hook. f. and *Aristolochia kwangsiensis* grown in Vietnam. The leaves of *A. setaceus, C. javanica* and *A. kwangsiensis* were

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collected from plants cultivated in Kỳ Sơn District, Nghệ An Province, Vietnam, in May 2014. The botanical identification of the plants was achieved by Dr. Dai. Voucher specimens DND 12, 431, 145 respectively were deposited at the Botany Museum, Vinh University, Vietnam.

2. Previous Studies

Anoectochilus setaceus Blume is a species in the genus *Anoectochilus*, which is a genus of about 50 orchids (family Orchidaceae). The plant is one of the rare medicinal orchids in Vietnam and has various functions in traditional medicine, such as anti-tumor, lipase decreasing, anti-diabetes and anti-hepatitis [1]. The chemical constituents and biological activities of some species in the genus *Anoectochilus* have been described [2-4] when compared to *A. setacues*. The antibacterial activity of helvolic acid which was isolated from *A. setacues* has been published [5]. However, the authors are not aware of any published report on the volatile constituents from the leaves or any other parts of *Anoectochilus* plants. *Codonopsis javanica* Hook. f. et Thoms is a perennial climber. The flowers are hermaphrodite and are a vital herb in Chinese folk medicine. The plant belongs to the Campanulaceae family [6]. *C. javanica* and other *Codonopsis* species have been used to treat diabetes and other diseases. A finding recently showed that fructose-induced hyperinsulinemia and associated oxidative stress could be attenuated by *C. javanica* root extracts [7]. Extract of *C. javanica* possess insecticidal action against *Aedes albopictus* [8]. No literature citation could be found on the chemical compositions of essential oils from the leaves or any other parts of *C. javanica*. *Aristolochia kwangsiensis* Chun et How ex CF Liang is a climbing shrub of the family Aristolochiaceae. Extracts from the plant have displayed some biological activities such as antimicrobial, antioxidant and anti-inflammatory [9]. The phytochemical compounds of *A. kwangsiensis* includes allantoin, aristolochic acid, β-sitosterol and 3,4-methylenedioxy-6,8-dimethoxy-1-methyl ester phenanthrene or 6-methoxy aristolochic acid methyl ester [10]. The volatile compositions from the leaves or any other parts of *A. kwangsiensis* have not been previously reported.

3. Present Study

The average yields of the hydrodistilled essential oils were 0.12%, 0.31% and 0.10% (v/w) respectively for the leaves of *A. setaceus, C. javanica* and *A. kwangsiensis*, calculated on a dry weight basis. All the oil samples were light yellow.

The GC analysis of essential oils was carried out using an Agilent Technologies HP 6890 Plus GC which was equipped with a flame ionization detector and HP-5MS column. Each analysis was performed in triplicate. Retention indices (RI) value of each component was determined relative to the retention times of a homologous n-alkane series (C6-C32), under the same operating conditions (Please see supporting information), with linear interpolation on the HP-5MS column as described previously [11].

The identified volatile constituents along with their percentages and retention indices calculated on HP-5MS column were shown in Table 1. Fifty compounds representing 91.4% of the total volatile compounds were identified in the leaf of *A. setaceus*. The main classes of compounds present in the leaf oil were monoterpene hydrocarbons (10.9%), oxygenated monoterpenes (17.0%), sesquiterpene hydrocarbons (19.6%) and oxygenated sesquiterpenes (43.9%). The major constituents present in the leaf oil of *A. setaceus* were α-cadinol (17.1%), (*E,E*)-farnesol (14.0%) and terpinen-4-ol (11.0%). To the best of our knowledge this is the first report on the essential oil compositions of these species. On the other hand, 64 constituents amounting to 97.1of the total oil content were identified in the leaf of *C. javanica*. The representative classes of comprising identified in the oil were mainly monoterpene hydrocarbons (44.2%), oxygenated monoterpenes (6.2%), sesquiterpene hydrocarbons (25.6%), oxygenated sesquiterpenes (15.4%), diterpenes (1.4%) and non-terpene compounds (4.7%). The significant compounds of the leaf of oil were β-pinene (20.8%) and α-pinene (15.4%). The authors are not aware of any published data on the volatile compounds of the leaf of *C. javanica,* and as such the present study may represent the first of its kind.

