In Vitro Cytotoxic Activity of the Essential Oil Extracted from *Artemisia Absinthium*

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ABSTRACT

Background: Essential oils are found to have multiple active components which can show in vitro cytotoxic action against various cancerous cell lines. This study reports the in vitro cytotoxic effects of the essential oil from Artemisia absinthium L. (Asteraceae) growing wild in Iran.

Methods: Water-distilled essential oil of A. absinthium collected from Ardabil, north-western Iran, was examined for its cytotoxic effects using a modified MTT assay. Air-dried aerial parts of A. absinthium was subjected to hydrodistillation using a clevenger-type apparatus. Cytotoxicity of the essential oil was measured against Hela and human healthy peripheral blood cells.

Results: The 50% cytotoxic concentrations were found to be 48.59 µg/ml and 5456.07 µg/ml for Hela cells and human lymphocytes, respectively. The volatile oil displayed good cytotoxic action against the human tumor cell line.

Conclusion: The IC_{50} shows that cytotoxicity of the oil against human tumor cell line is much higher than that required for healthy human cells. These results indicate low adverse effects for this oil. The findings of this study necessitate the need for further consideration of this essential oil in anti-neoplastic chemotherapy.

Keywords: Artemisia Absinthium, Cytotoxicity, Hela Cells, Lymphocyte.

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INTRODUCTION

Various phytochemicals from herbs and herbal oils with human health-promoting or biological properties are available that reduce the risk of chronic diseases [1]. Essential oils play an important role in the protection of the plants by exerting anti-bacterial, -viral, -fungal, -oxidative, -genotoxic, and free radical scavenging properties. In some cases, they act as insecticides [2]. The purpose of the present study was to determine the cytotoxic activity of the essential oil of *Artemisia absinthium* (Asteraceae) against human cancer cell lines, as well as its inhibitory effects against lymphocytes. The genus *Artemisia* has always been of great botanical and pharmaceutical interest and is useful in traditional medicines for the treatment of a variety of diseases and complaints [3,4]. *Artemisia absinthium* is a species of wormwood, native to temperate regions of Eurasia and northern Africa [5]. It grows naturally in vast areas of Iran. The components of leaves and flowering of *A. absinthium* are silica, two bitter substances (absinthin and anabsinthine), thujone, tannic and resinous substances, malic acid, and succinic acid [6]. The bitter taste of wormwood is because of sesquiterpene lactones (0.15-0.4%). Absinthin, artabsin, and guainolides are the major constituents in this plant [7,8]. *A. absinthium* is used medicinally as a tonic [7], anthelmintic [7], stomachic [9], antiparasitic [7],antiseptic [6,9] and choleretic [7],carminative [9], anti-inflammatory [9], mild antidepressant [9,10], cholagogue [11], and febrifuge agent [11]. The oil extracted from this plant can be used as a cardiac stimulant to improve blood circulation [12]. Pure wormwood oil is very poisonous, but with proper dosage, it poses little or no danger [12]. The oil is a potential source of novel agents for the treatment of *leishmaniasis* [13]. In our previous study, the composition of the leaves oil of *A. absinthium* was analyzed by GC and GC/MS. 1,8-Cineole (36.46%), borneol (25.99%), and camphor (10.20%) were found to be the major components among the 19 constituents characterized, comprising 100% of the total components detected [14]. Oxygenated

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monoterpenes (99.51%) were the main components in the leaves of *A. absinthium* collected from Iran [14]. The leaves essential oil of *A. absinthium* indicated significant activity against *Candida albicans*. Bactericidal kinetics of the oil extracted from *A. absinthium* leaves indicated that *C. albicans* is the most vulnerable [14]. In our previous study, antioxidative properties of the essential oil of *A. absinthium* leaves were determined by three methods: the ferric-reducing antioxidant Power (FRAP), radical-scavenging capacity of the oil or bleaching of 2,20-diphenylpicrylhydrazyl (DPPH), and β-Carotene-linoleic acid assay. The results suggest application of *A. absinthium* oil as a natural antioxidant agent [14].

