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Cytotoxic Effects of the Essential Oil from *Achillea wilhelmsii C. Koch*

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The cytotoxicity activity of the essential oils from the leaves of *Achillea wilhelmsii C. Koch* were studied on six tumor cell lines and a normal cell line with Lactate dehydrogenases (LDH) and trypan blue methods. The composition of the essential oil was also analyzed by Gas Chromatography–Mass spectrophotometery (GC-MS). The LDH test showed that the oil had marked cytotoxicity activity against all cancer cell lines. K562 (Human chronic myelogenous leukemia) and PC3 (Human prostate adenocarcinoma), were the most sensitive to the essential oil with IC_{50} value (the concentration of the essential oil causing 50% inhibition) of 12.62±1.3µg/ml and 15.88±2.4µg/ml, respectively. The least cytotoxic activity was exhibited on HUVEC (Human umbilical vein endothelial cell) and Hela (Human cervix carcinoma) cell lines with IC_{50} values of 19.85±3.2 and 46.34±2.7, respectively. Trypan blue assay approved these results. In conclusion, cytotoxic activities of the oil may be described by the presence of monoterpene derivatives. the results of this study suggest that the essential oil of *A. wilhelmsii* has a potential source for cancer therapy. As a significant result, HUVEC (as normal cells) exhibited the least sensitivity to cytotoxic effects of the oil, confirming its high selectivity and specialty against cancer cell lines.

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Introduction

The *Achillea* genus (*Asteraceae*) has more than 100 species, that are spread throughout the world, including Asia and Europe. In Iran, nineteen species of this genus have been identified and acknowledged for their essential oil [1]. *Achillea* genus is widely used in the folk medicine as sedative, menstrual regulator, anti-inflammation, anti helminthic, analgesic and also as a treatment for liver disorders, fever and cough [1]. Some studies have also demonstrated that the oils of *Achillea* species exhibit antimicrobial, antioxidant and cytotoxic activities [2-7]. *A. wilhelmsii* is a member of *Achillea* genus which is widely found in different parts of Iran. *A. wilhelmsii* has been the subject of several studies, namely antimicrobial, anti hypertensive, and anti hyperlipidemic. These effects may be attributed to high flavonoids and sesquiterpene lactones content [8,9]. Several studies have reported that the extract and oil of *Achillea* species exhibit cytotoxic effects against various cancer cell lines [6, 10, 11] but no cytotoxic activities of the essential oil of *A. wilhelmsi* have yet been found. At the present study, after obtaining the essential oil from the aerial parts of *A. wilhelmsii*, its cytotoxic effects were studied *in vitro* on six cancer cell lines (HL60, K562, Jurkat, PC3, HT29, and Hela) and normal cells (HUVEC), using Trypan blue exclusion and LDH assays. The composition of the essential oils from the aerial parts of *A. wilhelmsii* was also analyzed by GC–MS.

Materials and Methods

Experimental

Plant material

The aerial parts of *A. wilhelmsii* were collected in Kermanshah (West of Iran) in July 2012. The Specimens were identified by one of the authors (Prof. Bahrami) and vouchers were deposited at the herbarium of the College of Pharmacy (University of Medical Sciences, Kermanshah, Iran).The aerial parts of *A. wilhelmsii* were air dried and then powdered leaves were separately submitted to hydrodistillation using a Clevenger-type apparatus for 4h. Anhydrous sodium sulphate was used to dehydrate the essential oil and after filtration. The collected oil was then stored at a temperature of -20˚C until testing.

Analysis of the essential oil by GC–MS

The procedure of essential oil analysis has been reported in literature [12]

Cell lines and culture

The cell lines, PC3 (Human prostate adeno-carcinoma), HT29 (Human colon carcinoma), Hela (Human cervix carcinoma), K562 (Human chronic myelogenous leukemia), Jurkat (Adult T cell leukemia), HL60 (Human promyelocytic leukemia), HUVEC (Human umbilical vein endothelial cell) were purchased from the National Cell Bank of Pasteur Institute (Tehran). The cell lines were grown and maintained in a humidified incubator at 37 ºC and 5% CO_2 atmosphere in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100µg/ml streptomycin.

Trypan blue assay

To determine the cytotoxic effects of the essential oil of *A. wilhelmsii* on viability of the cell lines, approximately 0.75×10^5 cells/ml were cultured in a 6-well tissue culture plate and the oil was added at different concentrations (1, 10, 25, 50, 100, 250, 500, and 1000µg/ml). After 48h incubation, the cells were collected and resuspended in 0.4% trypan blue .The percentages of viable cells were counted and comprised with the control samples [13].

LDH assay

The cytotoxic effect of *A. wilhelmsii* was also examined using LDH assay [14]. Briefly, after the incubation of the cell lines with different concentrations of the oil (1, 10, 25, 50, 100, 250, 500, and 1000µg/ml) for 48h, the supernatant was removed. Then, 100 µl of the supernatant from each well was transferred to a new micro plate, and 100µl of LDH reagent mixed with catalyst (Roche Diagnostics Corporation, Indianapolis, IN, USA) was added to the wells and incubated for 30 min in dark at room temperature. Finally, the absorbance was read at 490nm with background subtraction at 630 nm using the Synergy HT multi detection Micro Plate Reader. For untreated cells, 1% triton X-100 with LDH reagent were added whose results were

considered as positive control; the media without cells considered as negative control.