Table 1. Essential oil constituents of the leaves of *A. setaceus, C. javanica* and *A. kwangsiensis**

S/N	Compound [a]	RI[b]	RI[c]	*A. setaceus*	*C. javanica*	*A. kwangsiensis*
1	α-Thujene	930	921	0.6	0.1	0.5
2	α-Pinene[e]	939	932	0.8	15.4	1.9
3	Fenchene	951	941	-	-	2.1
4	Camphene	953	946	2.6	1.1	0.2
5	Sabinene [e]	966	956	-	0.4	34.8
6	Verbenene	976	961	-	0.4	-
7	β-Pinene[e]	980	978	1.1	20.8	1.4
8	β-Myrcene	990	988	0.2	1.0	0.4
9	α-Phellandrene	1006	1004	0.1	0.6	-
10	δ -3-Carene	1011	1008	1.1	-	-
11	α-Terpinene	1017	1014	-	0.2	-
12	*o*-Cymene	1026	1024	1.8	-	1.7
13	β-Phellandrene	1028	1026	-	3.4	-
14	1,8-Cineole	1034	1032	-	0.6	-
15	(*E*)-β-Ocimene	1052	1044	-	0.2	-
16	γ-Terpinene	1061	1056	1.2	0.3	0.7
17	*cis*-Sabinene hydrate[e]	1067	1065	-	-	2.2
18	α-Terpinolene	1090	1089	0.4	0.3	-
19	Linalool	1100	1100	0.1	0.3	-
20	Nonanal	1106	1102	-	0.3	-
21	*allo*-Ocimene	1128	1128	1.0	-	-
22	Terpinene-1-ol	1139	1139	-	-	0.8
23	Camphor	1145	1141	0.1	2.0	-
24	*trans*-Pinocarvone[f]	1162	1164	-	0.2	-
25	Benzyl acetate	1165	1165	-	1.5	-
26	Borneol	1167	1167	4.1	-	-
27	*p*-Mentha-1,5-dien-8-ol[e]	1167	1170	-	0.6	-
28	Terpinen-4-ol[e]	1177	1177	11.0	-	8.6
29	*p*-Cymen-8-ol	1184	1188	0.1	-	1.3
30	α-Terpineol	1189	1187	1.0	-	-
31	Dodecane	1200	1200	-	1.0	-
32	Verbenone	1203	1195	-	0.5	-
33	Myrtenal	1204	1197	-	0.6	-
34	*trans*-Piperitol[e]	1205	1208	0.1	-	0.3
35	Pulegol	1214	1214	-	-	0.5
36	*trans*-Carveol	1217	1217	-	0.1	1.6
37	4,8-Dimethyl undecane	1218	1217	-	0.5	-
38	Fenchyl acetate[e]	1222	1228	0.4	-	-
39	Thymol methyl ether	1235	1235	-	-	0.4
40	2,5-Dimethyl -3-hexyne-2,5-diol [e]	1247	1249	-	-	6.7
41	Neral	1248	1249	-	0.1	-
42	*p*-Menth-1-en-7-al	1290	1281	-	-	0.1
43	*p*-Cymen-2-ol	1300	1298	0.1	-	-
44	Bicycloelemene	1327	1337	-	0.6	3.1
45	δ-Elemene	1340	1340	1.3	0.5	-
46	α-Cubebene	1351	1345	0.3	-	0.5
47	Neryl acetate	1362	1362	-	-	0.9
48	Isoledene	1373	1371	2.2	-	-
49	α-Copaene	1377	1374	1.6	0.4	-
50	β-Cubebene	1388	1387	0.1	0.5	-
51	Methyl cinnamate[e]	1389	1388	-	1.2	-
52	β-Elemene	1391	1389	0.4	1.0	0.5
53	α-Gurjunene	1412	1407	-	0.4	-
54	β-Maaliene	1413	1410	-	0.5	-
55	α-Cedrene	1414	1413	0.2	-	0.4
56	β-Caryophyllene	1419	1417	0.9	3.1	8.8

