The genus *Libanotis* L. belongs to the family Apiaceae. This genus consists of about 60 species widespread in Europe, Africa, Asia, and Oceania. The plants of this family possess peculiar botanical characteristics such as the typical umbellate inflorescences and are distributed widely from tropical to temperate regions where they often are used as spices or drugs [1,2].

According to the ancient literature, some of the *Libanotis* species such as *L. buchtormensis* and *L. laticalycina* have been reported to be used as traditional medicinal plants [2]. The various species of this genus are also believed to have anti-inflammatory, antipyretic, analgesic, and spasmolytic effects [2].

*L. transcaucasica* is a perennial plant and distributed in Europe, northwest of Iran and also Caucasia [3,4]. *Libanotis montana* var. *lasiopetala*, *seseli libanotis* var. *armeniacum*, and *seseli transcaucasicum* are the other names for this plant [3,5]. Sesquiterpene hydrocarbons [4], monoterpenic hydrocarbons [6] and coumarins [4,7] were isolated by previous phytochemical studies of *L. transcaucasica*.

The chemical constituents of the essential oil from *L. buchtormensis* have also been investigated before [2]. Previously, the compositions of different fractions of the essential oil from *L. transcaucasica* have been reported [6,8]. But to our best knowledge, there is not any published report on the analysis of phytochemical composition of the essential oil from *L. transcaucasica* by GC and GC-MS. In addition, no previous biological study has been performed on the *L. transcaucasica* essential oil. In the present work, chemical composition and cytotoxic activity of essential oil from *L. transcaucasica* were reported.

**Materials and Methods**

**Reagents and chemicals**

RPMI 1640, fetal bovine serum (FBS), trypsin and phosphate buffered saline (PBS) were purchased from Biosera (Ringmer, http://www.biosera.com). Acetic acid glacial, dimethyl sulphoxide, hexane, methanol were purchased from Merck (Darmstadt, http://www.merck.de). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), was obtained from Sigma-Aldrich (St. Louis, http://www.sigma-aldrich.com). Penicillin/streptomycin and doxorubicin were purchased from EBEWE Pharma (Unterach, http://www.ebewe.at) and Invitrogen (San Diego, http://www.invitrogen.com), respectively.

**Plant material and isolation procedure**

The plant material was collected in June 2008 from northwestern of Iran. The plant was identified at the Department of Biology, University of Shiraz, Iran and a voucher specimen was deposited at the herbarium of the Medicinal and Natural Products Chemistry Research Center, Shiraz, Iran (No. PC 87-6). The aerial parts of collected plant were air-dried at ambient temperature in the shade and grinded to powder. The powder (60 g) was subjected to hydro-distillation for 4 h using a distillation apparatus, the essential oil was obtained by hydro distillation of its air-dried aerial parts and analyzed by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Cytotoxic activity of the essential oil was assessed on four human cancer cell lines (HeLa, LS180, MCF-7 and Raji).

**Keywords:** *Libanotis transcaucasica*; Essential oil; Germacrene B; Cytotoxicity

**Introduction**

The genus *Libanotis* L. belongs to the family Apiaceae. This genus consists of about 60 species widespread in Europe, Africa, Asia, and Oceania. The plants of this family possess peculiar botanical characteristics such as the typical umbellate inflorescences and are distributed widely from tropical to temperate regions where they often are used as spices or drugs [1,2].

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**Results:** The yield of oil was 0.9% (w/w) and it had yellow color. Fifty-four compounds in *L. transcaucasica* representing 84.0% of the oil components were identified. Germacrene B (20.2%) was the most abundant compound in this oil, followed by isospathulenol (11.0%), germacrene D (9.2%) and kessane (5.5%). The volatile oil displayed weak to moderate cytotoxic activity in all evaluated human cancer cell lines (IC₅₀=0.9-0.2 mg/ml). The highest and the lowest cytotoxic effects were observed on Raji and LS180 cell lines, respectively.

**Conclusion:** Sesquiterpene hydrocarbons were identified as the main components of the essential oil (48.3%). The cytotoxic activity observed in the essential oil may be contributed to the existence of this group of hydrocarbons in the plant.

**Abstract**

**Background:** *Libanotis transcaucasica* Schischk. belonging to the family of Apiaceae is a perennial plant that is distributed in Europe, northwest of Iran and also Caucasia.

**Purpose:** Due to the interest on development of drugs from natural origins, we studied the components and cytotoxic activity of essential oil from *L. transcaucasica*.

**Methods:** The essential oil of *L. transcaucasica* was obtained by hydro distillation of its air-dried aerial parts and was analyzed by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Cytotoxic activity of the essential oil was assessed on four human cancer cell lines (HeLa, LS180, MCF-7 and Raji).

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**Identification of the oil components**

Gas chromatography (GC) analysis was carried out using an
Agilent 6890N chromatograph (FID) with an HP-5 column (30 mx0.25 mm; 0.25 µm film thickness). The oven temperature increased from 60 to 240°C at a rate of 3°C/min, the injector and detector temperature were 240°C and 250°C, respectively. Helium was used as the carrier gas with a flow rate of 0.9 ml/min. Relative percentage data were obtained from electronic integration of peak areas without the use of correction factor. GC-MS analysis of the essential oil was performed using an Agilent 7890A chromatograph operating at 70 eV ionization energy, 0.5 s/scan and the mass range of 35-400, equipped with a HP-5 capillary column (phenyl methyl siloxane, 30×0.25 mm², 0.25 µm film thickness) programmed as above with helium as the carrier gas with the flow rate of 0.9 ml/min and a split ratio of 1:20.

