In Vitro Biological Evaluation and Phytochemical Contents of Three Centaurea L. Species Growing from Eastern Anatolia in Turkey

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ABSTRACT

Centaurea L. species were used as medicinal plants among the people for treatment of the common cold, abscesses, peptic ulcers, hemorrhoid and diabetes etc.. In the present study, antiradical properties, phytochemical contents, antimicrobial and antiproliferative activities of three Centaurea species were investigated. Centaurea saligna (K.Koch) Wagenitz methanol (99.94%), Centaurea virgata Lam. methanol (98.23%) and water (98.10%) extracts were showed higher ABTS scavenging than trolox (96.79%). Centaurea kurdica Reichardt extracts showed lower activity than trolox for all the antiradical assays. Centaurea extracts exhibited antimicrobial activity against to some microorganisms. It was determined that these Centaurea species contain high amount of total flavonoid, phenolic and proanthocyanidin, phenolic acids, phytosterols and unsaturated fatty acids. Also, three Centaurea extracts showed very high antiproliferative property on LNCaP, HCT-116, MCF-7 cancer cell lines.

Doğu Anadolu, Türkiye’de Yetişen Üç Centaurea L. Türünün in vitro Biyolojik Değerlendirilmesi ve Fitokimyasal Özellikleri

ÖZET

Centaurea türleri halk arasında tıbbi bitkiler olarak soğuk algınlığı, apse, peptik ülser, hemorroid ve diyet altındaki hastalıklar tedavisinde kullanılmaktadırlar. Sunulan çalışmada, üç Centaurea türünün antiradikal özellikleri, fitokimyasal içerikleri, antimikrobiyal ve antiproliferatif aktiviteleri incelenmiştir. Centaurea saligna (K.Koch) Wagenitz metanol (99.94%), Centaurea virgata Lam. metanol (98.23%) ve su (98.10%) ekstraktları standart antioksidan trolokstan (96.79%) daha yüksek ABTS yok etme aktivitesi göstermiştir. Centaurea kurdica Reichardt ekstraktları trolokstan daha düşük antiradikal aktivite göstermiştir. Centaurea ekstraktları bazı mikroorganizmalara karşı antimikrobiyal aktivite göstermiştir. Centaurea türlerinin yüksek miktarda toplam flavonoid, fenolik ve proantosiyani, fenolik asitler, fitosteroller ve doymamış yağ asitleri içeriği belirlenmiştir. Ayrıca bu üç Centaurea ekstraktları MCF-7, HCT-116 ve LNCaP kanser hücre serileri üzerinde çok yüksek antiproliferatif özellik göstermiştir.

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INTRODUCTION

Plant-derived antimicrobials possess great therapeutic potentials and have been used for many years for the treatment of various infectious diseases (Iwu et al., 1999). Natural products can provide countless opportunities for the discovery of a new drug as pure compounds or herbal extracts owing to the fact that chemical diversity of these products have a very high potential. Recently, researchers have been looking for new ways to develop more effective drugs against microbial infections. Phytochemical compounds have antimicrobial effects and can be used in treating of microbial infections (Modi et al., 2012).

It was considered that plants are the oldest drugs used in the cancer therapy. The various reports indicated that the anticancer activity of medicinal plants caused by them contain antioxidant compounds. Indeed, the medicinal plants have lower costs, and easily available when compared to modern synthetic drugs. Therefore, the world of science is working hard for determining of the anticancer properties of plant-derived natural products, and their direct isolation and characterization of these natural products (Pandey and Madhuri, 2009; Prema et al., 2011; Wen et al., 2011).

The Centaurea genus is located in the Asteraceae family, and is represented by about 700 species. These genus members are annual, biennial and/or perennial herbaceous plants (Dittrich, 1977; Wagenitz and Hellwig, 1996). There are more than 180 Centaurea species in Turkey, and about 120 species of them are endemic (Davis, 1988). It is specified that a lot of Centaurea species are used in the treatment of common cold, abscesses, peptic ulcers, hemorrhoid and diabetes, and fresh shoots of some species are consumed as food among the people. In addition, many ethnopharmacological studies have shown that Centaurea species have antioxidant, antiradical, antibacterial, antimicrobial, antipyretic, antirheumatic, and antiinflammatory properties (Arif et al., 2004; Formisano et al., 2008; Ugur et al., 2009; Tekeli et al., 2010; Aktumsek et al., 2011; Zengin et al., 2012; Aktumsek et al., 2013a; Aktumsek et al., 2013b; Bruno et al., 2018).

