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Research Article

Centaurea cataonica Boiss. & Hausskn. ex Boiss. & Hausskn. INVESTIGATION OF BIOLOGICAL ACTIVITIES AND CYTOTOXIC ACTIVITY OF ENDEMIC PLANT

İrem Yılmaz[1,a,*], Ali Savaş Bülbül[2,b]

[1] *Kahramanmaraş Sütçü İmam Üniversitesi Fen Edebiyat Fakültesi Biyoloji Bölümü, Kahramanmaraş, Türkiye*
[2] *Bayburt Üniversitesi Uygulamalı Bilimler Fakültesi Acil Yardım Ve Afet Yönetimi Bölümü, Bayburt, Türkiye*

**Corresponding Author:*
E-mail: irem_ylmz@outlook.com

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a: ORCID 0000-0003-3836-8436, b: ORCID 0000-0002-2200-7348

ABSTRACT. In this study,the biological activities and cytotoxic effects of the endemic plant *Centaurea cataonica*. were investigated under in-vitro conditions. In the antimicrobial activity test, a totally of 6 bacterial samples were studied and the disc diffusion method was used. As a result of the antimicrobial test, it was observed that the plant material formed a zone diameter on all microorganisms. Moreover the antioxidant capacity test was performed using the 2,2-Diphenyl-1-picrylhydralysis (DPPH) radical quenching capacity. The results showed that the antioxidant capacity of the plant material increases from low to high concentrations. The cytotoxic acitivty was determined with two cell lines, human breast cancer cell line (MCF-7) and human umbilical vein endothelial cells (HUVEC) and MTS (Tetrazolium salt) viabilty test. It has been observed that plant material at high concentrations has a cytotoxic effect on cancerous cell lines.

Keywords: *Centaurea cataonica, MCF-7, HUVEC, MTS, DPPH*

INTRODUCTION

Turkey has wide plant diversity due to its climatic characteristics and location and it hosts approximately 3000 endemic species with 32% endemism rate [1]. *Centaurea cataonica* plant belongs to Asteraceae family and morphologically, this family includes annual, biennial, or herbaceous perennial plant forms and sometimes shrubs [2]. The endemism rate of *Centaurea* L. in Turkey has been reported as 60% [3]. It was also reported that the *Centaurea* genus, including Asteraceae family, spreads with 129 genera and 1156 species in Turkey [4]. *Centaurea* species are rich in cytotoxic sesquiterpene lactones and flavonoids in terms of bioactivity. Sesquiterpene lactones, are known to be compounds that are effective in this plant's cytotoxic and anti-inflammatory activity. However despite these features of the *Centaurea* species, studies on this species are very few and limited Turkey [5].

The intake of antioxidants with food or as a supplement can prevent many diseases such as cancer and cardiovascular diseases and delay aging. Antioxidant capacity tests are applied to foods and other materials to slow down these diseases ans aging. According to methods, this feature can be determined either with hydrogen atom transfer (HAT) or electron transfer (ET) procedures. In electron transfer methods the oxidant is tested by reducing the ability of the antioxidant by creating a color change [6, 7]. The DPPH (2,2-

diphenyl-1-picrylhydrazil), which isone of the ET-based methods, is commercially available stable organic nitrogen radical. It has a maximum absorbance at 515 nm [8].

In general terms, cancer is a complex disease based on the rapid and irregular proliferation of cells and their invasion of distant healthy tissues through metastasis. In addition to genetic susceptibility cancer can be formed by bacteria, viruses, environmental conditions, chemicals and bad habits [9]. According to the cancer incidence data of 2012, breast cancer ranks second after lung cancer with 1 million 670 thousand diagnoses globally. As in many parts of the world, breast cancer is the most common type of cancer among the female population in Turkey [10]. Progress in breast cancer incidence and death rate generally increases in proportion to age [11]. 44.5% of women diagnosed with breast cancer in Turkey are between the ages of 50-60 [10]. MTS (3-(4,5-dimethylthiazol-2-yl)-5 (3carboxymethoxyphenyl)-2-(4-sulphophenyl) measurement is a quantitative colorimetric method used to measure cytotoxicity based on the viability of metabolism under in-vitro conditions [12].

This study was performed with 6 bacteria, including *Enterobacter aerogenes* (ATCC13048), *Klebsiella pneumoniae, Salmonella typhimurium* strains as gram-negative and *Staphylacoccus aureus* (ATCC25923), *Bacillus subtilis* (DSMZ1971), *Enterococcus durans* strains as gram-positive. The experimental process was carried out by preparing three different concentrations from the plant sample. Considering the limitations of the literature studies on the endemic plant *Centaurea cataonica*, our study contributes to the literature studies.