Retention indices were determined by using retention times of n-alkanes that had been injected after the oil under the same chromatographic conditions according to the Van Den Dool method [9]. The compounds were identified by comparison of their mass spectra with the Wiley and Mass finder 3 libraries or with the published mass spectra [10].

Cytotoxicity assay

HeLa (human cervical adenocarcinoma), LS180 (human colon adenocarcinoma), MCF-7 (human breast adenocarcinoma) and Raji (human B lymphoma) cell lines were obtained from the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran. All cell lines were reported as one of the main constituent of the essential oils from other Libanotis species such as L. buchtormensis. As a rich source of Germacrene B, the plant genus can also be used as flavoring agent in the food and perfume industries [2]. Sesquiterpenoid hydrocarbons were also reported as one of the main constituent of the essential oils from other Libanotis species [2,15].

The chemical composition of the essential oil of L. transcaucasica contained 17 sesquiterpene hydrocarbons (48.3%), 11 oxygenated sesquiterpenes (22.7%), 13 monoterpenoid hydrocarbons (10.5%) and 10 oxygenated monoterpenes (2.2%). The main abundant component for L. transcaucasica was Germacrene B, which was in accordance with the previous report on other Libanotis species such as L. buchtormensis. As a rich source of Germacrene B, the plant genus can also be used as flavoring agent in the food and perfume industries [2]. Sesquiterpenoid hydrocarbons were also reported as one of the main constituent of the essential oils from other Libanotis species [2,15].

| Compound         | RI (%) | Compound         | RI (%) |
|------------------|--------|------------------|--------|
| Octane           | 865    | Methyl eugenol   | 0.2    |
| (2E)-Hexenal     | 885    | t-B-Caryophyllene| 0.1    |
| α-Thuene         | 930    | t-γ-Elemene      | 0.1    |
| α-Pinene         | 939    | t-α-Humulene     | 0.0    |
| Sabinene         | 975    | (E)-β-Farnesene  | 0.0    |
| β-Pinene         | 979    | t-γ-Murolene     | 0.0    |
| Myrcene          | 990    | Germacrene D     | 0.0    |
| α-Phellandrene   | 1002   | β-Selinene       | 0.0    |
| α-Terpene        | 1017   | γ-Selinene       | 0.0    |
| p-Cymene         | 1024   | 4-epi-cis-Dihyroagarofuran | 0.0 |
| Limonene         | 1029   | Germacrene A     | 0.0    |
| (Z)-β-Ocimene    | 1037   | Cadinene-β       | 0.0    |
| (E)-β-Ocimene    | 1050   | Kessane          | 0.0    |
| γ-Terpene        | 1059   | Germacrene B     | 0.0    |
| cis-Sabinene hydrate | 1070 | Mintonoxide     | 0.0    |
| Terpinolone      | 1088   | Spathulanol      | 0.0    |
| Linalool         | 1096   | Caryophyllene oxide | 0.0 |
| trans-Pinocamphone| 1162  | Copaen-4-α-ol-β | 0.0    |
| Terpinen-4-ol    | 1177   | Salvial-4(14)-en-1-one | 0.0 |
| α-Terpinol       | 1188   | Isospathulanol   | 0.0    |
| Myrtolene        | 1195   | Rosoradienol     | 0.0    |
| Isobornyl acetate| 1285   | 3-isothujopanone | 0.0    |
| Thymol           | 1290   | Acoronene B      | 0.0    |
| Dihydroedulan    | 1290   | Sesquiterpenoid hydrocarbons | 0.0 |
| Carvacrol        | 1299   | Oxygenated sesquiterpenes | 0.0 |
| Bicyclolene      | 1336   | Monoterpenoid hydrocarbons | 0.0 |
| β-Elemene        | 1338   | Oxygenated monoterpenes | 0.0 |
| α-Copaene        | 1376   | Other hydrocarbons | 0.0 |
| β-Cubebeene      | 1388   | Aliphatic aldehydes | 0.0 |
| Bourbonene-β     | 1388   | Total identified (%) | 0.0 |
| β-Elemene        | 1390   | 84.0             | 0.0    |

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The chemical composition of the essential oil of L. transcaucasica (% - 84.0

| Essential oil | HeLa cells | LS180 cells | MCF-7 cells | Raji cells |
|---------------|------------|-------------|-------------|------------|
| L. transcaucasica | 360 ± 83.6 | 304 ± 39.3 | 587.5 ± 62.8 | 295 ± 33.3 |
| Cisplatin     | 5.2 ± 1.2  | 4.6 ± 4.1   | 4.6 ± 2.9   | 3.3 ± 0.7  |

Values represent the mean of 4 experiments ± S.D.
The *in vitro* cytotoxic activities of the essential oil from *L. transcaucasica* were shown in Table 2. *L. transcaucasica* exhibited weak to moderate cytotoxic activity in the human cancer cell lines (Table 2).

As shown in Table 2, the most significant activity was observed against Raji cell lines and the lowest effect was observed on LS180 cell lines. The cytotoxic activity reported in the oil could also be contributed to the existence of some sesquiterpene hydrocarbons such as Germacrene B and Germacrene D [16,17].

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