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The Cytotoxic Effect of Fractions of Chloroform Extract from the Endemic *Achillea multifida* Aerial parts

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ABSTRACT

The objective of this study was to evaluate for the first time cytotoxic effects of fractions of chloroform extract from the endemic Achillea multifida aerial parts. The heptane, chloroform and methanol extracts from A. multifida aerial parts were prepared and screened for their cytotoxic effects. Among all the extracts chloroform extract showed the strongest cytotoxic activity. Therefore, chloroform extract was fractionated using column chromatography and obtained F-1, F-2, F-3, F-4, F-5 and F-6 fractions respectively. The cytotoxic activity of these fractions was determined on cancer (Human colon adenocarcinoma cancer cell line, HT29; Human cervix adenocarcinoma cancer cell line, HeLa; Human breast adenocarcinoma cancer cell line, MC-7) cell lines. The F-4 fraction showed a stronger cytotoxic effect and selectivity activity against HT-29 (74.3 %) and MCF-7 (73.67 %) tumour cell line and moderate activity against HeLa (52.33 %) cell lines. Thus, the fraction F4 of chloroform extract from A. multifida aerial parts might be a potential source of anti-cancer agent(s).

Keywords: *A. multifida*, cytotoxic activity, HeLa, HT-29, MCF-7.

INTRODUCTION

The genus *Achillea* of the family Compositae comprises 42 species and 47 taxa in Turkey, of which 23 taxa are endemic. The species of *Achillea* genus are known in Anatolia as "Civan perçemi", "Pireotu" and "Yılan çiceği" [1]. These species have useful properties and are used in cosmetics, fragrances and agriculture [2]. *Achillea* species comprise an important biological resource in Turkish folk medicine against gastro-intestinal complaints (stomach ache, abdominal pain, flatulence, diarrhea, hemorrhoids and inflammatory disorders (rheumatic pain, for maturation on abscess, and eye inflammations), for wound healing, as an emmenagogue, as a diuretic, against jaundice, and for many other complaints [3]. Aerial parts of different species of this genus are widely used in folk medicine for preparation of herbal teas with antiphlogostic and spasmolytic activity [4]. The species of this genus contain essential oils, phenolic compounds, sesquiterpene lactones and ionone glucosides [5]. The phenolic compounds such as flavonoids and phenol carbonic acids are considered to be one of the most important groups of pharmacologically active compounds present in the *Achillea* species [6]. Over the past decade, major advances have been made in investigating the cytotoxic effects of different *Achillea* species [7].

Cancer is the uncontrolled growth and spread of cells that may affect almost any tissue of the body. The most commonly diagnosed cancers worldwide were those of the lung (1.8 million, 13.0% of the total), breast (1.7 million, 11.9%), and colorectum (1.4 million, 9.7%). The most common causes of cancer death were cancers of the lung (1.6 million, 19.4% of the total), liver (0.8 million, 9.1%), and stomach (0.7 million, 8.8%). Lung, colorectal and stomach cancer are among the most common in the world for both men and women [8]. Plants have a long history of use in the treatment of cancer and they have provided some of the currently used effective anticancer agents such as vinblastine, vincristine, etoposide, teniposide, and paclitaxel. The analysis of the sources of new and approved drugs for the treatment of cancer shows that 60% of these drugs were shown to be of natural origin, being natural compounds or derivatives [9].

Achillea multifida (DC.) Boiss. is an endemic species of Turkish Flora. This plant grows on Uludağ Mountain in Bursa, Turkey. It is 10-30 cm tall, perennial, aromatic plant with green leaves and white-yellowish flowers, locally known as "ebülmülk or civanperçemi''[10].

To the best of our knowledge, there have been no reported cytotoxic effects of fractions of chloroform extract from *Achillea multifida* aerial parts. The aim of this study was to evaluate for the first time the cytotoxic effects of fractions of chloroform extract from *Achillea multifida* aerial parts.

MATERIALS AND METHODS

Collection of plant material: *Achillea multifida* aerial parts were collected from Uludağ Mountain in Bursa, Turkey, in August 2009 and was identified by Prof. Dr. Ertan Tuzlacı. Voucher specimens are deposited in the herbarium of the Faculty of Pharmacy, Marmara University (MARE); herbarium code numbers: MARE 11719.

Preparation of the extracts: The dried aerial parts of these plants were extracted using heptane, chloroform and methanol by maceration at room temperature until arriving at a colourless solution. The heptane, chloroform and methanol extracts were filtered and evaporated to dryness under reduced pressure at 50^{o} C in a rotary evaporator. The chloroform extract was fractionated using column chromatography and obtained F-1, F-2, F-3, F-4, F-5 and F-6 fractions respectively. These fractions were filtered and evaporated to dryness under reduced pressure at 50^{o} C in a rotary evaporator. The fractions were then transferred to vials and kept at +4 ^{o}C.

Cell culture: Human colon carcinoma cell line HT-29 (ATCC, HTB-38), breast cancer cell line MCF-7(ATCC, HTB-38), human cervical carcinoma cell line HeLa (ATCC, CCL-2) were used in this study for cytotoxicity experiments. The cells were maintained in DMEM, supplemented with 10% fetal bovine serum, 2 mM L- glutamine and 100 units mL^{-1} penicillin-streptomycin. The cells were incubated in a humidified 5% CO_2 incubator at 37° C. Extracts were dissolved in dimethyl sulfoxide (DMSO).