However, little information is available on the pharmacological and cytotoxic properties of *A. absinthium* essential oil. Therefore, the present investigation was undertaken to evaluate the cytotoxicity of essential oil from *A. absinthium* using Hela and human healthy peripheral blood cells.

MATERIALS AND METHODS

General

The major equipment types used were a clevenger apparatus and ELISA reader. Cell culture media and laboratory reagents were from Merck Co., Germany. Other chemicals were of analytical grade.

Plant Material

The leaves of *Artemisia absinthium L.* collected from Namin, Ardabil Province, in north-western Iran in July 2011. Voucher specimens were deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

Isolation of the Essential Oil

The aerial parts of *A. absinthium* were dried at room temperature for several days. Air-dried aerial parts of *A. absinthium* (100g) were separately subjected to hydrodistillation using a clevenger-type apparatus for 3 hours. After decanting and drying the oil over anhydrous sodium sulfate, the oil was recovered. The essential oil yield was 1.01% (w/w).

Cytotoxicity Assay

The human cervical carcinoma Hela cell line NCBI code No. 115 (ATCC number CCL-2) was obtained from Pasteur Institute, Tehran-Iran. The cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum, 1% (w/v) glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were cultured in a humidified atmosphere at 37 ℃ in 5% CO_2. Cytotoxicity was measured using a modified MTT assay. This assay detects the reduction of MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide] by mitochondrial dehydrogenase, to blue formazan product, which reflects the normal functioning of mitochondrial and cell viability [15]. Briefly, the cells (5×10^4) were seeded in each well containing 100µl of the RPMI medium supplemented with 10% FBS in a 96-well plate. After 24 hours of adhesion, a serial of doubling dilution of the essential oil was added to triplicate wells over the range of 1.0-0.005 µl/ml. The final concentration of ethanol in the culture medium was maintained at 0.5% (volume/volume) to avoid toxicity of the solvent [16]. After 2 days, 10 µl of MTT (5 mg/ml stock solution) was added and the plates were incubated for an additional 4 hours. The medium was discarded and the blue formazan, formed in the cells, was dissolved with 100 µl dimethyl sulphoxide (DMSO). The optical density was measured at 490 nm using a microplate ELISA reader. The cell survival curves were calculated from cells incubated in the presence of 0.5% ethanol. Cytotoxicity is expressed as the concentration of drug inhibiting cell growth by 50% (IC_{50}). All tests and analyses were run in triplicates and mean values were recorded.

RESULTS

Cytotoxicity Assay

Cytotoxicity was measured using a modified MTT assay (Figure 1). The essential oil displayed good cytotoxic action towards the human tumor cell line.

The cytotoxic effects of *A. absinthium* oil were tested using lymphocyte and Hela cells (Tables 1, 2 and Figures 2, 3).

