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Araştırma / Original Paper

In vitro Antiproliferative Activity of Endemic *Centaurea kilaea* Boiss. against Human Tumor Cell Lines

Ali Sen[1], Suna Turan Ozbas[2], Julide Akbuga[2], Leyla Bitis[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Marmara University, Istanbul - Turkey
[2]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Marmara University, Istanbul - Turkey

Yazışma Adresi / Address reprint requests to: Leyla Bitiş
Department of Pharmacognosy, Faculty of Pharmacy, Marmara University, Istanbul - Turkey
Elektronik posta adresi / E-mail address: leylabitis@marmara.edu.tr
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ÖZET

Endemik *Centaurea kilaea* Boiss. türünün insan tümör hücre dizilerine karşı *in vitro* antiproliferatif aktivitesi

Amaç: Bu çalışmada, *Centaurea kilaea* Boiss. (Asteraceae) bitkisinin ileride üzerinde antiproliferatif aktivite yönlendirmeli madde izolasyonu çalışması yapılacak olan aktif ekstresinin belirlenmesi amaçlanmaktadır. Bu nedenle *C. kilaea* türünün toprak üstü kısımlarından elde edilen heptan (CKH), kloroform (CKC) ve alt fraksiyonları (CKCSI, CKCSII, CKCSIII) ile metanol ekstresinin (CKM) farklı kanserli hücre hatları üzerinde aktiviteleri araştırılarak en iyi antiproliferatif aktivite gösteren ekstresinin ortaya çıkarılması planlanmaktadır.

Yöntem: Antiproliferatif aktivite üç insan kanser hücre hattı üzerinde (Hela: Serviks kanseri hücre dizisi, MCF-7: Meme kanseri hücre dizisi, PC-3: Prostat kanseri hücre dizisi) MTT deneyi aracılığıyla ölçüldü.

Bulgular: CKC, 53.07; 68.64 µg/ml $İK_{50}$ değerleriyle sırasıyla Hela ve MCF-7 hücre dizilerine karşı en yüksek antiproliferatif aktiviteyi sergilemiştir. CKC ve CKM ise PC-3 hücre hattı üzerinde en yüksek antitümoral aktivite göstermişlerdir (sırasıyla $İK_{50}$: 73.92; 70.11 µg/ml). Aynı zamanda aktif kloroform ekstresinin alt fraksiyonunun (CKCSII), 60.75 ve 60.70 µg/ml $İK_{50}$ değeriyle sırasıyla Hela ve MCF-7 hücre dizilerine karşı orta derecede antiproliferatif aktiviteye sahip olduğu bulunmuştur.

Sonuç: Bu çalışma CKC ve CKCSII ekstrelerinin, yeni antitümoral aktiviteye sahip bileşiklerin biyoaktivite yönlendirmeli fraksiyonlama aracılığıyla izolasyonu için iyi birer aday olduklarını gösterir. Aynı zamanda bulgularımız, başka *Centaurea* türleri üzerinde yapılmış olan çalışmalarla benzerlik göstererek *Centaurea* türlerinin antikanser aktiviteye sahip oldukları gerçeğini doğrulamaktadır. Ayrıca aktif çıkan ekstre farklı hücre hatları üzerinde de denenebilir.

Anahtar sözcükler: *Centaurea kilaea*, antiproliferatif aktivite, MTT

ABSTRACT

In vitro antiproliferative activity of Endemic *Centaurea kilaea* Boiss. against Human Tumor Cell Lines

Objective: In this study, it is aimed to determine the active extract on which substance isolation through bioactivity-guided method is to be performed in the future. Therefore, it is planned to reveal the extract exhibiting the highest antiproliferative activity by investigating the activities of heptane (CKH), chloroform (CKC), sub-fractions of active chloroform (CKCSI, CKCSII, CKCSIII) and methanol extracts (CKM) obtained from the aerial parts of *Centaurea kilaea* Boiss. (Asteraceae) on different cancerous cell lines.

Method: Antiproliferative activity was measured against three human cancer cell lines (Hela; cervix adenocarcinoma, MCF-7; breast adenocarcinoma, PC-3; prostate adenocarcinoma) using MTT assay.