Cytotoxicity: The cytotoxic activity of the fractions of chloroform extract from *Achillea multifida* aerial parts were assayed using the MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) protocol. Briefly, the cells were seeded at a density of 1 × 10^{4} cells/mL density in 96-well plates and allowed to adhere for 24 h in CO_2 incubator at 37° C. After the incubation, the extracted samples were added to the cells to a final concentration of 100 µg mL^{-1}. An untreated group was used as a negative control. Then the plates were incubated at 37° C, in 5% CO_2, humidified atmosphere for 24 h. After an incubation period, the medium was aspirated and the cells washed in PBS. The medium was replaced with 100 µL fresh medium and 10 µL MTT solution (5 mg mL^{-1}) was added to each well. After four hours of incubation, 100 µL permeabilization buffer was added, to solubilize the formazan crystals for 18 h. Absorbance was then determined at 540 nm by an ELISA plate reader. The average values were

determined from triplicate readings and substract from the average values of the blank. The percentage viability was calculated as: % Viability = [OD of treated cells/OD of control cells] × 100 [11].

RESULTS AND DISCUSSION

The cytotoxic activity of fractions of chloroform extract from *Achillea multifida* aerial parts was determined on cancer (Human colon adenocarcinoma cancer cell line, HT29; Human cervix adenocarcinoma cancer cell line, HeLa; Human breast adenocarcinoma cancer cell line, MC-7) cell lines. The results are summarized in Table 1.

Table 1. *In-vitro* cytotoxic effects of fractions of chloroform extract from *Achillea multifida* aerial parts on human MCF-7, HeLa and HT-29 cancer cell lines

	Fractions (100 µg mL^{-1})					
Human cancer cell lines	F-1	F-2	F-3	F-4	F-5	F-6
	Cell death (%)					
HeLa (cervical)	22.04	26.18	14.66	52.33	9.65	6.44
MCF-7 (breast)	9.06	33.66	10.59	73.67	15.77	9.36
HT-29 (colon)	10.7	43.24	-40.76	74.3	13.57	6.65

The F-4 fraction showed a stronger cytotoxic effect and selectivity activity against HT-29 (74.3 %) and MCF-7 (73.67 %) tumour cell line and moderate activity against HeLa (52.33 %) cell lines. The F-2 fraction exhibited moderate activity against HT-29 (43.24 %) tumour cell line but this fraction showed little activity against MCF-7 (33.66 %) and HeLa (26.18 %) cell lines. The F-1 fraction exhibited little activity against HeLa (22.04 %), MCF-7 (9.06 %) and HT-29 (10.7 %) cell lines. The F-3 fraction exhibited little activity against HeLa (14.66 %) and MCF-7 (10.59 %) cell lines but this fraction had no any activity against HT-29 (-40.76 %). The F-5 fraction exhibited little activity against HeLa (9.65 %), MCF-7 (15.77 %) and HT-29 (13.57 %) cell lines. The F-6 fraction showed little activity against HeLa (6.44 %), MCF-7 (9.36 %) and HT-29 (6.65 %) cell lines.

Therefore, after examining the toxic effects on different normal cell line of F-4 fraction it is believed that this F-4 fraction will be useful to plant-derived pharmaceutical manufacturing (especially anti-cancer effective) in the future.

On the other hand, to the best of our knowledge, there are only two reports in literature on the endemic *A. multifida* [10,12]. It was reported that the composition of the water-distilled essential oil of *A.multifida* was analysed by GC and GC/MS. Fifty-eight compounds were identified representing 93.9 % of the total oil. α-Thujone, β-thujone, sabinene, and camphor were characterised as the main constituents. The essential oil was tested for its antimicrobial activity using a micro-dilution assay resulting in the inhibition of human pathogenic bacteria and yeast. It showed good inhibitory activity against Gram (-) human pathogens *E. aerogenes* (MIC :62.5 µg/mL), *P. aeruhinosa* (MIC:125 µg/mL) and the yeast *C. albicans* (MIC: 62.5 µg/mL) [10]. Also, the antifungal and antibacterial activities of n-hexane, chloroform and methanol extracts from *A. multifida* flower head have been reported before [12]. In this study, the chloroform extract of *A. multifida* showed moderate activity against *S. aureus* (ATCC 6538/P) and *S. epidermidis* (MIC=50 µg/mL for each). In addition this extract showed antibacterial activity against hemorrhagic *E.coli* (MIC=100 µg/mL). All of the extracts were found inactive against *C. albicans*.
To the best of our knowledge, there have been no reports of cytotoxic effects of fractions of chloroform extract from *Achillea multifida* aerial parts. Hence, in the present studies the F-1, F-2, F-3, F-4, F-5 and F-

6 fractions of chloroform extract from *A.multifida* were tested cytotoxic activity. The fraction F-4 of chloroform extract showed the highest cytotoxic activity. Preliminary phytochemical screening of this fraction showed the existence of bioactive compounds such as flavonoids, coumarins, phenolic acid. Bioactive compounds in F-4 fraction were not identified in this study. In further studies, cytotoxic compounds will be isolated (column chromatography, thin layer chromatography, chromatotron) and identified (UV, IR, NMR) from F-4 fraction. These identified compound(s) could be useful as antitumour agents in the future.

APPLICATIONS

The fraction F4 of chloroform extract from *A. multifida* aerial parts is applicable as potential source of anti-cancer agent(s).

CONCLUSIONS

The fraction F4 of chloroform extract showed stronger cytotoxic effect and selectivity activity than the other fractions. Therefore, after examining the toxic effects on different normal cell line of F-4 fraction it is believed that this F-4 fraction a might be a potential source of anti-cancer agent(s).

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