As far as we know, there is no report on the antiradical and antiproliferative properties of Centaurea saligna (K.Koch) Wagenitz and Centaurea virgata Lam. species. Yet, there is more information about antiradical (Aktumsek et al., 2011), antimicrobial (Guven et al., 2005) and phytochemical properties (Aktumsek et al., 2011) of Centaurea kurdica Reichardt, the antimicrobial properties (Tekeli et al., 2008) of Centaurea virgata Lam. in the literature.

The aim of the present study was to investigate i) the antiradical activities; ii) the antimicrobial properties; iii) the antiproliferative properties; iv) phytochemical compositions of C. virgata, C. kurdica, C. saligna water, ethanol, methanol and acetone extracts.

MATERIALS and METHODS

Chemicals and standards

All standards and chemical compounds were purchased from Sigma-Aldrich.

Extraction procedures

Centaurea kurdica Reichardt, Centaurea virgata Lam. and Centaurea saligna (K.Koch) Wagenitz flowers were collected in June-September of 2016 from Elazig, Turkey. Voucher specimen numbers were Turkoglu 4865, 4866 and 4867, respectively. Voucher specimen was stored in the herbarium of Firat University, Science Faculty, Department of Biology, Elazig, Turkey. The flowers were dried at dark and room temperature. Flowers were pulverized using a mechanic grinder, and then 100 g of the powdered samples was extracted with 1000 mL of solvent (water, ethanol, methanol and acetone). These were centrifuged at 5000 rpm. After centrifuging and filtrating of solvents, the supernatants were concentrated with a rotary evaporator. All extractions were repeated three times. The standard antioxidants and extracts were dissolved in DMSO (for HPLC grade) at the concentration of 1000 μg/mL (Keser, 2014).

Determination of Antiradical Activities

The ABTS⁺⁻, hydroxyl and DPPH radical scavenging activities (RSAs) were determined by the methods of Re et al. (1999), Halliwell et al. (1987) and Brand-Williams et al. (1995), respectively. The antiradical activity tests were done at 500 μg/mL concentration for the extracts and standard antioxidant. All tests were repeated thrice and the average values were computed. The radical scavenging activity percentages (RSA%) for each sample was estimated by the following equation:

\[ RSA\% = \frac{[A_0 - A_1]}{A_0} \times 100 \]

Where, A₀ and A₁ are the absorbance of control and the sample, respectively.

Determination of Phytochemical Compounds

Total Phenolic Contents

These contents were determined according to Slinkard and Singleton’s method (1977). The results were expressed as gallic acid equivalent.

Total Flavonoid Content

The total flavonoid contents were performed according to Kim et al.’s method (2003). The catechin was used as a standard.
Proanthocyanidin Content
The proanthocyanidin contents were determined according to method described by Amaeze et al. (2011). The catechin was used as a standard.

Flavonoids and Phenolic Acids Analyses
The flavonoids and phenolic acids in the Centaurea extracts were determined using the method of Zu et al. (2006). The results of the analyses were expressed as mg/g.

Fatty Acids Analyses
Fatty acids in the Centaurea extracts were analyzed by GC according to Christie’s method (1992). The results were expressed as percent.

Vitamins and Phytosterols Analyses
The phytosterols and vitamins were extracted from Centaurea kurdica Reichardt, Centaurea virgata Lam. and Centaurea saligna (K.Koch) Wagenitz according to the HPLC method of Sánchez-Machado et al. (2002) and Lopez-Cervantes et al. (2006). The results were expressed as mg/g.