Table 1 Continued…

57	Aristolene	1428	1428	-	0.7	-
58	*trans*-α-Bergamotene	1435	1432	0.1	-	2.5
59	γ-Elemene	1437	1437	-	-	0.8
60	Aromadendrene	1441	1439	0.7	-	0.2
61	α-Humulene	1454	1452	0.9	2.4	-
62	(*E*)-β-Farnesene	1455	1455	-	-	0.4
63	β-Cadinene	1475	1474	1.0	-	-
64	γ-Gurjunene	1477	1479	0.6	-	-
65	γ-Muurolene	1480	1480	-	0.5	-
66	Germacrene D	1485	1484	-	2.8	-
67	α-Amorphene	1485	1485	1.4	0.6	-
68	β-Selinene	1486	1486	-	1.4	1.1
69	δ-Selinene[f]	1493	1493	1.5	0.7	-
70	Zingiberene	1494	1493	-	1.0	-
71	Cadine-1,4-diene	1496	1496	0.6	0.8	-
72	α-Muurolene	1500	1500	-	0.2	-
73	β-Bisabolene	1506	1506	0.5	-	-
74	α-Farnesene	1506	1507	-	0.2	-
75	*cis*-(Z)-α-Bisabolene epoxide[e]	1515	1515	-	-	0.7
76	β-Agarofuran[e]	1520	1516	-	4.3	-
77	*endo*-1-Bourbonanol[f]	1520	1522	-	0.8	-
78	δ-Cadinene	1525	1522	3.6	2.4	-
79	α-Cadinene	1535	1533	0.6	-	-
80	β-Calacorene	1546	1541	0.5	-	-
81	α- Agarofuran	1554	1556	-	0.6	-
82	Elemol	1550	1548	0.4	-	-
83	(*E*)-Nerolidol	1563	1561	-	0.4	-
84	Spathulenol	1578	1577	6.5	-	1.4
85	Caryophyllene oxide	1583	1581	0.9	0.8	1.4
86	Globulol	1585	1584	-	1.1	0.4
87	α-Cyperol	1599	1600	0.1	-	-
88	Guaiol	1601	1600	-	1.8	-
89	Longiborneol	1606	1607	-	0.9	-
90	β-Oplopenone[e]	1608	1607	-	0.9	-
91	Aromadendrene epoxide	1623	1620	-	-	1.9
92	τ-Muurolol	1646	1640	-	1.1	-
93	γ-Eudesmol	1650	1643	0.8	-	-
94	β-Eudesmol	1651	1645	-	2.1	-
95	α-Cadinol	1654	1652	17.1	1.4	-
96	Bulnesol	1672	1666	-	0.8	-
97	α-Bisabolol	1685	1685	3.6	-	0.6
98	Valerenol	1712	1711	0.5	-	-
99	(*E,E*)-Farnesol	1748	1747	14.0	0.9	-
100	Benzyl benzoate	1760	1760	-	1.4	-
101	β-Costol	1780	1778	-	-	0.2
102	Guaiazulene[g]	1790	1790	0.6	-	-
103	Farnesyl acetate	1848	1846	-	2.4	-
104	Phytol	2125	2124	-	1.4	-
	Monoterpene hydrocarbons			10.9	44.2	43.7
	Oxygenated monoterpenes			17.0	6.2	16.2
	Sesquiterpene hydrocarbons			19.6	25.6	18.3
	Oxygenated sesquiterpenes			43.9	15.4	6.8
	Diterpenes			-	1.4	-
	Non-terpenes			-	4.7	6.7
			Total	**91.4**	**97.5**	**2.0**

*SD: % 0.1, [a]Elution order on HP-5MS column; [b]Retention indices on HP-5MS column; [c]Literature retention indices [11]; [d] Standard deviation (SD ±); [e]Co-injection with authentic compounds; [f] Correct isomer not identified; [g] Tentative identification; - Not identified; *A. sel*, *A. setaceus* leaf; *C. jal, C. javanica* leaf; *A. kwl*, *A. kwangsiensis* leaf

On chemotaxonomic consideration, the essential oils of *C. pilosula* [12] contained aromatic compounds, those of *C. clematidea* [13], *C. thalictrifolia* [14] and *C. cordifolioidea* [15] consisted mainly of fatty acids while terpenes dominates in the oil of *C. javanica* (present study). Thirty-seven constituents totaling 92.2 ± 0.01 % of the total oil contents were identified in the leaf of *A. kwangsiensis*. This comprises of monoterpene hydrocarbons (43.7%), oxygenated monoterpenes (16.7%), sesquiterpene hydrocarbons (18.3%), oxygenated sesquiterpenes (6.8%) and non-terpenes (6.7%). The components occurring in higher amounts in the leaf oil were sabinene (34.8 ± 0.02%), β-caryophyllene (8.8 ± 0.01%) and terpinen-4-ol (8.6 ± 0.01%). As earlier stated the authors are not aware of any report on the composition of the leaf oil of *A. kwangsiensis*. The oils of *Aristolochia* may be classified into those containing monoterpene hydrocarbons such as *A. kwangsiensis*, *A. trilobata* [16] and *A. gibertii* [21]; oxygenated monoterpenes found in *A. indica* [17] and *A. asclepiadifolia* [25]; sesquiterpene hydrocarbons present in *A. impudica* [19], *A. papillaris* [23] and *A. rodriguesia* [23]; oxygenated sesquiterpenes as seen in *A. ovalifolia* [18] and *A. argentina* [22]. There are essential oils containing abundance of sesquiterpene hydrocarbons and oxygenated sesquiterpenes common to A. *gibertii* [24] as well oils containing non-terpenes compound found in *A. cymhifera* [23].

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Supporting Information

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