Results: CKC exhibited the greatest antiproliferative activity with IC_{50} of 53.07; 68.64 µg/ml against Hela and MCF-7 cells, respectively, while CKC and CKM showed the highest antitumor activity against PC-3 cell (73.92; 70.11 µg/ml, respectively). Also, a sub-fraction II of active chloroform extract (CKCSII) demonstrated moderate antiproliferative activity with IC_{50} values of 60.75 and 60.70 µg/ml against Hela and MCF-7 cells, respectively.

Conclusion: The results show that CKC and CKCSII are good candidates for further bioactivity-guided fractionation in the search for new active antitumor compounds. Also, these findings confirm other results that have been reported in the literature relating to antiproliferative activities of different *Centaurea* species. Furthermore, active extracts would be tested on different tumor cell lines.

Key words: *Centaurea kilaea*, antiproliferative activity, MTT

INTRODUCTION

New drugs exhibiting antitumor activity are needed worldwide due to escalating cancer cases. This requirement could be met either by producing new molecules or by isolating new substances from the plant. The great majority of the drugs used in cancer treatment are mainly obtained either from substances isolated from plants, from modification of natural molecules or from main structure of compounds of the natural origin. Therefore; it is so important to maintain research on the plants exhibiting anticancer activity in the discovery of new molecules (1).

Centaurea kilaea Boiss. (Asteraceae) is one of the 205 taxon of the genus *Centaurea* growing wild in Turkey (2-4).

It is reported that *Centaurea* species are rich in flavonoids and sesquiterpene lactones in a previous study (5). In traditional medicine, *C. species* are used for fever, menstrual disorders, vaginal candidiasis, the treatment of liver, kidney and ulcer diseases, as antidiarrheal, stomachic, tonic, appetitive, antidiabetic, antipyretic, also as a diuretic and expectorant (6,7). Antiproliferative activity studies conducted on some *Centaurea* species in recent years have found that various extracts of these species significantly have antitumor activity (8-13). In this study, the different extracts and fractions of active extract from aerial parts of *C.kilaea* were tested for antiproliferative activity against three human cancer cell lines (Hela; cervix adenocarcinoma, MCF-7; breast adenocarcinoma, PC-3; prostate adenocarcinoma) using MTT assay.

MATERIALS AND METHODS

Plant Material

Plant samples were collected in the flowering periods from the Catalca region of Istanbul in 2009 and identified by Dr. Gizem Bulut, a botanist of the Faculty of Pharmacy, University of Marmara. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Marmara University (MARE No: 11712).

Extraction

Dried aerial parts of *C. kilaea* (1595 g) were extracted separately in n-heptane, chloroform and methanol using maceration method. Also, active chloroform extract (20 g) was subjected to a silica gel column (800 g) and eluted by gradient elution (Hexane-$CHCl_3$-CH_3OH) to afford 20 fractions. Fractions were combined according to their TLC behaviour to yield CKCSI (F4-10), CKCSII (F11-14) and CKCSIII (F15-20). All extracts were filtered, concentrated and dried under vacuum on a rotary evaporator at 40°C and stored in a refrigerator for further analysis.

MTT test

The extracts (CKH, CKC, CKM, CKCSI, CKCSII, CKCSIII) were tested for their cytotoxic activities. Cell viability and cytotoxic activity profile of the extracts were analyzed using the MTT assay (14-16). MTT is cleaved to formazan by the "succinate-tetrazolium reductase" system (EC 1.3.99.1) which belongs to the mitochondrial respiratory chain and is active only in viable cells. Different cell lines were used for the determination of cytotoxic activity [HeLa; (ATCC®, CCL-2™), MCF-7 (ATCC®, HTB-22™), PC-3; (ATCC®, CRL-1435™)]. The cells were cultured with DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (Fetal bovine serum), 1% L-Glutamine and antibiotic solutions (penicillin-streptomycin-amphotericin).