Determination of Antimicrobial Properties
*Bacillus megaterium* DSM 32, *Escherichia coli* ATCC 25922, *Proteus vulgaris* FMC 1, *Bacillus subtilis* IMG 22, *Listeria monocytogenes* SCOTTA, *Klebsiella pneumoniae* FMC 5, *Staphylococcus aureus* COWAN 1, *Pseudomonas aeruginosa* DSM 50071 bacteria and *Candida albicans* FMC 17 yeast were employed as test organisms. Collins and Lyne’s method (1989) were used for the antimicrobial tests using the disc diffusion method. All the antimicrobial tests were repeated three times. All the results were compared with nystatin (30 mg/disc) and streptomycin sulfate (10 mg/disc) used as standards.

Determination of Antiproliferative Properties
The prostate cancer (LCaP), colon cancer (HCT-116) and breast cancer (MCF-7) cell lines were used in the present study. These cell lines were retrieved from American Type Culture Collection (ATCC). The water, ethanol, methanol and acetone extracts of *C. virgata*, *C. kurdica* and *C. saligna* were screened for their antiproliferative properties against three cancer cell lines. These cells were treated with different concentrations (1, 5, 10, 25, 50, 75 and 100 µg/mL) of *C. virgata*, *C. kurdica* and *C. saligna* extracts, then they were incubated for 24 h. Effects of the % cell viability of *C. virgata*, *C. kurdica* and *C. saligna* extracts were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Denizot and Lang, 1986; Mosmann, 1983).

Statistical Analyses
SPSS Statistics software was used for statistical analysis. The antiradical results were evaluated using the analysis of variance and the means were compared by Duncan’s multiple range tests. For antiproliferative activity tests, normal distribution was obtained using Kolmogorov Smirnov test (p<0.05). The IC$_{50}$ values were calculated by using % cell viabilities of extracts.

RESULTS and DISCUSSION

**Antiradical Properties**
The antiradical properties of *Centaurea virgata* Lam., *Centaurea kurdica* Reichardt and *Centaurea saligna* (K.Koch) Wagenitz extracts are presented in Table 1. *C. saligna* methanol (99.94%), *C. virgata* methanol (98.23%) and *C. virgata* water (98.10%) extracts were showed higher antiradical activity than standard antioxidant trolox (96.79%) in the ABTS radical scavenging activity (RSA) test. *C. virgata* methanol (98.46%) extract was showed higher antiradical activity than standard antioxidant trolox (94.89%) in the OH RSA test. In the DPPH RSA test, trolox (97.33%) had the highest scavenging activity among all the extracts.

The scavenging activities of all the samples at 500 µg/mL concentration for the ABTS are sorted as follows: *Centaurea saligna* methanol (CSM) > *Centaurea virgata* methanol (CVM) > *Centaurea virgata* water (CVW) > Trolox > *Centaurea saligna* water (CSW) > *Centaurea virgata* ethanol (CVE) > *Centaurea virgata* acetone (CVA) > *Centaurea kurdica* methanol (CKM) > *Centaurea kurdica* acetone (CKA) > *Centaurea saligna* acetone (CSA) > *Centaurea kurdica* ethanol (CKE) > *Centaurea saligna* ethanol (CSE) > *Centaurea kurdica* water (CKW).

The scavenging activity values of all the samples at the 500 µg/mL concentration for the OH are sorted as follows: CVM > Trolox > CSM > CVA > CKM > CVE > CVW > CKE > CKA > CKW > CSE > CSW > CSA. The scavenging activity of all the samples at the 500 µg/mL concentration for the DPPH is sorted as follows: Trolox > CVM > CVW > CKM > CKW > CSM > CVE > CSW > CVA > CSE > CKE > CKA > CSA.