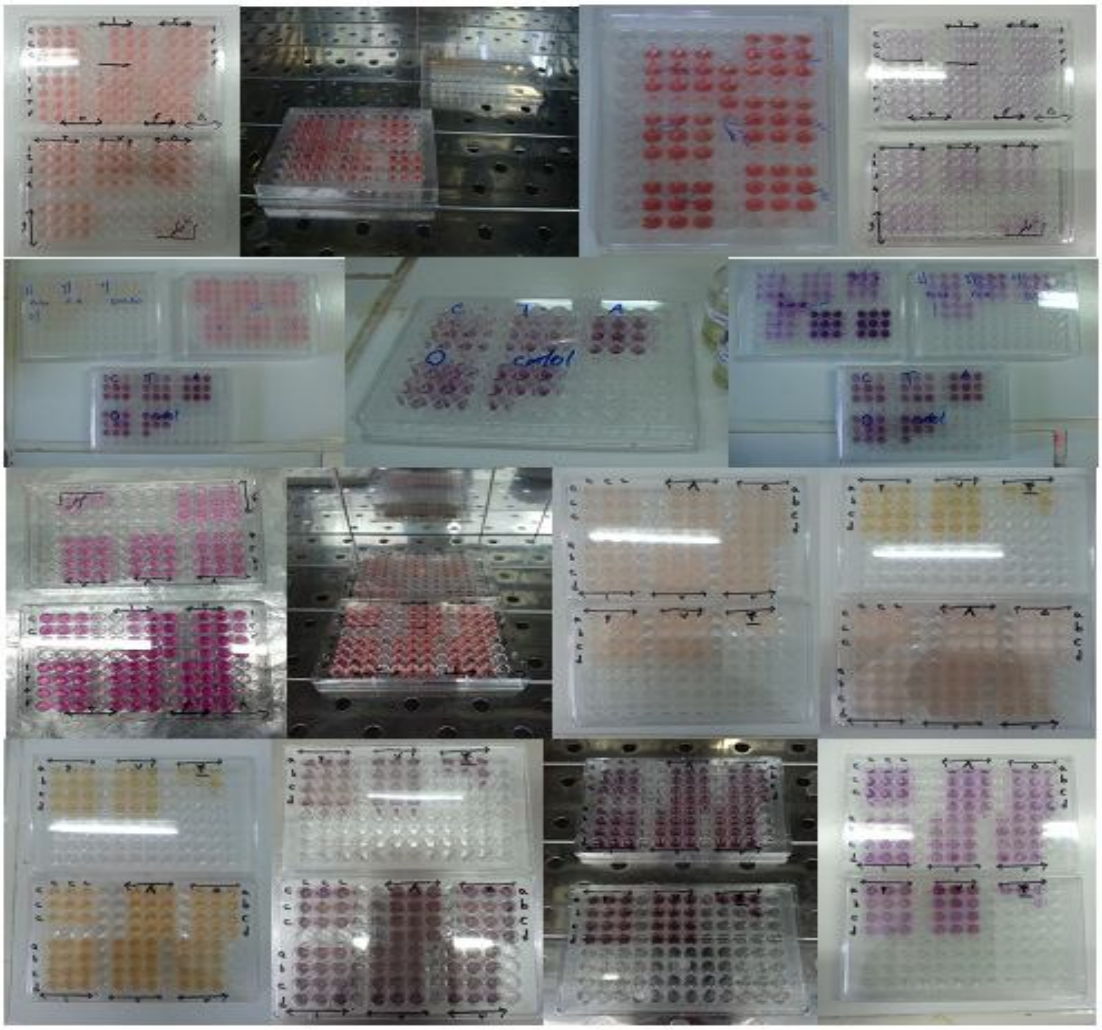

Figure 1. Cytotoxicity tests on Hela and lymphocyte cells.

Table 1. Cytotoxicity assay of *A. absinthium* oil on lymphocyte cells.

Oil Dilutions (µg/ml)	% Viable Lymphocyte cells	% Lymphocyte cells Death
control	100	0
700	75.89 ± 3.53	24.10
1400	69.89 ± 5.66	30.10
2800	61.92 ± 9.18	38.07
5600	50.13 ± 9.18	49.86
IC_{50} (µg/ml)	**5456.07**	

Table 2. Cytotoxicity assay of *A. absinthium* oil on Hela cells.

Oil Dilutions (µg/ml)	% Viable Hela cells	% Hela cells Death
control	**100**	**0**
7	89.22 ± 4.92	10.78
14	87.01 ± 3.85	12.99
28	72.95 ± 8.51	27.04
35	62.07 ± 7.52	37.93
42	53.70 ± 5.28	46.30
56	44.09 ± 7.42	55.90
IC_{50} (µg/ml)	**48.59**	

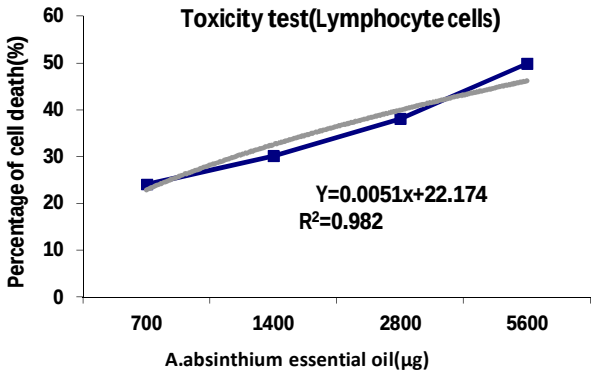

Figure 2. Cytotoxicity test on lymphocyte cells.

