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Research Article

Identification of essential oil components in Chinese endemic plant *Achnatherum inebrians*

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Abstract

The chemical composition of the essential oil from the seeds and leaves of a Chinese endemic plant *Achnatherum inebrians* were analyzed for the first time by gas chromatography coupled with mass spectrometry (GC-MS). Forty-six and twenty-four volatile components were identified from leaves and seeds representing 93.74% and 85.13% of oils, respectively. The major components detected in the leaf oil were 3-hexen-1-ol (9.45%), hexahydrofarnesyl acetone (7.61%), phytol (6.74%), 2H-pyran-3-ol (6.62%), benzyl alcohol (6.58%) and 2-methoxy-3-(2-proprnyl)-3-allyl-2-methoxyphenol (6.30%). However, n-butyl acetate (12%), benzene methanol (8.01%), 2H-pyran-3(4H)-one (6.95%), acetophenone (6.86%), 4-hydroxymethylacetophenone (6.42%) were the major components of the seed oil. Furthermore, various bioactive compounds were identified in both essential oils at different amounts, such as benzaldehyde, coumarin, and phytol.

Keywords: GC-MS, chemical composition, volatile components, bioactive compounds, China.

Introduction

Essential oils are natural complex mixtures of volatile compounds characterized with strong odor that are stored in cavities, canals, epidermic cells, glandular trichromes and secretory cells within several plant organs, including flower, bud, seed, leaves, herbs, fruit, bark and roots [1]. Essential oils play a significant role in the protection of plants against herbivores by reducing their appetite for such plants and they also may attract some insects to favour the dispersion of pollens and seeds [2]. Due to their large variety of secondary metabolites, including terpenes, phenols, alcohols, acetones, acids, esters and aldehydes, several essential oils have long been researched for their insecticidal properties [3], antioxidant [4], antiviral [5], antibacterial [6], antifungal [7] and anticancer activities [8]. So far, more attention has been paid to the use of essential oils against food spoilage microorganisms, because the side effects of chemical preservatives can cause respiratory and health problems, which led to the research for new antimicrobial agents from plants to enhance the safety of food products [9]. *Achnatherum inebrians* (Hance) Keng is an important perennial herbaceous bunch grass which belongs to the genus Achnatherum, Poaceae, Gramineae family. This plant is commonly known as "Medical Herb" [10]. *Achnatherum inebrians* generally grows at 2000 to 3000 m of mountain grasslands, slopes, bushes and mountain shrub of Inner Mongolia, Gansu, Ningxia, Qinghai, Xinjiang, Tibet and Sichuan where it has caused grassland degradation and environmental damage [11, 12, 13]. Many studies have shown that *Achnatherum inebrians* is usually infected with the endophyte *Neotyphodium gansuense* which provides to this bunch grass a powerful ability owing to an increased tolerance to drought, salt [14], cold [15], heavy-metals [16, 17] and pathogens and fungi pests [18]. Furthermore, studies on the chemical composition of Endophyte-infected (E+) and Endophyte-non infected (E-) of this plant have been investigated, and results showed that Endophyte-infected (E+) contain a high level of ergonine and evergonine [19, 20], which are probably the main causes of intoxication of livestock in China [21]. Furthermore, *Achnatherum inebrians* could also be used as medicinal herbs. It has been shown that this poisonous weed has some biological activities, such as anti-bactericidal, insecticidal and anti-tumor effects [22, 23]. To the best of our knowledge, no chemical composition of the essential oils from the seeds and leaves of this plant have been reported until now. Due to this reason, we find it necessary to make an investigation of the essential oil composition extracted from seeds and leaves of *Achnatherum inebrians*.

Figure 1. ***Achnatherum inebrians*** **in flower and growing in typical grassy upland country.**

Materials and Methods

Plant material

Fresh plants of *Achantherum inebrians* were collected during September 2012 from Yuzhong (E: 104° 09`, N: 35° 52`, elevation: 1874 m) in Gansu, China. The plant was identified by the Institute of Botany at the Chinese Academy of Science. Voucher specimens were deposited at the herbarium of the Institute.

Pretreatment

Freshly harvested plants were subjected to sorting by hand for the elimination of pests which could potentially contaminate the chemical nature of the essential oil. The samples were washed with water then shade dried in the open air.