Zengin et al. (2018) determined that *C. saligna* ethyl acetate, methanol and water extracts were highly scavenged DPPH and ABTS radicals. Ayaz et al. (2017) showed that *C. virgata* extract has DPPH radical scavenging activity. Uysal et al. (2013) represented that *C. persica*, *C. polyclada* and *C. consanguinea* ethanol and acetone extracts were scavenged DPPH radical rate of 38.22, 7.96, 43.23, 13.08, 24.46 and 4.09%, respectively. Zengin et al. (2010) showed that *C. pulchella*, *C. patula* and *C. tchihatcheffii* methanol extracts were scavenged...
DPPH radical rate of 63.60, 55.08 and 51.13%, respectively. In another study, Aktumsek et al. (2011) determined that C. kurdica, C. rigida, C. amanicola, C. cheirolopha and C. ptosimopappoides methanol extracts were scavenged DPPH radical rate of 75.23, 69.34, 65.63, 79.52 and 70.45%, respectively. We found that C. kurdica methanol extract was scavenged DPPH radical in proportion as 86.38%. Our activity result was higher than aforementioned study results. Aktumsek et al. (2013a) showed that C. polypodifolia, C. pyrrhoblephara and C. antalyanse methanol and water extracts were scavenged DPPH radical in rate of 76.09, 87.81, 56.27, 76.14, 52.57 and 80.74%, respectively; were scavenged ABTS radical 93.42, 73.86, 91.13, 61.83, 90.65 and 76.24%, respectively.

**Phytochemical Composition**

The total proanthocyanidin, total flavonoid and total phenolic contents of C. virgata, C. kurdica and C. saligna extracts are summarized in Table 1. The phenolic acids and flavonoid contents of C. virgata, C. kurdica and C. saligna are shown in Table 2. The phytochemical, lipid soluble vitamins, and fatty acids content of C. virgata, C. kurdica and C. saligna are presented in the Table 2.

The total flavonoid amounts of all the samples as mg catechin equivalent/g extract are sorted as follows: CVM > CSM > CVA > CKM > CSW > CVE > CVW > CSE > CSA > CVA > CK > CKW. The total phenolic compound amounts of all the samples as mg gallic acid equivalent/g extract are sorted as follows: CSA > CVM > CVA > CVE > CSM > CSW > CKM > CSE > CK > CV > CVM > CVE > CV > CVA > CK > CKM > CK > CVA.

The phenolic acids, flavonoid phytoesters, fatty acids and lipid soluble vitamin contents of C. virgata, C. kurdica and C. saligna are shown in Table 2.

Zengin et al. (2018) determined that C. saligna ethyl acetate, methanol and water extracts were included 26.21 mg GAE/g, 23.03 mg GAE/g and 30.18 mg GAE/g (respectively) total phenolic compounds; 25.81 mg RE/g, 43.16 mg RE/g and 6.33 mg RE/g (respectively) total flavonoid compounds. Ayaz et al. (2017) showed that C. virgata was included 699.86 mg GA/g dry weight (dw) total phenolic compounds, 292.67 mg GA/g dw total flavonoid. Aktumsek et al. (2011) detected that C. kurdica was included 135.71 mg GA/g total phenolic, 165.21 mg RE/g total flavonoid, 37.57% palmitic acid (16:0), 5.22% stearic acid (18:0), 7.05% oleic acid (18:1), 13.90% linoleic acid (18:2), 17.87% linolenic acid (18:3), 52.14% total saturated and 47.86% total unsaturated fatty acids.

In this study, it was detected that C. kurdica is included 31.36% total saturated and 68.64% total unsaturated fatty acids.

### Table 1. ABTS**, OH•, DPPH• radicals scavenging activities, total flavonoid, total proanthocyanidin and total phenolic compounds values of C. kurdica, C. virgata and C. saligna extracts

