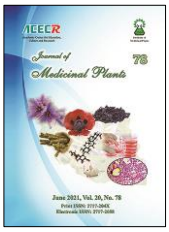
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Research Article

Cytotoxic activity of *Centaurea albonitens* Turrill aerial parts in colon and breast cancer cell lines

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ABSTRACT

Background: Cancer is the second cause of death in developed countries. Colon and breast cancers are among the most prevalent ones. Research focusing on finding new natural products with fewer side effects to fight cancer is increasing. **Objectives:** The present study aimed to evaluate the cytotoxicity of *Centaurea albonitens* Turrill methanol extract and its fractions against colon and breast cancer cell lines and a normal cell line of bovine kidney cells. **Methods:** The methanol extract and petroleum ether, chloroform and aqueous fractions were provided from the aerial parts of *C. albonitens* by maceration method in three days. Each day the solvent was refreshed. Colon (HT-29) and breast (MCF-7) cell lines were treated with the extract/fractions for 48 h for evaluating the cytotoxic activity by MTT assay. The apoptotic induction potential was also evaluated with the Hoechst 33258 staining method. **Results:** The most considerable effect was reported from the chloroform fraction with IC_{50} values of 25.6 and 25.1 µg/mL in MCF-7 and HT-29 cells, respectively. In Hoechst staining, condensed chromatin of the apoptotic cells was observed in both cell lines. **Conclusion:** *Centaurea albonites* can be suggested for further cancer research studies.

1. Introduction

Nowadays, many research projects are focused on finding effective treatments for cancer [1]. Empirical studies suggest that herbal and natural dietary compounds can be introduced as cancer inhibitors and plant-derived

Abbreviations: MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MCF-7, Breast cancer cell line; HT-29, Human colorectal adenocarcinoma cell line

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compounds with antioxidant activity can play a preventive role in cancer. [2]. Treatments such as chemotherapy and radiotherapy cause undesirable side effects [3]; therefore, the use of medicinal plants in the treatment of cancer can be helpful due to their probable fewer side effects. Many species of *Centaurea* contain sesquiterpene lactones and flavonoids with cytotoxic and antioxidant effects. They have also shown anti-inflammatory and antibacterial properties [4, 5]. *Centaurea* has about 500 species worldwide and several species of this genus have been introduced for cancer treatment beside their antimicrobial, anti-diabetic and anti-rheumatic properties [6] due to the presence of sesquiterpene lactones and flavonoids components. Previous studies have shown the cytotoxic effects of *C. albonitens* (Asteraceae) in acute lymphoblastic leukemia cells while no considerable cytotoxicity was reported in normal cells .The extract of this species, alone and in combination with vincristine, has demonstrated considerable cytotoxicity in NALM-6 (acute lymphoblastic leukemia), REH (acute lymphocytic leukemia), NB4 (acute promyelocytic leukemia) and KMM-1 (myeloma) cell lines [7, 8]. Considering the previous reports about cytotoxic effects of other species of the genus and also the above reported cytotoxicity in some cancerous cell lines, in the present study, we evaluated the cytotoxic effect of *C. albonitens* extracts and fractions in HT-29 and MCF-7 cancer cell lines.

2. Materials and methods

2.1. Plant Collection

Aerial parts of *C. albonitens* Turrill were collected from Hamadan, Iran. It was authenticated by botanists at Traditional Medicine and Materia Medica Research Center (TMRC) and the herbarium number TMRC 3234 was registered for the voucher specimen.

2.2. Extraction

The methanol extract was prepared in three days (plant solvent ratio of 1:10) through the maceration method. Every 24 hours, the extract was filtered and fresh solvent was added. Fractionation was performed by petroleum ether, chloroform and water. The extracts and fractions (except for the aqueous fraction) were condensed using a rotary evaporator. The aqueous fraction was dried by freeze drying method.

2.3. Cell Lines

HT-29 (human colorectal adenocarcinoma cell line) and MCF-7 (breast cancer cell line) were provided from the Pasteur Institute of Iran.