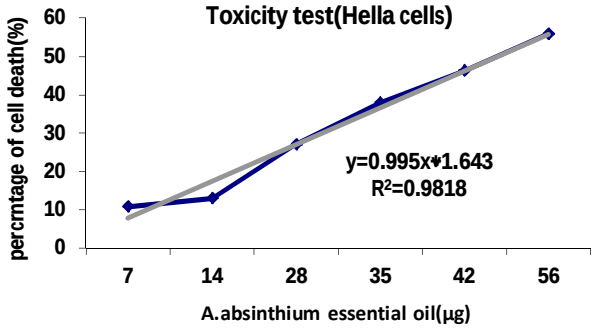

Figure 3. Cytotoxicity tests on Hela cells.

At a concentration of 56 µg/ml, the obtained oil destructed Hela cells by 55.90% (Table 2) while at a concentration of 5600 µg/ml, it destructed lymphocyte cells by 49.86% (Table 1). At lower doses, the oil was still toxic to the Hela cells.

DISCUSSION

The volatile oil displayed good cytotoxic action towards the human tumor cell line. Regarding the cytotoxic activity based on MTT assay, the IC_{50} values for Hela and lymphocyte cells were calculated to be 48.59 µg/ml and 5456.07 µg/ml, respectively (Table 3, Figure 4). The IC_{50} showed that cytotoxicity of the oil towards human tumor cell line was much higher than that required for human healthy cells. These results indicate low adverse side effects for the oil. Cancer chemo prevention is defined as the use of chemicals or dietary components to block, inhibit, or reverse the development of cancer in normal or pre-neoplastic tissues. A large number of potential chemo-preventive agents have been identified, and they function by mechanisms directed at all major stages of carcinogenesis [17]. Essential oil constituents have a very different mode of action in bacterial and eukaryotic cells. For bacterial cells, they are having strong bactericidal properties, whereas in eukaryotes they modify apoptosis and differentiation, interfere with the post-translational modification of cellular proteins, and induce or inhibit some hepatic detoxifying enzymes. Therefore, essential oils may induce very different effects in prokaryotes and eukaryotes [18]. Based on this study, *A. absinthium* essential oil is reported to be cytotoxic. Some reports support the relationship between cytotoxicity and antioxidant activity [18]. In our previous research, the results suggested the application of *A. absinthium* oil as a natural antioxidant agent [14]. In spite of the limitations of all *in vitro* studies with respect to *in vivo* impact, the present results are very promising as far as anti-neoplastic chemotherapy is concerned. This further forms a firm basis for future research. Although all *in vitro* experiments hold limitations with regard to possible *in vivo* efficacy, the results of this study deserve attention regarding the possible anti-neoplastic chemotherapy and form a basis for future research. Even though this essential oil might not be ideal for the treatment of human cancers, the oil tested certainly deserves some further investigation.

Table 3. Comparison of cytotoxicity in Hela and lymphocyte cells through IC_{50} value.

Essential oil	IC_{50} Normal lymphocytes (µg/ml)	IC_{50} Hela cells (µg/ml)
A. absinthium	5456.07	48.59

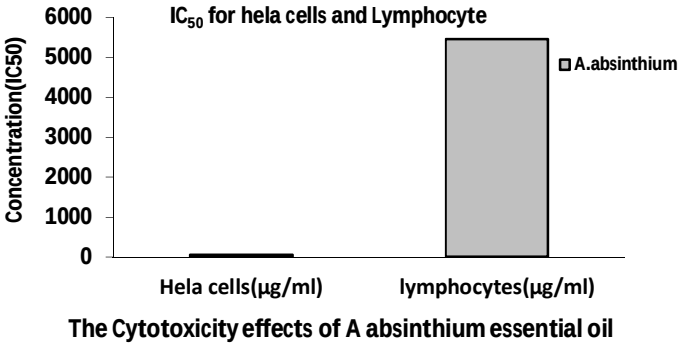

Figure 4. IC_{50} for Hela and lymphocyte cells.