| Samples | ABTS• Scavenging | OH• Scavenging | DPPH• Scavenging | Total Flavonoid | Total Proanthocyanidin | Total Phenolic |
|---------|------------------|----------------|------------------|-----------------|------------------------|---------------|
|         | (µg CE/g)        | (µg CE/g)      | (µg CE/g)        | (µg/100 µg/mL)  | (µg/100 µg/mL)         | (µg/100 µg/mL) |
| CKW     | 42.09±1.25       | 67.56±0.63c    | 72.57±0.23c      | 207.43±1.22     | 139.67±0.76            | 29.70±0.09    |
| CKE     | 54.21±0.99d      | 78.48±0.45b    | 85.45±1.12f      | 343.31±1.36     | 166.33±0.88            | 17.90±0.17    |
| CKM     | 66.75±1.07b      | 81.88±0.36d    | 96.38±0.95d      | 1213.28±2.54    | 293.00±1.26            | 23.89±0.33    |
| CKA     | 65.04±0.88c      | 73.38±0.81c    | 72.51±0.20e      | 391.69±1.09     | 263.00±1.07            | 7.82±0.22     |
| CVW     | 98.10±0.33a      | 78.97±0.42d    | 88.34±0.73h      | 1014.34±3.07    | 260.78±0.86            | 58.36±1.11    |
| CVA     | 70.15±1.07b      | 80.10±0.55b    | 97.24±1.24e      | 1173.46±2.54    | 457.44±1.46            | 50.12±0.55    |
| CVM     | 98.23±0.27a      | 98.46±0.09b    | 93.80±0.13c      | 1965.75±3.69    | 834.11±1.72            | 57.90±0.63    |
| CSE     | 91.98±0.97b      | 83.34±0.58a    | 53.38±0.52b      | 2143.33±3.01    | 745.22±1.91            | 34.01±0.34    |
| CSW     | 94.81±0.23a      | 62.84±1.34c    | 56.48±1.18c      | 1175.36±1.68    | 303.00±0.90            | 106.27±0.19   |
| CSM     | 50.43±1.34d      | 66.56±1.22c    | 47.58±1.55d      | 513.54±0.87     | 278.56±0.74            | 57.53±0.08    |
| CSA     | 99.94±0.00a      | 85.14±0.75b    | 71.96±1.08b      | 1561.25±1.97    | 398.49±0.59            | 97.74±0.55    |
| Trolox  | 60.04±1.02c      | 49.32±2.95d    | 21.09±2.02e      | 395.87±0.34     | 1084.19±1.36           | 35.02±0.11    |

There was not statistically difference among in the same letter groups: p<0.001. The antiradical activity results were calculated for 500 µg/mL concentrations. Total flavonoid and total proanthocyanidin results were expressed as µg catechin equivalent/g extract, total phenolic compound results were expressed as mg gallic acid equivalent/g extract.

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**Tablo 1. C. kurdica, C. virgata ve C. saligna ekstraktlarının ABTS•⁺, OH•, DPPH• radikali yok etme aktiviteleri, total flavonoid, total proantotsiyaniyanidin ve total fenolik bileşik değerleri**
Table 2. Flavonoids, phenolic acids, lipid soluble vitamins, phytosterols, fatty acid contents of C. kurdica, C. virgata and C. saligna extracts

| Flavonoids and Phenolic Acids | C. kurdica | C. virgata | C. saligna |
|-------------------------------|------------|------------|------------|
| Rutin (Rutin)                | 0.80±0.10  | nd         | 1.05±0.05  |
| Myricetin (Myrisetin)         | nd         | 0.60±0.05  |            |
| Morin (Morin)                | 0.30±0.05  | 1.00±0.15  | 0.05±0.00  |
| Quercetin (Kuersetin)         | 0.75±0.05  | 1.40±0.10  | 0.05±0.00  |
| Kaempferol (Kaempferol)       | 0.85±0.15  | nd         | 0.05±0.00  |
| Catechin (Kateşin)            | 59.45±1.05 | 119.65±1.15| nd         |
| Naringin (Naringin)           | nd         | 0.90±0.10  |            |
| Naringenin (Naringenin)       | nd         | 0.30±0.05  | 0.05±0.00  |
| Resveratrol (Resveratrol)     | 3.30±0.20  | 12.05±0.30 | 0.15±0.00  |
| Vanillic Acid (Vanillik Asit) | 104.95±0.55| 18.95±0.40 | 47.35±1.35 |
| Gallic Acid (Galik Asit)      | 1384.65±2.35| 2633.80±2.55| 11.40±0.90|
| Hydroxycinnamic Acid          | 2.35±0.15  | nd         | 0.25±0.05  |
| Caffeic Acid (Kafeik Asit)    | 14.70±0.70 | 310.90±1.05| 34.30±2.35 |
| Ferulic Acid (Ferulik Asit)   | 659.30±1.50| nd         | 237.00±2.00|
| Rosmarinic Acid (Rosmarinik Asit) | 439.65±1.85 | nd | nd |
| Retinol (Retinol)             | nd         | 0.03±0.00  | 0.05±0.00  |
| α-Tocopherol (α-Tokofero)     | 0.40±0.05  | 0.25±0.05  | 0.05±0.00  |
| δ-Tocopherol (δ-Tokofero)     | 0.15±0.00  | 0.20±0.00  | 2.05±0.10  |
| Vitamin K (Vitamin K)         | 0.15±0.00  | 6.70±0.35  | 0.90±0.05  |
| Vitamin D (Vitamin D)         | 0.05±0.00  | 0.50±0.05  | 0.75±0.05  |
| β-Sitosterol (β-Sitosterol)    | 5.20±0.25  | nd         | nd         |
| Ergosterol (Ergosterol)       | 13.05±0.25 | 86.50±1.15 | 20.25±0.95 |
| Stigmasterol (Stigmasterol)   | 11.00±0.60 | 5.60±0.10  | 17.55±0.70 |