MATERIALS AND METHODS

Materials

The identification and supply of the plant sample were provided by Dr. Yusuf Ziya Kocabaş from Kahramanmaraş Sütçü Imam University, Department of Herbal and Animal Production. MCF-7 (Human Breast Cancer Line) and HUVEC (Human Umbilical Vein Endothelial Cells) cell lines used in the cytotoxicity stage were obtained from Kahramanmaraş Sütçü Imam University Faculty of Agriculture cell culture laboratory.

Methods

Plant Extraction

The plant sample, which was dried in the laboratory, was extracted by siphoning three times in a soxhlet device with methanol as the primary extraction solvent. While applying the method, 1/10 (w/v) ratio was used.

Disk Diffusion Method

Three concentrations of sample (20mg/ml, 10mg/ml and 5mg/ml) were prepared and cultivated on Müeller Hilton Agar (MHA). Then, the prepared plant concentrations were loaded on sterile discs with a diameter of 6 cm and kept in an incubator at 37ºC for 24 hours. After 24 hours, discs loaded with plant samples were placed on bacteria inoculated with sterile swaps on MHA at regular intervals and kept in the ıncubator at 37ºC for 24 hours. The study was carried out in triplicate. Zone diameters were measured after the completion of the time.

Minimum Inhibition Concentration (MIC)

The activity of plant extracts against microorganisms was tested by disk diffusion. Additionally, the Minimum Inhibition Concentration (MIC) test was applied on 96-well microplates to determine the lowest inhibition concentrations of these plant samples on microorganisms. Plant sample and bacterial strains were added to the relevant wells in the microplates and the microplate was incubated at 37ºC for 18-24 hours in incubator. At the end of the incubation process, the microplate was read in the microplate reader at 550 nm.

Minimum Bactericidal Concentration (MBK)

This test was performed to observe whether there is microorganism growth in three concentrations of the plant sample after the MIC test. For the MBK test, samples were taken from the wells in the microplate with the help of sterile loops and cultivated on MHA, which is a solid medium. For one day, the cultivated petri dishes were kept in incubator at 37ºC for one day. At the end of the incubation process, the concentration that killed all the microorganisms in the petri dishes was measured as the MBK value.

Antibiofilm Activity

Biofilms are communities of microorganisms found in the extracellular matrix on surfaces where the necessary moisture is present [13]. After MIC and MBK processes, microplate containing bacteria and plant extract was incubated at 37ºC for 48 hours. After the incubation process was completed, all the wells in the plate were emptied and washed with distilled water (dH_2O) then, and they were left to dry. After these procosses, 130 µl of methanol (95%) was added to all the wells in the drying plate and left for fixation for 15 minutes. The wells were again completely emptied and left at room temperature to dry. 125 µl of 0.1% crystal violet solution was added to the dried plate wells and kept at room temperature for 10 minutes. After this the plates were emptied and washed with distilled water and left to dry. 33% glacial acetic acid was added to the wells containing gram -positive bacteria and 200 µl of 95% ethanol solution was added to the wells containing gram harmful bacteria and left for 15 minutes. At the end of 15 minutes, measurements were done by spectrophotometer device at 550 nm.

Determination of DPPH Free Radical Antioxidant Capacity

To determine the antioxidant capacity, DPPH (2,2-diphenyl-1-picrylhydralysis) method, which is one of the methods based on electron transfer, was applied. DPPH and BHT (2,6-Di-tert-butyl-4-methylphenol) were prepared as 0.1Mm solvent in DMSO (10%). Three concentrations of plant samples (0.01mg/ml, 0.1mg/ml and 1mg/ml) were prepared. The analysis was carried out in 96-well microplates and was repeated in 3 replicates. The absorbance values were read at a wavelength of 517 nm on the microplate, which was kept in the dark for 30 minutes at room temperature and under aseptic conditions.

Cell culture

MCF-7(Human Breast Cancer Line) and HUVEC(Human Umbilical Vein Endothelial Cells) cell lines were grown in 75cm_2 flasks in 15ml of medium containing FBS (fetal bovine serum) at 37°C and 5% CO_2 atmosphere.

