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The Chemical Composition and Biological Activity of the Essential Oil from the Underground Parts of *Ferula tadshikorum* (Apiaceae)

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Abstract: The underground parts of *Ferula tadshikorum* M. Pimen were collected from the southern part of Tajikistan. The essential oils were obtained by hydrodistillation and analyzed by GLC-FID and GLC-MS. A total of 26 compounds were identified representing 94.4 % of total oil composition. The essential oil was dominated by the sulfur-containing compounds (*Z*)-1-propenyl *sec*-butyl disulfide (37. 3%), (*E*)-1-propenyl *sec*-butyl disulfide (29.9%), (*E*)-1-propenyl 1-(methylthio)propyl disulfide (16.8%), and propyl *sec*-butyl disulfide (4.8%). The antioxidant, antimicrobial and cytotoxic activities of the essential oil was evaluated. To our best knowledge, this is the first report concerning the chemical composition and biological activity of the essential oil obtained from the underground parts of *F. tadshikorum*.

Keywords: *Ferula tadshikorum*; essential oil composition; disulfides.

1. Introduction

The genus *Ferula* L. includes about 130 species in the family Apiaceae, the majority of species are widespread in Asia [1]. There are thirty-seven *Ferula* species present in Tajikistan. *F. tadshikorum* M. Pimen is an endemic plant of southern Tajikistan [2]. In recent years, due to the production of an

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oleo-gum-resin (exudate) from *Ferula* species as asafoetida, interest in *F. tadshikorum* has also increased. The oleo-gum-resin is used for needs of the medical industry and as flavoring spice for various foodstuff. Approximately, the annual volume of exported oleo-gum-resin from *Ferula* species in Tajikistan is 150 tons [3]. The harvest in the wild is harmful and endangers populations of asafetida-containing *Ferula* species, including *F. tadshikorum*.

Ferula species have been used as herbs since ancient time in traditional medicine. Avicenna recommended *Ferula* for treatment of skin diseases (vitiligo), tuberculosis, pains in joints, against worms, inflammation of stomach and intestines, and as an antidote for toxic salts and compounds [4]. In Tajik traditional medicine, asafoetida is used as an antispasmodic, antihelminthic, and carminative agent. *Ferula* is used to treat tumors and to rejuvenate the body [5].

Tadzhiferin and tadzhikorin, two terpenoid coumarins, have been isolated from the fruit of *F. tadshikorum* [6]. Later, deacetyltadzhikorin also was isolated from the acetone extract of the roots of *F. tadshikorum* [7]. Asafoetida has a characteristic sulfurous odor and a bitter taste, due to presence of sulfur-containing compounds [8]. Regarding the chemistry and bioactivity of the essential oil of *F. tadshikorum,* to our best knowledge, until now, there have been no published reports on volatile secondary metabolites of the underground parts of *F. tadshikorum*.

2. Materials and Methods

2.1. Plant Materials

The underground parts of *F. tadshikorum* M. Pimen were collected near the Guli Bodom village, Yovon region of Tajikistan (1000 m above sea level), on 15 June 2016, during the period of plant harvesting. The plant was identified by comparison with voucher specimen (MW0594029) which are deposited in the herbarium of the Lomonosov Moscow State University (Mosquensis University). The fresh samples from the underground parts of *F. tadshikorum* were cut into small pieces and hydrodistilled for 3 h to give the yellow essential oil, with 1.5 % yield.

2.2. Gas-liquid Chromatography

The gas chromatographic analysis of the essential oil was performed using a GC-2010 plus gas chromatograph (Shimadzu), equipped with a non-polar ZB-5 fused bonded column (Phenomenex) and flame ionization detector (FID). The column parameters were 30 m length, 0.25 mm inner diameter and 0.25 μm film thickness. The carrier gas was helium with a flow rate of 1.5 mL/min with the split mode. The operating temperature conditions were initial temperature 120°C for 2 min isothermal followed by linear temperature increase until 320°C at a rate of 8°C / min, and then for 10 min at isothermal mode at 320°C. Detector and injector temperatures were 320°C and 310°C, respectively. GC solution by Shimadzu was used for recording and integration.