| Fatty Acids · Yağ Asitleri (%) | C. kurdica | C. virgata | C. saligna |
|--------------------------------|------------|------------|------------|
| 16:0                           | 21.98±0.82 | 22.29±0.29 | 20.14±1.47 |
| 16:1                           | 2.45±0.12  | 6.62±0.32  | 4.72±0.49  |
| 18:0                           | 9.38±0.11  | 5.91±0.16  | 6.96±0.57  |
| 18:1                           | 26.34±0.86 | 20.27±0.89 | 21.18±1.65 |
| 18:2                           | 28.77±0.91 | 28.08±0.95 | 29.76±2.02 |
| 18:3                           | 11.08±0.22 | 9.03±0.19  | 17.24±1.34 |
| 20:5                           | nd         | 7.80±0.11  | nd         |
| Saturated FA (Doymuş Yağ Asitleri) | 31.36    | 28.20     | 27.10     |
| Unsaturated FA (Doymamış Yağ Asitleri) | 68.64    | 71.80     | 72.90     |

nd: not detected

Ayaz et al. (2017) showed that C. virgata is included 5.75% palmitic acid (16:0), 2.65% stearic acid (18:0), 18.40% oleic acid (18:1), 62.99% linoleic acid (18:2), 0.49% linolenic acid (18:3), 9.97% total saturated and 89.10% total unsaturated fatty acids. In our study, it was observed that C. virgata was included 28.20% total saturated and 71.80% total unsaturated fatty acids.

Antimicrobial Properties

The antimicrobial property results of C. virgata, C. kurdica and C. saligna water, ethanol, methanol and acetone extracts are summarized in Tables 3-5.

It was observed that C. kurdica water extract has an antimicrobial activity on only P. aeruginosa, P. vulgaris, S. aureus bacteria and C. albicans yeast; ethanol and methanol extracts have an antimicrobial activity on P. vulgaris, E. coli, L. monocytogenes, P. aeruginosa, S. aureus, K. pneumoniae, B. subtilis and B. megaterium bacteria, and C. albicans yeast; the acetone extract has an antimicrobial activity only P. vulgaris, E. coli, B. subtilis, P. aeruginosa, S. aureus and B. megaterium bacteria, and C. albicans yeast.

It was determined that C. virgata water, ethanol, methanol and acetone extracts have an antimicrobial property on only P. aeruginosa, P. vulgaris, B. megaterium, S. aureus and B. subtilis bacteria, and C. albicans yeast.
It was concluded that *C. saligna* water extract has an antimicrobial activity on only *K. pneumoniae* and *B. subtilis* bacteria; acetone extract has an antimicrobial activity only *K. pneumoniae, L. monocytenes, S. aureus* and *B. megaterium* bacteria; ethanol and methanol extracts have an antimicrobial activity on *P. vulgaris, E. coli, L. monocytenes, P. aeruginosa, K. pneumoniae, B. megaterium, S. aureus* and *B. subtilis* bacteria, and *C. albicans* yeast.