Statistical analysis

Statistical analysis was performed by Excel 2007 and SPSS (V=11.5).The results were expressed as mean + S.E after triple measurement.

Results and discussion

Chemical composition of the essential oil

Eighty-six compounds were identified which constituted 98.85% of total essential oil from aerial parts of *A. wilhelmsii* . The major components were ρ- Cymene (23.35%), 1,8-cineole (20.83%), Dihydrocarvone (19.13%), camphor (6.67%) and verbanol acetate (3.53). The complete results of the essential oil analysis has been reported in our previous study[12]

Cytotoxicity assay

The cytotoxic activities of *A. wilhelmsii* were examined on the six human cancer cell lines and a normal cell line by trypan blue exclusion and lactate dehydrogenase (LDH) assays. LDH is an enzyme which exists in all cells and is released in culture medium when the cells are suffering apoptosis [15]. The results of present study proved that the oil has marked cytotoxic effects on all the six cancer cell lines which the IC_{50} values shown in Table 1. Furthermore, the cytotoxic effects of the essential oil on three leukemia cell lines were significantly stronger than other cell lines. K562 was the most sensitive to the essential oil with an IC_{50} value of 12.62±1.3µg/ml.

Among the other cancer cell lines, PC3 had high sensitivity with IC_{50} values of 15.88±2.4µg/ml. The minimum cytotoxic activity was exhibited on HUVEC and Hela cell lines with IC_{50} values of 19.85±3.2 and 46.34±2.7, respectively. As a result, HUVEC (as normal cells) exhibited the least sensitivity to cytotoxic effects of the oil, confirming its high selectivity and specialty against cancer cell lines. In the present study, the essential oil of *A. wilhelmsii* showed high and low cytotoxic activities against K562 and Hela, respectively (Fig. 1). The reason for the differences of IC50 values for different cell lines are related to type of cell lines.

Table 1. IC_{50} values (µg\ml) of cytotoxic effects of the essential oil from *A. wilhelmsii* on different cell lines by LDH assay. Reported results represent mean ± standard deviation from triplicate tests.

cell lines	Jurkat	K562	HL60	HT29	Hela	PC3	Huvec
IC50	14.63±2.2	12.42±1.3	13.06±2.4	16.37±1.1	46.34±2.7	15.88±2.4	19.85±3.2

Cytotoxic activities of the oil may be related to the presence of monoterpene derivatives such as ρ-Cymene (23.35%), 1,8-cineole (20.83%), Dihydrocarvone (19.13%), camphor (6.67%), α-pinene (1.81%), α-terpineol (1.26%) that existed in essential oil of *A. wilhelmsii* [12]. The monoterpene compounds can show cytotoxic activity by several mechanisms; among them are: cell membrane damage, coagulation of the cytoplasm, depolarization of mitochondria membrane, perturbation in ionic Ca^{++} cycle, and anti genotoxicity effect [16]. Eucalyptol showed protection of cells against genotoxicity [17] and α-terpineol showed anticancer activities against human melanoma M14 cell line [6]. The studies on cytotoxic activities of other *Achillea* spp approved our results. As the essential oil of flowers (FL) and vegetative parts (VP) of *Achillea ligustica* have shown cytotoxic effects. The high cytotoxic effects were observed on B16-F1 cell line with IC_{50} values of 0.220±0.022 mg/ml and 0.459±0.067 mg/ml for FL and VP oil, respectively [6].

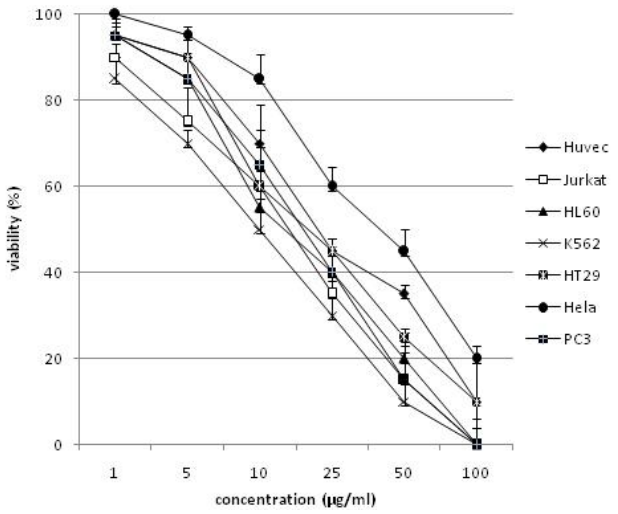

Fig. 1 . Effects of the essential oil from *A. wilhelmsii* on the studied cell lines after 48 h as investigated by trypan blue exclusion. Each data shown is one representative example of three independent experiments, expressed as the percentage of control and standard deviations.

In conclusion, the essential oil from aerial parts of *A. wilhelmsii* showed high cytotoxic activities on different cancer cell lines. The results of this study suggest that the possibility of using the essential oil of *A. wilhelmsii* as a potential source for cancer therapy. Our results can be considered as the first report on *in vitro* cytotoxicity of the essential oil of *A. wilhelmsii*.

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Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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