Cytotoxicity testing in vitro was done by the method of modified Woerdenbag et al. (15,16). The MTT metabolic assay was carried out in seeded at the density of $1x10^4$ cells/well in 96-well flat-bottom cell culture plates with 100 µL of opti-MEM and 24-48 hours incubation at 37°C, 5% CO_2.. The following day, media was aspirated and the extracts were dissolved in DMSO and diluted with medium before they were added to the cell cultures at the different concentrations (7,5-100 µg/ml). Cells were incubated for 48 hrs at 37°C, 5% CO_2. After the incubation period 10µL of the MTT labelling reagent [final concentration 0.5 µg/mL (Cell proliferation kit MTT, Roche, Germany)] was added to each well. The cultures were incubated for 4-12 hours in a humidified atmosphere (e.g. 37°C, 5.0% CO_2) and 100µL of the solubilization buffer was added into each well. The plate was allowed to stand overnight in the incubator in a humidified atmosphere (e.g. 37°C, 5.0% CO_2), the formazan precipitates were solubilized. Absorbance of the formazan product was measured spectrophotometrically at 550 and 690 nm.

Statistical Analysis

The data were reported as means±standard deviations and analysed by one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison tests using GraphPad Prism 5. Differences between means at $p<0.05$ level were considered significant.

RESULTS

The antiproliferative activity of different extracts of *C.kilaea* were evaluated on three cancer cell lines (Hela, MCF7 and PC-3). All extracts at the concentration of 100 µg/ml inhibited three cancer cell lines growth about by 50% compared to a control ($p<0.0001$) (Figure 1).

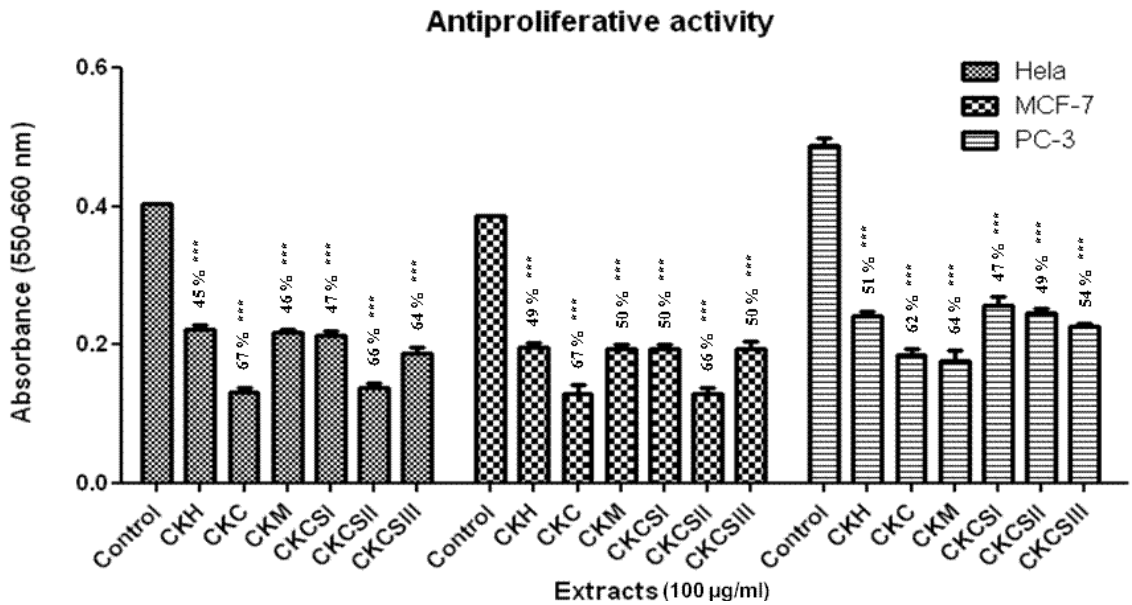

Figure 1: The effects of *C.kilaea* on proliferation of cancer cells at the concentration of 100 µg/ml

***p<0.0001 vs control
- The percentage values on graph indicate rate of growth inhibition.
- **Abbreviations; CKH**: Heptane extract, **CKC:** Chloroform extract, **CKM:** Methanol extract, **CKCSI:** Subfraction-I of chloroform extract, **CKCSII:** Subfraction-II of chloroform extract, **CKCSIII:** Subfraction-III of chloroform extract, of *Centaurea kilaea*.