Isolation of essential oils

The essential oils were extracted from the dried plant material (seeds and leaves) by hydro distillation for 3 hours using Clevenger type-apparatus according to the Europe pharmacopoeia [24]. The essential oils were separated from water by diethyl-ether, then dried over anhydrous sodium sulphate and concentrated by a rotary-evaporator. The essential oils obtained were stored in sealed glass vials and conserved at 4°C until analysis by GC-MS.

Gas Chromatography–Mass Spectrometry

The GC-MS analysis of the leaves and seeds essential oils was performed with a Shimadzu GC/MS QP2010SE (Japan), which was equipped with a capillary column (fused silica 30m×0.25mm id, coated with 0.25µm film) Rtx-5MS. The column oven temperature was programmed from 35°C to 80°C, at 2°C /min, held isothermal for 2 min then up to 250°C and then held isothermal for 3 min. The flow rate of the Carrier gas (He) was set at 3ml/min, the flow rate of the carried gas (He) was 3 ml/min, the detector interface temperature 250°C and ionization energy 70 eV. The oil components were identified as the relative amount (%) of individual components of the oil and expressed as percent peak area relative to total peak area from MS analyses and by NBS 75, Wiley 7n and mass finder databases.

Results and Discussion

The essential oils obtained were light yellow liquids with a characteristic odor. The yield of essential oil from leaves was 2.28-fold (V/W) %, higher than that found in seeds. Table 1 shows the yield and the chemical composition identified by GC/MS in order to their elution on the Rtx-5MS capillary column of both essential oils from leaves and seeds. Forty six volatile components were identified from leaves and twenty four volatile components were identified from seeds. The chemical class distribution of volatile compounds identified from leaves and seeds revealed the presence of 6 classes of compounds, including alcohols, aldehyde, ketones, phenols, terpenoids, fatty acids and other compounds. In addition, alcohols were the major class of constituents of both essential oils (leaves and seeds) at a rate of 28.01% and 19.43% respectively. The main volatile components identified in the essential oils from leaves are 3-hexen-1-ol(9.45%), hexahydrofarnesyl acetone (7.61%), phytol (6.74%), 2H-pyran-3-ol (6.62%), benzyl alcohol (6.58%), and 2-methoxy-3-(2-proprnyl)-3-allyl-2-methoxyphenol (6.30%). Furthermore, hydrocarbon and oxygenated monoterpene, sesquiterpenes, and o diterpene, such as, β-cyclocitral (0.67%), cis-geraniol (0.20 %), and β-ionone (1.09%), hexahydrofarnesyl acetone (7.61%), (E,E)- farnesyl acetone (0.60%) and phytol (6.74%) were identified in the leaf oils. On the other hand, the main constituents of seed essential oils were n-butyl acetate (12%), benzene methanol (8.01%), 2H-pyran-3(4H)-one (6.95%), acetophenone (6.86%), 4-hydroxymethylacetophenone (6.42%). In addition, oxygenated monoterpene, diterpenes were also present in the seed oils, including, β-damascenone (1.14%), β-cyclocitral (0.12%), β-ionone-5, 6-epoxide (2.77%) and phytol (1.78%). The chemical composition of both leaves and seed essential oils reveals the presence of a wide range of bioactive components, which may provide strong antimicrobial activity to those essential oils. Moreover, many researchers have reported the antimicrobial activity of essential oils is attributed to the presence of volatile components such as terpenes, phenol and aldoketones, which have been shown to exhibit strong antimicrobial activity [25, 26]. However, no other reports on the essential oil composition of *Achnatherum inebrians* have been investigated. Thus, it would be very difficult to compare our results with others.

Table 1. Yield and essential oil composition of *Achnatherum inebrians* (leaves and seeds) identified by GC-MS.