CONCLUSION

The IC_{50} shows that cytotoxicity of the oil towards human tumor cell line is much higher

than that required for healthy human cells. These results indicate low adverse effects for the oil. From the previously mentioned results, it can be concluded that the *A. absinthium* essential oil may be exploited as a health-promoting agent that can conveniently find its appropriate therapeutic applications. The results of this study deserve attention to possible anti-neoplastic chemotherapy and form a basis for future research.

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REFERENCES

1. Curin Y, Andriantsitohaina R. Polyphenols as potential therapeutical agents against cardiovascular diseases. Pharmacological reports. 2005;57:97-107.
2. Yousefzadi M, Riahi-Madvar A, Hadian J, Rezaee F, Rafiee R, Biniaz M. Toxicity of essential oil of Satureja khuzistanica: In vitro cytotoxicity and anti-microbial activity. Journal of immunotoxicology. 2013;11(1):50-5.
3. Rustaiyan A, Masoudi S. Chemical constituents and biological activities of Iranian Artemisia species. Phytochemistry Letters. 2011;4(4):440-7.
4. Firouzni A, Vahedi H, Sabbaghi F, Bigdeli M. Composition of the essential oil of Artemisia ciniformis, A. kopetdaghensis, and A. khorasanica in Iran. Chemistry of natural compounds. 2008;44(6):804-6.
5. Linnaeus Cv. Species plantarum, 2 vols. Salvius, Stockholm. 1753.
6. Tariku Y, Hymete A, Hailu A, Rohloff J. In vitro evaluation of antileishmanial activity and toxicity of essential oils of Artemisia absinthium and Echinops kebericho. Chemistry & biodiversity. 2011;8(4):614-23.
7. Mills S, Bone K. The essential guide to herbal safety: Elsevier Health Sciences; 2005.
8. Wichtl M. Herbal drugs and phytopharmaceuticals: a handbook for practice on a scientific basis: Medpharm GmbH Scientific Publishers; 2004.
9. Hoffmann D. Medical herbalism: the science and practice of herbal medicine: Inner Traditions/Bear & Co; 2003.
10. Mahmoudi M, Ebrahimzadeh M, Ansaroudi F, Nabavi S, Nabavi S. Antidepressant and antioxidant activities of Artemisia absinthium L. at flowering stage. African Journal of Biotechnology. 2009;8(24): 7170-5.
11. Fiamegos YC, Kastritis PL, Exarchou V, Han H, Bonvin AM, Vervoort J, et al. Antimicrobial and efflux pump inhibitory activity of caffeoylquinic acids from Artemisia absinthium against gram-positive pathogenic bacteria. PLoS One. 2011;6(4):e18127.
12. Lust John ND. *The Herb Book. Bantam Books*.1979.
13. Taherkhani M, Rustaiyan A, Rasooli I, Taherkhani T. Chemical composition, antimicrobial activity, antioxidant and total phenolic content within the leaves essential oil of Artemisia absinthium L. growing wild in Iran. African Journal of Pharmacy and Pharmacology. 2013;7(2):30-6.
14. Lau C, Ho C, Kim C, Leung K, Fung K, Tse T, et al. Cytotoxic activities of Coriolus versicolor(Yunzhi) extract on human leukemia and lymphoma cells by induction of apoptosis. Life Sciences. 2004;75(7):797-808.
15. Sylvestre M, Legault J, Dufour D, Pichette A. Chemical composition and anticancer activity of leaf essential oil of Myrica gale L. Phytomedicine. 2005;12(4):299-304.
16. Samarth R, Panwar M, Kumar M, Kumar A. Protective effects of Mentha piperita Linn on benzo [a] pyrene-induced lung carcinogenicity and mutagenicity in Swiss albino mice. Mutagenesis. 2006;21(1):61-6.
17. Hou J, Sun T, Hu J, Chen S, Cai X, Zou G. Chemical composition, cytotoxic and antioxidant activity of the leaf essential oil of Photinia serrulata. Food Chemistry. 2007;103(2):355-8.
18. Lazutka J, Mierauskiene J, Slapšyte G, Dedonyte V. Genotoxicity of dill (Anethumgraveolens L.), peppermint (Menthaxpiperita L.) and pine (Pinussylvestris L.) essential oils in human lymphocytes and Drosophila melanogaster. Food ChemToxicol .2001;39:485-92.