Table 1: The antiproliferative activity (IC50 µg/ml) of various extracts of *C.kilaea* on cancer cell lines

Extracts/fractions	IC_{50} (µg/ml)*		
	MCF-7	Hela	PC-3
CKH	90.82±1.70	103.8±1.51	90.25±2.08
CKC	68.64±1.37	53.07±0.70	73.92±2.12
CKM	91.53±1.11	102.1±3.01	70.11±1.65
CKCSI	90.17±3.15	98.28±1.48	100.9±3.86
CKCSII	60.70±2.07	60.75±1.40	96.08±1.64
CKCSIII	89.81±3.89	83.51±0.80	86.62±0.67

- Each value in the table is represented as mean±SD (n=3)
- *IC_{50} is the concentration that causes 50% growth inhibition.
- **Abbreviations; CKH**: Heptane extract, **CKC:** Chloroform extract, **CKM:** Methanol extract, **CKCSI:** Subfraction-I of chloroform extract, **CKCSII:** Subfraction-II of chloroform extract, **CKCSIII:** Subfraction-III of chloroform extract, of *Centaurea kilaea*.

According to the American National Cancer Institute (NCI), the IC_{50} values < 20 µg/ml, 20-100 µg/ml and > 100 µg/ml are considered as active, moderately active and inactive, respectively (17). All extracts, except for CKH and CKM against Hela, CKCSI against PC-3, represented moderate antiproliferative activity.

A low IC_{50} value, the concentration that causes 50% growth inhibition, indicates high activity. As shown in Table 1, CKC exhibited the greatest antiproliferative activity with IC_{50} of 53.07; 68.64 µg/ml against Hela and MCF-7 cells, respectively, while CKC and CKM showed the highest antitumor activity against PC-3 cell (73.92; 70.11 µg/ml, respectively). Also, CKCSII demonstrated moderate antiproliferative activity with IC_{50} values of 60.75 and 60.70 µg/ml against Hela and MCF-7 cells, respectively.

DISCUSSION

In a study performed by a team of researchers from NCI, 7500 plant extracts were monitored and found that the great majority of active extracts had been obtained from species belonging to family Asteraceae, which is rich in sesquiterpene lactones (18).

In recent years, studies on the antiproliferative activity

of *Centaurea* species, a member of Asteraceae family have been frequently conducted. In previous antiproliferative activity studies performed on different *Centaurea* species, it was found that chloroform extracts of these species, which are rich in flavonoids and terpenoids, generally had significant anti-cancer activity.

Csupor et al. (2009) reported that various extracts obtained from different parts of *C.biebersteinii* and *C.jacea*, especially chloroform fractions of aqueous methanol extract, exhibit high antiproliferative activity on Hela, MCF-7, A431 human cancer cell lines (8).

In bioactivity studies done on *C. arenaria* by Csapi et al. (2010) and *C. bruguierana* subsp. *belangerana* by Rajabi et al. (2009), chloroform fractions of aqueous methanol extracts have been demonstrated to have antitumor activity (10,11).

In another study related to antiproliferative activity-guided isolation of active compounds from *C. jacea*, it was discovered that compounds responsible for anticancer activity are in the form of flavonoid (centauredin, cirsiliol, isokaempferide, apigenin, hispidulin) and sesquiterpene lactone (cnicin and 4'-acetylcnicin) (9).

Erel et al. (2011) found out that cinicin, a sesquiterpene lactone frequently existed in *Centaurea* species, isolated from chloroform extract of aerial part of *C. calolepis* showed high degree of antiproliferative activity on LLC-PK_{11} (pig kidney epithelial cell lines), SK-MEL (human malignant melanoma cell lines) and BT-549 (human ductal carcinoma) with IC_{50} values 23.3, 14.0 and 18.3 µM, respectively (19).

In our present study, CKC showed similarity tothe studies of Csupor et al. (2009) and Forgo et al. (2012) by exhibiting the best antiproliferative activity. Highly methoxylated flavonoids and terpenoids (sesquiterpene lactones and triterpenes) have been isolated so far in our ongoing isolation studies on CKCSII. We believe that these compounds in CKCSII may be responsible for the antiproliferative activity. Therefore, in our future studies, we aim to detect the compounds responsible for activity, firstly by considering the structure elucidation of these substances and then, testing the effects of them, singularly or in combination, normal and cancerous cell lines.

CONCLUSION

The results show that CKC and CKCSII are good candidates for further activity-guided fractionation in the search for new active antitumor compounds. Also, these findings confirm other results that have been reported in the literature relating to antiproliferative activities of different *Centaurea* species. Furthermore, active extracts would be tested on different tumor cell lines.

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