2.3. Gas Chromatographic-Mass Spectral Analysis

GLC-MS was performed using a Shimadzu GCMS-QP2010 Ultra operated in the electron impact (EI) mode (electron energy = 70 eV), scan range = 40–400 atomic mass units, scan rate = 3.0 scans/s, and GC-MS solution software. The GC column was a ZB-5 fused silica capillary column with a (5% phenyl)-polymethylsiloxane stationary phase and a film thickness of 0.25 μm. The carrier gas was helium with a column head pressure of 552 kPa and flow rate of 1.37 mL/min. Injector temperature was 250°C and the ion source temperature was 200°C. The GC oven temperature program was programmed for 50°C initial temperature, temperature increased at a rate of 2°C/min to 260°C. A 5% *w*/*v* solution of the sample in CH_2Cl_2 was prepared and 0.1 μL was injected with a splitting mode (30:1). Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [9], and stored in our in-house library [10].

2.4. Antioxidant, Antimicrobial and Cytotoxicity Assays

The antioxidant activity of the essential oil was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-[3-ethylbenzthiazoline-6-sulfonic acid] (ABTS) and ferric reducing antioxidant power (FRAP) assays as described previously [11]. The essential oil was stabilized in Tween-80 and screened against methicillin-resistant *Staphylococcus aureus* (MRSA) NCTC 10442 and *Escherichia coli* ATCC 25922 bacteria [12]. Cytotoxicity of essential oil against CCRF-CEM (human T lymphoblast leukemia) and CEM/ADR5000 (adriamycin-resistant leukemia) cancer cell lines was determined by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Antioxidant, antimicrobial and cytotoxicity assays were analyzed as previously described [13].

3. Results and Discussion

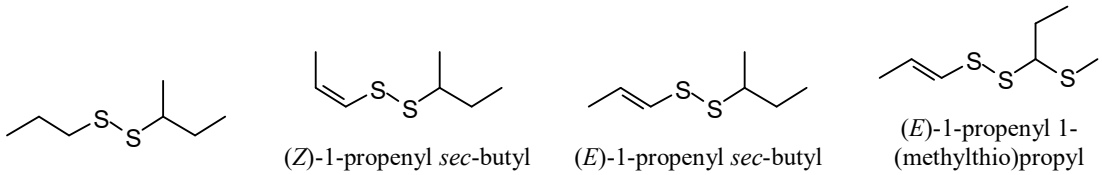
3.1. Essential Oil Composition

The essential oil was and analyzed by GLC-FID and GLC-MS. A total of 26 compounds were identified representing 94.4 % of total oil composition (Table 1). The essential oil was dominated by the sulfur-containing compounds (*Z*)-1-propenyl *sec*-butyl disulfide (37.3%), (*E*)-1-propenyl *sec*-butyl disulfide (29.9%), (*E*)-1-propenyl 1-(methylthio)propyl disulfide (16.8%), and propyl *sec*-butyl disulfide (4.8%) (Figure 1).

Table 1. Chemical composition of the essential oil of the underground parts of *Ferula tadshikorum*.

RI[a]	RI[b]	Compound	%
860	860	2-Methyloctane	0.2
900	900	Nonane	t
925	926	Tricyclene	t
932	939	α-Pinene	0.8
949	954	Camphene	0.1
968	973	2-Pyrone	0.2
978	979	β-Pinene	0.4
989	990	Myrcene	0.1
995	---	Methyl *sec*-butyl disulfide	t
1010	1010	2,3,5-Ttrimethylthiophene	t
1017	1017	α-Terpinene	t
1024	1024	*p*-Cymene	t
1029	1029	Limonene	t
1035	1037	(*Z*)-β-Ocimene	0.3
1045	1050	(*E*)-β-Ocimene	0.5
1058	1059	γ-Terpinene	t
1108	1103	Dipropyl disulfide	0.1
1164	1164	Propyl *sec*-butyl disulfide	4.8
1169	1169	(*Z*)-1-Propenyl *sec*-butyl disulfide	37.3
1174	---	(*E*)-1-Propenyl *sec*-butyl disulfide	29.9
1212	1212	Di-*sec*-butyl disulfide	0.5
1421	1421	(*Z*)-1-Propenyl 1-(methylthio)propyl disulfide	2.5
1427	1427	(*E*)-1-Propenyl 1-(methylthio)propyl disulfide	16.8
1452	1456	(*E*)-β-Farnesene	t
1503	1505	(*E*,*E*)-α-Farnesene	0.3
		Terpenoids	2.5
		Sulfur-containing compounds	91.8
		Others	0.2
		Total identified	94.4