Uysal *et al.* (2013) showed that *C. polyclada, C. persica* and *C. consanguinea* ethanol and acetone extracts have antimicrobial activity on the *K. pneumoniae, S. aureus, L. monocytenes, B. subtilis, E. coli, P. vulgaris* bacteria and *C. albicans* yeast. In another study, Sarker *et al.* (2012) determined that *C. persica* methanol extract show antimicrobial effect on....

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### Table 3. The antimicrobial activities of *C. kurdica* extracts (mm zone)

| Microorganism (Mikroorganizma) | CKW | CKE | CKM | CKA | Standard |
|-------------------------------|-----|-----|-----|-----|----------|
| *E. coli*                     | nd  | 12  | 16  | 14  | 10       |
| *P. vulgaris*                 | 10  | 10  | 12  | 10  | 10       |
| *P. aeruginosa*               | 10  | 9   | 11  | 8   | 15       |
| *L. monocytenes*              | nd  | 10  | 11  | nd  | 8        |
| *K. pneumoniae*               | nd  | 10  | 11  | nd  | 9        |
| *B. subtilis*                 | nd  | 10  | 10  | 8   | 9        |
| *B. megaterium*               | nd  | 11  | 10  | 9   | 12       |
| *S. aureus*                   | 9   | 10  | 12  | 8   | 12       |
| *C. albicans*                 | 9   | 10  | 12  | 10  | 10       |

Streptomycin sulfate (10 mg/disc) and Nystatin (30 mg/disc) were used as standard antibiotic discs. The diameter of the paper discs was 6 mm.

**nd**: not determined

### Table 4. The antimicrobial activities of *C. virgata* extracts (mm zone)

| Microorganism (Mikroorganizma) | CVW | CVE | CVM | CVA | Standard |
|-------------------------------|-----|-----|-----|-----|----------|
| *E. coli*                     | nd  | nd  | nd  | nd  | 10       |
| *P. vulgaris*                 | 12  | 13  | 13  | 10  | 10       |
| *P. aeruginosa*               | 11  | 12  | 12  | 9   | 15       |
| *L. monocytenes*              | nd  | nd  | nd  | nd  | 8        |
| *K. pneumoniae*               | nd  | nd  | nd  | nd  | 9        |
| *B. subtilis*                 | 11  | 12  | 12  | 9   | 9        |
| *B. megaterium*               | 12  | 13  | 13  | 10  | 12       |
| *S. aureus*                   | 11  | 13  | 13  | 10  | 12       |
| *C. albicans*                 | 8   | 9   | 9   | 8   | 10       |

Streptomycin sulfate (10 mg/disc) and Nystatin (30 mg/disc) were used as standard antibiotic discs. The diameter of the paper discs was 6 mm.

**nd**: not determined

### Table 5. The antimicrobial activities of *C. saligna* extracts (mm zone)

| Microorganism (Mikroorganizma) | CSW | CSE | CSM | CSA | Standard |
|-------------------------------|-----|-----|-----|-----|----------|
| *E. coli*                     | nd  | 8   | 9   | nd  | 10       |
| *P. vulgaris*                 | nd  | 8   | 9   | nd  | 10       |
| *P. aeruginosa*               | nd  | 8   | 9   | nd  | 15       |
| *L. monocytenes*              | nd  | 8   | 10  | 8   | 8        |
| *K. pneumoniae*               | 8   | 9   | 11  | 8   | 9        |
| *B. subtilis*                 | 9   | 8   | 9   | nd  | 9        |
| *B. megaterium*               | nd  | 9   | 10  | 8   | 12       |
| *S. aureus*                   | nd  | 8   | 10  | 8   | 12       |
| *C. albicans*                 | nd  | 8   | 9   | nd  | 10       |

Streptomycin sulfate (10 mg/disc) and Nystatin (30 mg/disc) were used as standard antibiotic discs. The diameter of the paper discs was 6 mm.