2.4.Evaluation of cytotoxicity by MTT assay

MTT assay is a colorimetric method based on the transformation of yellow crystals of tetrazolium by succinate dehydrogenase enzyme to insoluble purple formazan crystals. This method has high reliability, accuracy and sensitivity. The amount of color produced is directly related to the number of cells that are metabolically active.

To investigate the cells by MTT assay, HT-29 and MCF-7 cells were seeded with 9000, and 9500 cells/well in 96-well plates, respectively. The plates were kept in the incubator (37 $^{\circ C}$ and 5 % CO_2) for 24 hours. The cells were then treated with serial dilutions of the extracts and fractions for 48 hours. Afterwards, the medium was removed and the cells were exposed to MTT

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solution. Four hours later, the medium was replaced with DMSO. The final color intensity of formazan crystals created by healthy cells was recorded at 570 nm. Tamoxifen was used as the positive control [9, 10]. The relative cell viability (%) related to control wells containing cells, cell culture medium and DMSO 1 % was calculated by [A] samples/[A] control × 100. Where [A] samples is the absorbance of test sample and [A] control is the absorbance of wells containing cells + medium + DMSO, 1 %. To calculate IC_{50} values, viability (%) versus concentrations was graphed using the Microsoft Excel software.

2.5. Evaluation of cell nucleus morphology using Hoechst 33258

Hoechst 33258 can pass through the cell membrane and stain the content of the nucleus of the cell. Since chromatin is condensed in apoptosis, the glowing nucleus of the cells will be detected by fluorescent microscopy.

The cells were cultured in each 96-plates for investigating the induction of apoptosis. The cells were exposed to the extracts/fractions (at the concentration of IC_{50} from MTT assay). DMSO 1 % served as the negative control. The cells were incubated for 48 hours and then examined [11].

3. Results

The petroleum fraction did not dissolve in the medium and was excluded from further analysis. The cytotoxic effect of *C. albonitens* methanol extract and chloroform and aqueous fractions has been shown in Figures 1 and 2. Each test was performed with three repetitions. The IC_{50} values are presented in Table 1.

Figures 3-6 show cell nucleus morphology by Hoechst33258 staining. As shown in fluorescent microscopy images, dense and shining DNA can be observed in cells treated with chloroform extract (the most effective sample).

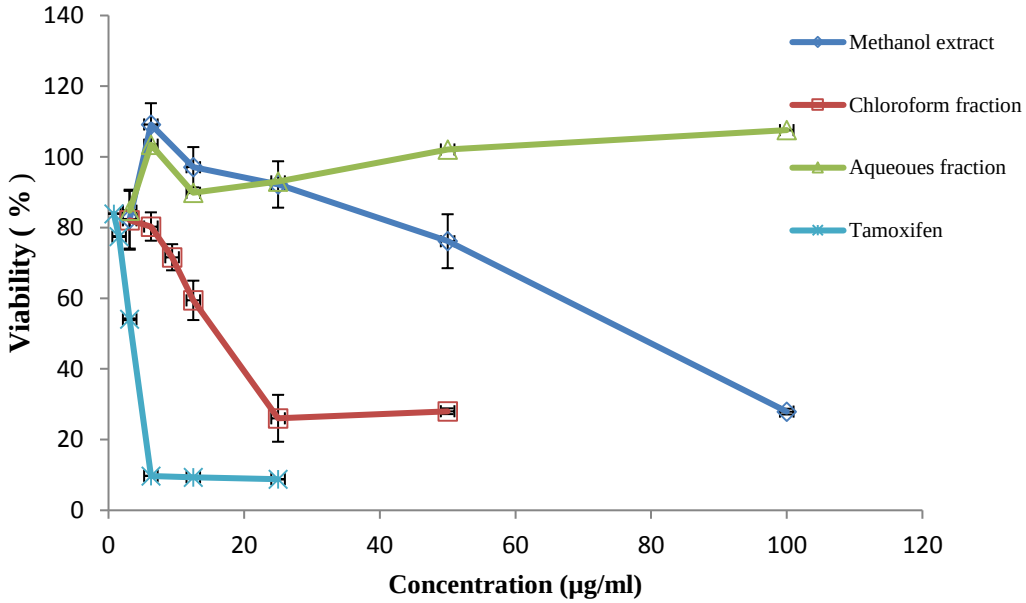


Fig. 1. Cytotoxic effects of *C. albonitens* extract and fractions in MCF-7 cells