[a] Retention Index (RI) determined with reference to a homologous series of *n*-alkanes on a ZB-5 column.
[b] Retention Index from the databases [9,10]. t:trace



Propyl *sec*-butyl disulfide | (*Z*)-1-propenyl *sec*-butyl disulfide | (*E*)-1-propenyl *sec*-butyl disulfide | (*E*)-1-propenyl 1-(methylthio)propyl disulfide

Figure 1. Structure of the major components of the essential oil of *Ferula tadshikorum*.

According to literature reports, the sulfur-containing compounds are the major components of the essential oils of many *Ferula* species, including *F. assa-foetida* [14], *F. fukanensis* [15], *F. latisecta* [16,17], *F. persica* [18,19] and *F. sinkiangensis* [15]. The most prevalent sulfur-containing compounds in the *Ferula* oils were (*Z*)-1-propenyl *sec*-butyl disulfide and (*E*)-1-propenyl *sec*-butyl disulfide [20], which is in agreement with the *F. tadshikorum* essential oil composition.

3.2. Antioxidant, Antimicrobial and Cytotoxic Activities

The essential oil of *F. tadshikorum* exhibited low antioxidant activity as compared to the positive control, caffeic acid. The results of the DPPH, ABTS and FRAP analyses are represented Table 2. Median inhibitory concentration (IC_{50}) for radical scavenging of the essential oil was 17.8 mg/mL for DPPH and 8.2 mg/mL for ABTS, respectively. The ferric reducing antioxidant power value was 1072.4 µM Fe(II)/mg of the oil sample.

Table 2. Antioxidant and cytotoxic activities of the essential oil of *Ferula tadshikorum*.

Sample	DPPH IC_{50}, mg/mL	ABTS IC_{50}, mg/mL	FRAP µM Fe(II)/mg of samples	CCRF-CEM IC_{50}, µg/mL	CEM/ADR 5000 IC_{50}, µg/mL
F. tadshikorum essential oil	17.8	8.2	1072.4	21.6	142.5
Positive control	0.002[a]	0.0015[a]	2380[a]	0.25[b]	1.4[b]

[a]Caffeic acid, [b]doxorubicin

The antimicrobial activity of the essential oil was screened against one Gram-positive and one Gram-negative bacteria (MRSA NTCT 10442 and *E. coli* ATCC 25922). The essential did not exhibit antimicrobial activity at a concentration 20 mg/mL. Our results indicate that the essential oil has weak antioxidant and antimicrobial activities, similar to other sulfur-containing *Ferula* oils [21].

The cytotoxicity of the oil was tested against CCRF-CEM and CEM/ADR5000 cancer cell lines: IC_{50} values were 21.6 µg/mL for CCRF-CEM, and 142.5 µg/mL for CEM/ADR5000 cell lines. CEM/ADR5000 over-expresses the ABC transporter p-gp. The reduced cytotoxity of the essential oil suggests that it contains substrates of p-gp, which would be rapidly pumped out of the cells. Bagheri and co-workers reported that methanol extracts of different *Ferula* species and the oleo-gum-resin of *F. assa-foetida* exhibited cytotoxic effect with IC_{50} values in the range of 6-321 µg/mL [22].

In conclusion, this work is the first report concerning the chemical composition and biological activity of the essential oil of the underground parts of *F. tadshikorum*. The chemical compositions of the essential oil were dominated by the sulfur-containing compounds (*Z)*-propenyl *sec*-butyl disulfide, (*E)*-propenyl *sec*-butyl disulfide and (*E*)-1(1-propen-1-yl)-2(2-thiopent-3-yl) *trans* disulfide. These components have been found in essential oils of several other *Ferula* species as major constituents. The cytotoxicity of the essential oil against CCRF-CEM and CEM/ADR5000 cancer cell lines is in agreement with its traditional use as antitumor drug.

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