**nd**: not determined
the E. coli, Ugur et al. (2009) suggested that C. ensiformis ethanol extract shows antimicrobial effect on the S. aureus, E. coli, B. subtilis, P. aeruginosa, S. epidermidis and S. mutans bacteria. Guven et al. (2005) specified that C. kurdica ethanol and acetone extracts exhibit antimicrobial property on the P. vulgaris, P. aeruginosa, B. cereus, E. coli, L. monocytogenes, B. subtilis and K. pneumoniae bacteria with C. albicans yeast; Tekeli et al. (2008) detected that C. virgata have antimicrobial effect on the Salmonella enteritidis and E. coli bacteria.

**Antiproliferative Properties**

The antiproliferative property results of C. virgata, C. kurdica and C. saligna water, ethanol, methanol and acetone extracts on the LNCaP, HCT-116 and MCF-7 cancer cell lines are shown in Tables S7-S15. The IC₅₀ values of all the extracts are presented in Table 6 and Figure 1 for the antiproliferative activity.

Table 6. The IC₅₀ values of C. kurdica, C. virgata and C. saligna extracts on the MCF-7, HCT-116 and LNCaP cancer cell lines for the antiproliferative activity assay (μg/mL)

| Samples (Örnekler) | MCF-7       | HCT-116      | LNCaP     |
|-------------------|-------------|--------------|-----------|
| CKW               | 12.32±1.07  | 5.89±0.38    | 2.01±0.11 |
| CKE               | 8.38±0.68   | 5.03±0.43    | 1.48±0.13 |
| CKM               | 9.54±0.96   | 6.90±0.46    | 2.31±0.19 |
| CKA               | 9.93±0.79   | 3.49±0.29    | 2.46±0.33 |
| CVW               | 6.39±0.91   | 2.55±0.17    | 0.97±0.05 |
| CVE               | 1.96±0.12   | 3.82±0.36    | 2.21±0.31 |
| CVM               | 5.61±0.41   | 2.91±0.33    | 1.91±0.18 |
| CVA               | 6.98±0.54   | 3.02±0.27    | 1.88±0.09 |
| CSW               | 26.13±2.43  | 2.74±0.25    | 15.72±1.82|
| CSE               | 4.90±0.39   | 1.73±0.18    | 1.90±0.15 |
| CSM               | 28.13±2.69  | 1.43±0.10    | 1.19±0.08 |
| CSA               | 8.91±0.63   | 1.64±0.11    | 0.40±0.02 |

Figure 1. The IC₅₀ values of C. kurdica, C. virgata and C. saligna extracts on the MCF-7, HCT-116 and LNCaP cancer cell lines after 24-hour treatment for the antiproliferative activity assay (μg/mL)

*Şekil 1. Antiproliferatif aktivite testi için C. kurdica, C. virgata ve C. saligna ekstraktlarının MCF-7, HCT-116 ve LNCaP kanser hücre serileri üzerinde 24 saatlik uygulama sonrasında IC₅₀ değerleri (μg/mL)*
C. virgata ethanol extract (1.96±0.12 µg/mL) has better antiproliferative activity for the MCF-7 cell lines than all the other extracts: C. saligna methanol extract (1.43±0.10 µg/mL) has better antiproliferative activity for the HCT-116 cell lines than all the other extracts: C. saligna acetone extract (0.40±0.02 µg/mL) has better antiproliferative activity for the LNCaP cell lines than all the other extracts.

To our best knowledge, there is no report about antiproliferative properties in Centaurea virgata Lam., Centaurea kurdica Reichardt and Centaurea saligna (K.Koch) Wagenitz species. For this reason, this study may be the first report about the antiproliferative properties of these plants.

CONCLUSION
This study purposed to assess radical scavenging activity, phytochemical composition, antimicrobial activities and antiproliferative activities of the water, ethanol, methanol and acetone extracts of Centaurea virgata Lam., Centaurea kurdica Reichardt and Centaurea saligna (K.Koch) Wagenitz. These results showed that these plant extracts have important antiradical, antimicrobial and antiproliferative properties. Moreover, these plants contain phytochemical compounds (flavonoids, phenolics, proanthocyanidins, fatty acids, vitamins, sterols), which are important and beneficial for health.

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Statement of Conflict of Interest
Authors have declared no conflict of interest.

Author’s Contributions
The contribution of the authors is equal.

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