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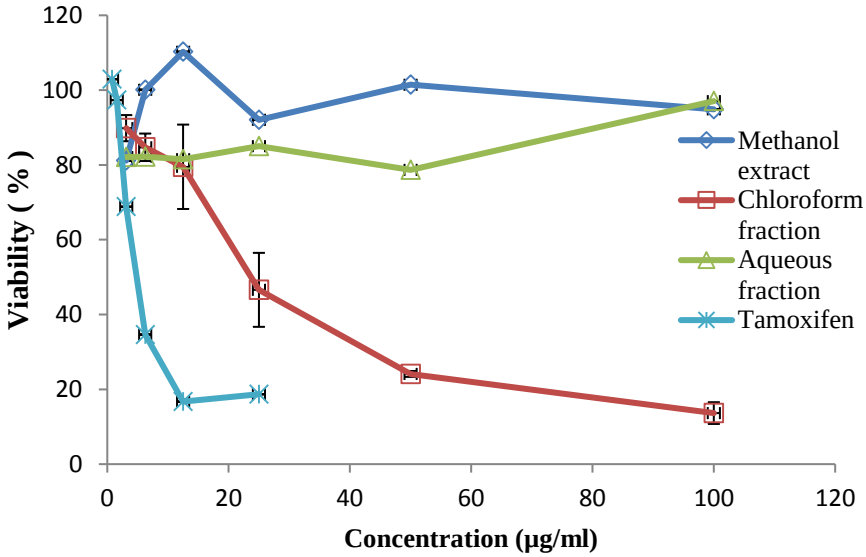


Fig. 2. Cytotoxic effects of *C. albonitens* extract and fractions in HT-29 cells

Table 1. IC_{50} values of *C. albonitens* extract and fractions in cell lines

No.	Sample	IC_{50} ± SD (µg/mL)	
		HT-29	**MCF-7**
1	Methanol extract	-	69.6 ± 5.7
2	Petroleum ether fraction	*	*
3	Chloroform fraction	25.1 ± 3.9	25.6 ± 6.6
4	Aqueous fraction	-	-
5	Tamoxifen	4.9 ± 0.0	4.33 ± 0.1

-: Cytotoxic activity was not observed
*: The sample did not dissolve in the medium

