AkiNik

American Journal of Essential Oil and Natural Product

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AJEONP

American Journal of Essential Oil and Natural Product

Chemical composition and antiproliferative activity of the essential oil of *Galagania fragrantissima* Lipsky (Apiaceae)

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AJEONP 2013; 1 (1): 11-13

Received 20-7-2013
Accepted: 28-7-2013

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ABSTRACT

Galagania fragrantissima Lypsky (Apiaceae) is native to central Asia and is used as a spice for soups and other dishes. The plant has not been previously examined phytochemically. The aim of this research was to characterize the essential oil of this plant, which has a pleasant spicy odor, and to examine the potential cytotoxic activity of the oil. The aerial parts of *G. fragrantissima* were collected from the Yovon region of Tajikistan. The essential oil was obtained by hydrodistillation and analyzed by gas chromatography – mass spectrometry (GC-MS). The *in-vitro* cytotoxicity of the oil was determined on three different human tumor cell lines using the MTT method. Nine different compounds were identified in the oil accounting for 98.8% of the composition. *G. fragrantissima* oil was rich in unsaturated aldehydes, with (2*E*)-dodecenal (83.6%) dominating. The essential oil showed cytotoxic activity against HeLa, Caco-2, and MCF-7 tumor cell lines with IC_{50} values of 0.206, 0.074, and 0.058 mg/mL, respectively. The cytotoxicity of *G. fragrantissima* oil is most likely due to the major component, (2*E*)-dodecenal, which can react with a variety of nucleophiles, either from proteins or DNA.

Keywords: Essential Oil Composition; (2E)-Dodecenal; (2E)-Dodecenol; Cytotoxicity.

1. Introduction

Galagania fragrantissima Lypsky belongs to the genus *Galagania* (Apiaceae), a genus that has been included together with *Muretia* in the genus *Elaeosticta*, a group comprising 24 European and Asian species [1]. It is distributed in Afghanistan, Kyrgyzstan, Uzbekistan and Tajikistan. The leaves and young shoots are used as a spice for soups and other dishes [2]. The essential oil, which may be important for the perfumery industry, has a light yellow color and exhibits a spicy pleasant odor. The oil yield was 0.16-0.3% [3]. Because information about the chemical composition of *Galagania fragrantissima* was missing, we have analyzed its essential oil using high-resolution capillary gas chromatography – mass spectrometry (GC-MS) which is the method of choice for such analyses. In addition, we have analyzed the potential cytotoxicity of the essential oil against three human tumor cell lines.

2. Materials and methods

2.1 Plant Material

The aerial parts of *Galagania fragrantissima* Lypsky were collected during its flowering stage on 17 July 2012 near the Chormaghzak village, Yovon region of Tajikistan, (38.2447 N, 69.1021 E, 1300 m above sea level). A voucher specimen (accession number N6028) has been deposited in the herbarium of the Institute of Botany, Plant Physiology and Genetics of the Tajikistan Academy of Sciences. The fresh samples were hydrodistilled for 2 h; the yields of the essential oils were between 0.05 and 0.15 %.

2.2 Gas Chromatography – Mass Spectrometry (GC-MS)

The essential oil was analyzed by GC-MS using an Agilent 6890 GC with an Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 40-400 amu, and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a 5% phenyl-polymethylsiloxane stationary phase, film thickness of 0.25 μm, a length of 30 m, and an internal diameter of 0.25 mm.

The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. Inlet temperature was 200°C and interface temperature 280°C. The GC oven temperature program was programmed as follows: 40°C initial temperature, hold for 10 min; increased at 3°C/min to 200°C; increased 2°/min to 220°C. A 1 % w/v solution of the sample in CH_2Cl_2 was prepared and 1 µL was injected using a splitless injection technique.

Identification of the oil components was based on their Kovats 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 [4] and stored in the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.080]. The percentages of each component are reported as raw percentages based on total ion current (= 100%) without standardization.

2.3 Cytotoxicity Screening

The potential cytotoxic effects of the essential oil of *Galagania fragrantissima* in three human tumor cell lines (HeLa, Caco-2, and MCF-7) were assayed by the MTT assay. The cells were seeded at a density of 2×10^4 cells/well. The essential oil was serially double diluted in DMSO from 5 mg/ml to 0.01 mg/ml, and 100 µl liquid of each concentration was applied to the wells of a 96-well plates. Cells were incubated with the essential oil for 24 h before the medium was removed and replaced with fresh medium containing 0.5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The formazan crystals were dissolved in DMSO 4 h later; the absorbance was measured at 570 nm with a Biochrom Asys UVM 340 Microplate Reader.