No.	Compound	RT	Leaf oil (%)	Seed oil (%)
1	Acetic acid-1-methyl ethyl ester	3.22	1.82	-
2	l- Homoserine	4.05	1.71	-
3	n-Butyl acetate	8.34	-	12.00
4	p-Dioxane-2,5-dione	8.35	3.22	-
5	2-Ethyl-1.3-dioxalane	8.54	-	3.42
6	Furfural	9.30	0.88	-
7	3-Hexenol	11.08	9.45	-
8	2-Hexenol	11.99	0.34	-
9	Benzaldehyde	18.94	1.85	1.54
10	6-Methyl-5-hepten- 2-one	21.31	0.24	-
11	(E,E)-2,4-Heptadienal	23.25	0.29	-
12	3-Methyl-2,5-furanedione	23.48	0.34	-
13	Benzyl alcohol	24.94	6.58	4.47
14	Benzene acetaldehyde	25.52	1.54	3.37
15	Benzene methanol	27.00	-	8.01
16	Acetophenone	27.15	-	6.86
17	**α-Methyl-α-[4-methyl-3-pentenyl]**oxirane methanol	27.84	0.79	-
18	2H-Pyran-3(4H)-one	30.40	2.75	-
19	Benzene ethanol	30.85	3.12	6.95
20	Ethyl 3-(acetyloxy) butanoate	31.36	0.63	-
21	Camphor	33.31	0.91	-
22	p-Ethyl phenol	34.29	2.84	-
23	2H-Pyran-3-ol	34.49	6.62	-
24	Diethyl ester-Succinic acid	34.72	0.28	0.33
25	Caprin aldehyde	35.27	0.67	0.36
26	β -Cyclocitral	35.45	0.67	0.12
27	Coumaran	35.51	0.47	1.33
28	Cis- geraniol	35.60	0.20	-
29	(1E)-1-Phenylethanone oxime	35.85	-	1.14
30	p-Isopropyl benzaldehyde	35.98	0.17	-
31	2,3 Dimethoxy toluene	36.34	2.40	-
32	Acetophenone oxime	36.60	-	4.43
33	4-Hydroxy-3-methylacetophenone	36.82	2.03	6.42
34	2-Methoxy-3-(2-propenyl)-phenol	37.29	6.30	-
35	3-Hydroxy-2,4,4-trimethylpentyl ester	37.48	0.78	-
36	β -Damascenone	37.57	-	1.14
37	4-(2,2-Dimethyl-6-methylenecyclohexyl) butanal	37.59	2.27	-
38	Cis-4,7,10,13,16,19-Docosahexanoic acid	37.63	3.80	5.70
39	Trans- geranylacetone	38.13	0.76	-
40	β –Ionone	38.43	1.09	-
41	β-Ionone-5,6-epoxide	38.45	0.97	2.77
42	Dicyclohexyl ketone	38.66	1.60	2.73
43	1H-Cycloprop[e]azulen-7-ol	39.20	1.11	-
44	Cis-isoeugenol	40.15	0.87	-
45	Hexahydrofarnesyl acetone	40.64	7.61	-
46	Phthalic acid diisobutyl ester	40.76	1.28	4.25
47	(7Z,10Z,13Z)-7,10,13-Hexadecatrienal	40.94	0.94	-
48	(E,E)- Farnesyl acetone	41.02	0.60	-
49	Palmitic acid methyl ester	41.06	0.97	-
50	Palmitic acid	41.24	1.91	1.36
51	Phthalic acid dibutyl ester	41.29	0.58	4.56
52	Linoleic acid methyl ester	42.09	1.42	-
53	Phytol	42.15	6.74	1.87
Total (%)			93.74	85.13
Essential oil yield (% w/w)			0.016	0.007

Conclusion

In the present study, the chemical composition of essential oils extracted from the leaves and seeds of the Chinese endemic plant *Achnatherum inebrians* have been investigated for the first time. The yield of essential oil obtained from leaves was 2.28-folds more than that obtained from the seeds, and the structural analysis by GC/MS revealed the presence of 46 and 24 volatile compounds from leaves and seeds, respectively. Alcohol components were the major constituents of both essential oils (leaves and seeds) at a rate of 28.01% and 19.43%, respectively. Diverse classes of bioactive compounds have been identified in both essential oils, including aldehyde, ketones, phenols, monterpenoids, diterpenoids and sesquiterpenoids, which may make these essential oils a promising source of antimicrobial activities. Further studies are needed to evaluate the antimicrobial activity of these essential oils.

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