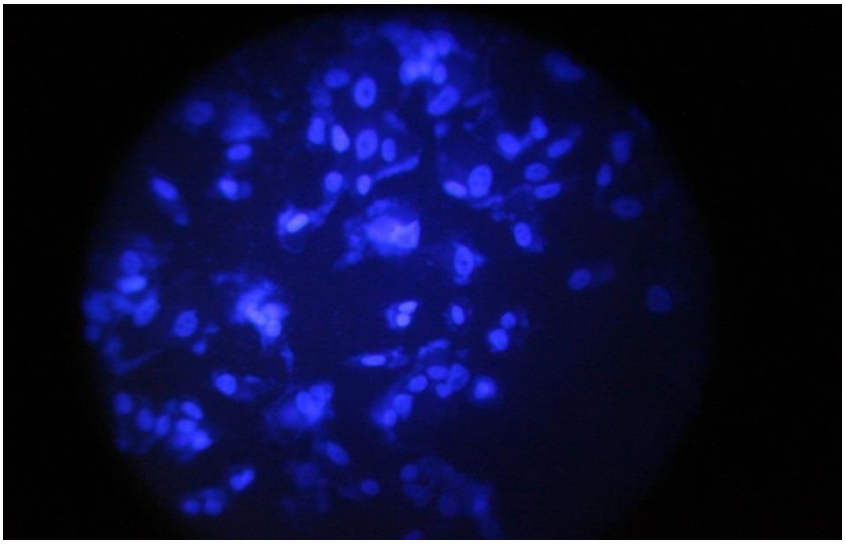


Fig. 3. MCF-7 cells treated with DMSO 1 % as the control

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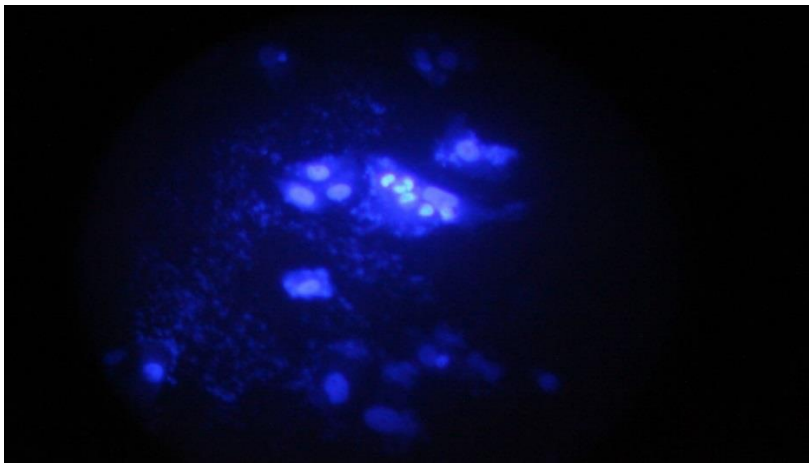


Fig. 4. Change in the morphology of MCF-7 cells treated with 25 µg/mL of *C. albonitens* chloroform fraction