Cell Count

To calculate the number of cells per milliliter of total cell suspension, a thoma slide with an area of 1 mm^2, a depth of 0.1 mm, divided into 25 small squares, was used so that the total volume could be calculated. Before the cells were treated with the plant sample, they were counted as 5000 cells per well in 96-well culture dishes and waited for 24 hours for the cells to adhere to the surface they were on.

Application of Centaurea cataonica

Centaurea cataonica dissolved in 10% DMSO was applied on the cells in 96-well culture dishes at 4 different concentrations as 0, 0.01, 0.1 and 1 mg/ml.

MTS Test

10µl of MTS solution was applied to each well and left for 2 hours at 37ºC for cells to metabolize MTS. After 2 hours, the colour change was observed and absorbance values were taken at 490 nm wavelength.

Statistical analysis

The GraphPad Prism 6 program was used for statistical analysis (ANOVA). Statistically, $p \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Results

The disk diffusion method was used to determine the antimicrobial activity of *Centaurea cataonica*. It was observed that the plant material formed a zone diameter on all bacterial strains at 5 mg/ml, 10 mg/ml and 20 mg/ml concentrations. 10% DMSO was used as the control group, and Ipinem (IPM) 10mg/ml was used as the antibiotic. In the control groups, it was measured that DMSO did not create a zone diameter, while IPM formed the most oversized zone diameter. The highest activity on plant material at all concentrations was observed on *Staphylacoccus aureus* ATCC 25923 and *Salmonella typhimurium* strains. Zone diameters are shown in Figure 1, and the results are recorded in millimeters (mm) in Table 1.

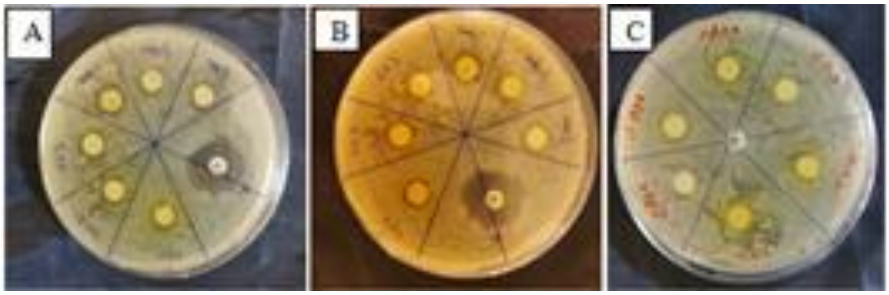

Fig. 1. *Centaurea cataonica antimicrobial zone diameters (**A:** Enterobacter aerogenes ATCC 13048; **B:** Klebsiella pneumoniae; **C:** Staphylococcus aureus ATCC 25923)*

Table 1. *Centaurea cataonica antimicrobial zone diameters (Disc diameters not removed 6mm) (-): There is no inhibition zone. (IPM10): Rope 10mg/ml. DMSO (10%))*

Centaurea cataonica					
	5 mg/ml	**10 mg/ml**	**20 mg/ml**	**DMSO**	**IPM10**
Enterobacter aerogenes ATCC 13048	**7**	**9**	**10**	**-**	**24**
Klebsiella pneumoniae	**10**	**10**	**12**	**-**	**20**
Enterococcus durans	**11**	**12**	**13**	**-**	**22**
Salmonella typhimurium	**11**	**13**	**15**	**-**	**25**
Staphylococcus aureus ATCC 25923	**12**	**14**	**15**	**-**	**22**
Bacillus subtilis DSMZ 1971	**11**	**12**	**14**	**-**	**24**

The MIC results supported the antimicrobial activity, and the maximum effect was observed on *Salmonella typhimurium* strain. The results are shown in Table 2.

Table 2. *Centaurea cataonica MIC*

Centaurea cataonica	
Enterobacter aeorogens ATCC 13048	**5 ± 0,669796**
Klebsiella pneumoniae	**5 ± 0,626028**
Enterococcus durans	**5 ± 0,87697**
Salmonella typhimurium	**2.50 ± 0,72926**
Staphylococcus aureus ATCC 25923	**5 ± 0,706004**
Bacillus subtilis DSMZ 1971	**5 ± 0,632054**

Antibiofilm capacity is shown in Table 3. According to this table, the highest effect was observed at 20mg/ml on *Salmonella typhimurium* strain with a 46.28% ratio.