3. Results & Discussion

The chemical composition of the essential oil of *Galagania fragrantissima* was analyzed by GC-MS (Table 1). Nine components representing 98.8% of the total oil were identified. The main constituents of the essential oil were aliphatic aldehydes and alcohols such as (2*E*)-dodecenal (83.6 ± 4.7%), (2*E*)-dodecenol (7.8 ± 1.9%), (2*E*)-tetradecenal (3.4 ± 0.7%), and dodecanal (2.3 ± 0.8%). Kim *et al.* [5] had described the microbial metabolism of (2*E*)-dodecenal which resulted in two microbial metabolites: (2*E*)-dodecenol and (3*E*)-dodecenoic acid.

The cytotoxicity of the oil was tested against HeLa, Caco-2 and MCF-7 cancer cell lines. Dose-dependent cytotoxicity of *Galagania fragrantissima* essential oil is shown in Figure 1. IC_{50} values were 0.206 mg/mL for HeLa, 0.074 mg/mL for Caco-2 and 0.058 mg/mL for MCF-7 cell lines. The cytotoxicity is most likely due to the major component (2*E*)-dodecenal – it is very electrophilic and can react with a variety of nucleophiles, such as amino groups either from proteins or DNA [6]. The other aldehydes are also reactive and can form Schiff bases with free amino groups. Alkylation of amino groups of DNA bases could potentially lead to mutations [7].

Kano *et al.* [8] noted that alkenals (C_{10}-C_{16}) have anti-deforming activity in Raji cells carrying the genome of Epstein-Barr virus (EBV). The activity is correlated with the length of carbon chain of the unsaturated aldehydes. (2*E*)-dodecenal disappeared immediately from the blood; probably it quickly binds to blood proteins.

4. Conclusions

The essential oil composition of *Galagania fragrantissima* has been presented for the first time. The oil is rich in α,β-unsaturated aldehydes, which accounts for the cytotoxic activity observed.

5. Acknowledgments

FSS is grateful to the DAAD-UCA Scholarship Program for a generous research/travel grant. WNS is grateful to an anonymous private donor for the gift of the GLC-MS instrumentation.

Table 1: Composition of the essential oil of *Galagania fragrantissima* determined by GC-MS[1]

RI	Compound	%
1206	Decanal	0.5±0.3
1261	(2*E*)-Decenal	Trace
1400	(4*E*)-Dodecenal	1.0±0.6
1411	Dodecanal	2.3±0.8
1453	Unidentified	0.9±0.4
1473	(2*E*)-Dodecenal	83.6±4.7
1480	(2*E*)-Dodecenol	7.8±1.9
1589	1-Hexadecene	0.1±0.1
1613	Tetradecanal	0.1±0.1
1673	(2*E*)-Tetradecenal	3.4±0.7
	Total identified	98.8

[1]The composition is based on the mean (± standard deviations) for three separate injections of the essential oil.

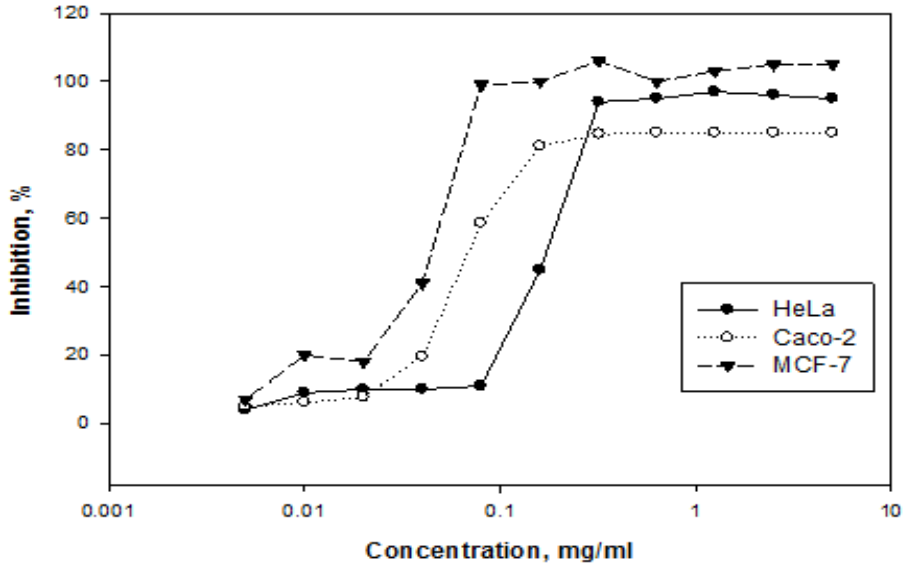

Fig 1: Dose-dependent inhibition of cell proliferation of *Galagania fragrantissima* essential oil in HeLa, Caco-2 and MCF-7 cell lines.

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