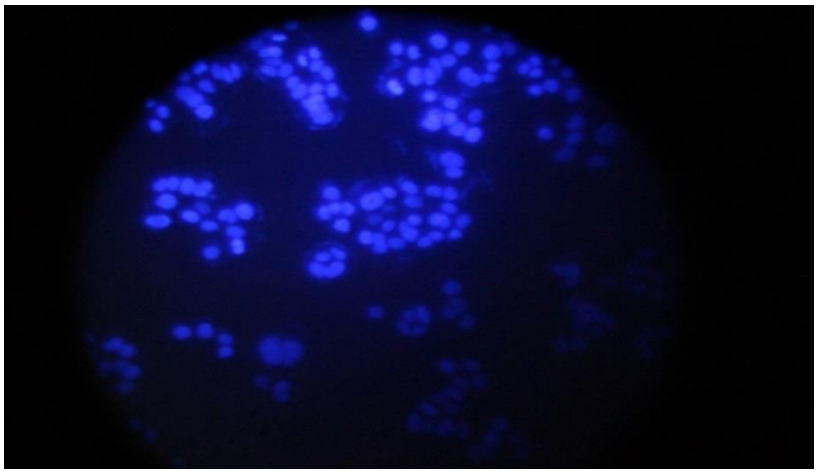


Fig. 5. HT-29 cells treated DMSO 1 % as the control

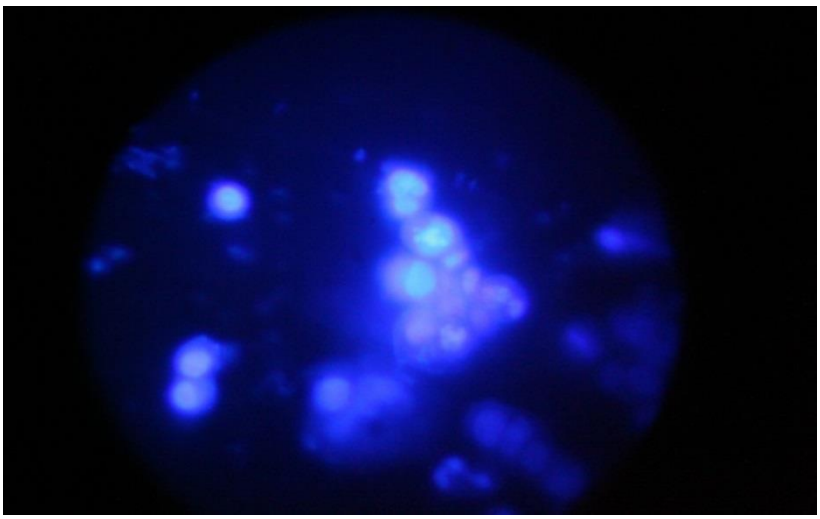


Fig. 6. Change in the morphology of HT-29 cells treated with 25 µg/mL of *C. albonitens* chloroform fraction

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4. Discussion

Medicinal plants are valuable sources for developing new drugs in cancer treatment. According to previous reports about the cytotoxic effect of *Centaurea* species and their potential in cancer research, the cytotoxic effect of *Centaurea albonitens*, a species with little record of cytotoxic investigation, was evaluated in the present study.

Several cytotoxic research projects have been conducted on other species of *Centaurea* in different cell lines [12, 13]. Studies about compounds from *C. montana* [14], cytotoxic and apoptotic effects of *C. ainetensis* in HCT-116 colon cell line and animal model [15], effectiveness of *C. schischkinii* seed extract in colorectal cancer cell line (CaCo-2) [16] and the effects of *C. bornmuelleri* and *C. huber-morathii* methanol extracts in CaCo-2 cells indicating the cytotoxic effect [17] are some examples.

There are not many studies about the effects of *Centaurea* species on HT-29 cells; however, researchers have evaluated the effects of different species of *Centaurea* on MCF-7 cell line some of which have been discussed here. In a study conducted in Turkey, the effect of *C. kilaea* was investigated on three cell lines of Hela (cervical adenocarcinoma), MCF-7 (breast adenocarcinoma) and PC-3 (prostate adenocarcinoma) using MTT assay and the chloroform fraction showed the highest anti-proliferation activity in Hela and MCF-7 cells [18]. The ethyl acetate fraction of *C. bruguierana* led to arrest in the G1 phase of the cell cycle and nuclear fragmentation and induction of apoptosis in MCF-7 cells [19]. A compound from *C. cyanus* caused subG1 and G1 arrest in the cell cycle of MCF-7 cells. This compound, named 13ASA, was introduced for further investigations in breast cancer studies [20]. *Centaurea aegyptiaca* also showed cytotoxic activity against MCF-7 cells in MTT assay [21]. The biological effects of *C. baseri* from Turkey were investigated and the extract demonstrated potent selective cytotoxicity in MCF-7 and some other cell lines namely PANC-1, A549, and C6 glioma cells [22].

The results of the present study indicated that the chloroform fraction of *C. albonitens* reduced the viability of both breast cancer (MCF-7) and colon (HT-29) cell lines. The IC_{50} was 25.0 µg/mL in MCF-7 cell line and 25.1 µg/mL in HT-29 cell line suggesting that the plant can show similar potency for cytotoxicity in both breast and colon cancer cells. Unlike the chloroform fraction, the aqueous fraction did not show cytotoxicity in any of the cell lines. This implies that the observed toxicity may not be attributed to flavonoids because these compounds are polar and should be present in the aqueous fraction. Inactivity of the aqueous fraction and effectivity of the chloroform fraction suggest that semi-polar compounds such as sesquiterpene lactones, which are characteristic of the Asteraceae family, can be the effective components in cytotoxic activity. Similar to our study, Ostad et al. evaluated the cytotoxicity of another *Centaurea* species (*C. bruguierana* ssp. *belangerana*) and found the chloroform fraction to be the most potent fraction against colon and breast cancerous cell lines [23]. Chloroform extracts of *C. polyclada* and *C. athoa* have also shown cytotoxic effects on some cell lines including BT-549, KB and SK-OV-3 [24]. In a bioassay guided isolation study, compounds with different structures including flavone, sesquiterpene and

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lignan-type structures from the chloroform extract of *C. arenaria* were found to be effective against HeLa, MCF7 and A431 cell lines [25]. These results confirm that chloroform fraction of different species of *Centaurea* contain effective compounds that can induce toxicity. These compounds should be isolated and evaluated in more profound studies.