Table 3. *Centaurea cataonica antibiotic activity results*

	20 mg/ml	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**
Enterobacter aerogenes ATCC 13048	**-**	**-**	**-**	**-**	**-**
Klebsiella pneumoniae	**-**	**-**	**-**	**-**	**-**
Enterococcus durans	**23,04**	**12,5**	**8,98**	**-**	**-**
Salmonella typhimurium	**46,28**	**45,04**	**20,24**	**-**	**-**
Staphylococcus aureus ATCC 25923	**7,48**	**-**	**-**	**-**	**-**
Bacillus subtilis DSMZ 1971	**20,37**	**13,27**	**4,26**	**-**	**-**

The antioxidant capacity was determined by DPPH method. BHT (Butylated Hydroxytoluene) with known antioxidant capacity was used as control. The results showed that the concentration is proportional to antioxidant capacity as the antioxidant

capacity increases from low to high concentrations. The antioxidant capacity results are shown in Figure 2.

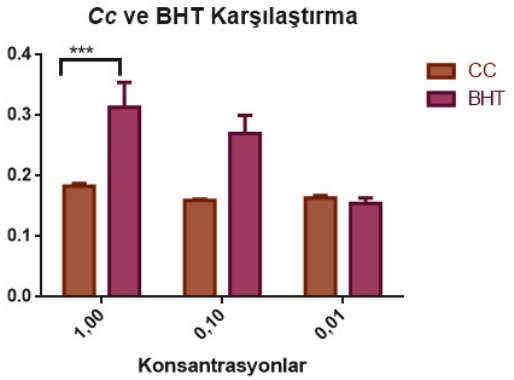

Fig. 2. *Centaurea cataonica and BHT's DPPH % sweeping capacity*

MTS method was preferred to measure cytotoxicity. The cytotoxic effects of the plant material on MCF-7 and HUVEC cell lines were statistically analyzed at concentrations of 0 , 0.01 , 0.1 , and 1 mg/ml. According to the statistical analysis result performed on the MCF-7 cell line, the first significant reductions were observed at 1 mg/ml ($p<0.05$). on the other hand, the results of statiscial analyses HUVEC cell line showed a low decrease in cell viability even at the highest concentration of 1mg/ml. Figure 3 shows MCF-7 and HUVEC cell lines comparatively.

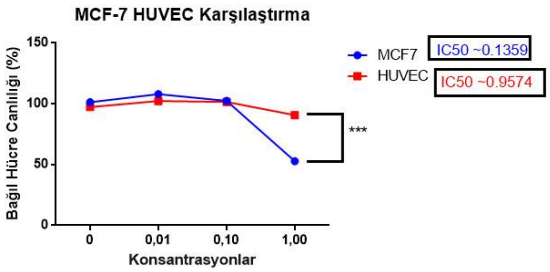

Fig. 3. *The comparative cytotoxic effect of Centaurea cataonica plant material on the MCF-7 and HUVEC cell lines (*Represents values below $p<0.05$, which is seen as significant as a result of statistical analyses.)*

Discussion and Conclusion

C. cataonica for Romaschenko et al. They investigated the chromosome numbers of the species with their studies in 2004 [14]. Kaya investigated *Centaurea derdereifolia* Wagenitz and *C. saligna* (C. Koch) Wagenitz species from taxonomic and palynological perspectives in 1985. Aydin et al. (2013) *Centaurea* L. and *Psephellus* Cass. investigated the genomic DNA of 7 endemic species belonging to the genus by using the stem and leaf anatomy [15].

As a result of the researches on *Centaurea* genera, essential oils were obtained from plant samples within the species. The antimicrobial activities of the essential oils obtained

were tested. There are not many literature studies for the endemic plant *Centaurea cataonica*, and when the studies on its close species are examined, it has been determined that it shows antioxidant capacity with the DPPH method for the determination of antioxidant capacity [16].

In studies on *Centaurea* strains, essential oils were obtained from plant samples and their antimicrobial activities were determined by disk diffusion and agar health diffusion methods [16]. It was determined that the essential oils showed antimicrobial activity. Not many studies were found in the literature on *Centaurea cataonica*. However its close species showed antioxidant capacity by DPPH method for its determination of antioxidant capacity.

In this study, *Centaurea cataonica* endemic plant was tested on a total of 6 bacterial strains and it was observed that in had an antimicrobial effect on pathogenic microorganisms. Furthermore, the antioxidant capacity was also found by the DPPH method. The cytotoxicity of the MTS viability assay was compared on two cell lines. Based on the scarcity of the literature, we think that our study contributes to the knowledge of the literature and can be a source for future studies.

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