5. Conclusion

In the present study, *C. albonitens* showed the toxic effect on MCF-7 and HT-29 cell lines. Considering that the cytotoxic compounds that have been previously isolated from *Centaurea* species were mostly sesquiterpene lactones, the observed effects can be somewhat related to these compounds. Isolation and purification of effective constituents may result in active compounds for cancer studies. Also, considering the results of the Hoechst staining, it is necessary to investigate the probability of apoptosis and its mechanism in future studies.

Author contributions

M. M. supervised the study; S. E. and M. HM. conducted the cytotoxicity and apoptosis studies; SM. M. was involved in extraction methods; M. A. performed the experiments.

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Conflicts of interest

The authors have no competing interests to declare.

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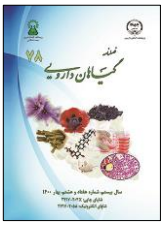
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سمیت سلولی قسمت‌های هوایی گیاه گل گندم درخشان در رده‌های سلولی سرطانی کولون و پستان

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اطلاعات مقاله

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MCF-7

چکیده

مقدمه: سرطان دومین عامل مرگ و میر در کشورهای توسعه‌یافته است. از شایع‌ترین سرطان‌ها می‌توان به سرطان پستان و کولون اشاره نمود. امروزه تحقیقات برای یافتن ترکیبات طبیعی با اثرات جانبی کم و همچنین ارزان جهت درمان سرطان رو به افزایش است که در این راستا بررسی اثرات سمیت سلولی از اولین گام‌ها به شمار می‌رود. **هدف:** در مطالعه‌ی حاضر اثر سمیت سلولی عصاره متانولی و فرکشن‌های حاصل از آن از گیاه گل گندم درخشان با نام علمی (*Centaurea albonitens* Turrill) بر رده‌های سلولی سرطانی کولون (HT-29)، و پستان (MCF-7) مورد بررسی قرارگرفته است. **روش بررسی:** عصاره متانولی و فرکشن‌های پترولیوم اتری، کلروفرمی و آبی قسمت‌های هوایی گیاه، در طی ۳ روز متوالی به روش ماسراسیون تهیه شدند. هر روز حلال تازه استفاده گردید. رده‌های سلولی به مدت ۴۸ ساعت تحت تیمار با غلظت‌های مختلف عصاره و فرکشن‌ها قرار گرفتند. سمیت سلولی به روش MTT ارزیابی شد و احتمال القاء آپوپتوز توسط رنگ‌آمیزی هوخست مورد بررسی قرار گرفت. **نتایج:** قابل توجه‌ترین اثر در فرکشن کلروفرمی با IC_{50} برابر با ۲۵/۶ و ۲۵/۱ میکروگرم در میلی‌لیتر به ترتیب در رده‌های سلولی MCF-7 و HT-29 مشاهده شد. در رنگ‌آمیزی هوخست نیز کروماتین متراکم و درخشان در هر دو رده‌ی سلولی مشاهده شد. **نتیجه‌گیری:** با توجه به اثرات سمیت سلولی مشاهده شده در این مطالعه، گیاه فوق کاندید مناسبی جهت ادامه مطالعات در زمینه داروهای ضدسرطان می‌باشد.

مخفف‌ها: MTT، ۳-(۵،۴- دی متیل تیازول-۲- ایل)-۵،۲- دی فنیل تترازولیوم بروماید؛ MCF-7، رده سلولی سرطان پستان؛ HT-29، رده سلولی ادنوکارسینومای کولورکتال انسان

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