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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.

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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, hemoroit, diyabet vb. hastalıkların 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 flavonoit, fenolik ve proantosiyanidin, fenolik asitler, fitosteroller ve doymamış yağ asitleri içerdiği 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 $ABTS^{\cdot+}$, 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:

$RSA\% = [(A_0 - A_1)/A_0] \times 100$

A_0 and A_1 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 done using according to 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 (LNCaP), 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 phytosterols, 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 µg catechin equivalent/g extract are sorted as follows: CVM > CSM > CVA > CKM > CSW > CVE > CVW > CSE > CSA > CKA > CKE > CKW. The total proanthocyanidin amounts of all the samples as µg catechin equivalent/g extract are sorted as follows: CSA > CVM > CVA > CVE > CSM > CSW > CKM > CSE > CKA > CVW > CKE > CKW. The total phenolic compound amounts of all the samples as mg gallic acid equivalent/g extract are sorted as follows: CSW > CSM > CVW > CVM > CSE > CVE > CSA > CVA > CKW > CKM > CKE > CKA.

The phenolic acids, flavonoid phytosterols, 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 GAE/g total phenolic, 165.21 mg RE/g total flavonoid, 37.59% 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. $ABTS^{+\cdot}$, $OH^{\cdot}$, $DPPH^{\cdot}$ radicals scavenging activities, total flavonoid, total proanthocyanidin and total phenolic compounds values of *C. kurdica*, *C. virgata* and *C. saligna* extracts

Tablo 1. C. kurdica, C. virgata ve C. saligna ekstraktlarının $ABTS^{+\cdot}$, $OH^{\cdot}$, $DPPH^{\cdot}$ radikali yok etme aktiviteleri, total flavonoit, total proantosiyanidin ve total fenolik bileşik değerleri

Samples *Örnekler* (500 µg/mL)	$ABTS^{+\cdot}$ Scavenging *$ABTS^{+\cdot}$ Yok Etme* (%)	$OH^{\cdot}$ Scavenging *$OH^{\cdot}$ Yok Etme* (%)	$DPPH^{\cdot}$ Scavenging *$DPPH^{\cdot}$ Yok Etme* (%)	Total Flavonoid *Total Flavonoit* (µg CE/g)	Total Proanthocyanidin *Total Proantosiyanidin* (µg CE/g)	Total Phenolic *Total Fenolik* (µg GAE/g)
CKW	42.09±1.25^{e}	67.56±0.63^{c}	72.57±0.23^{c}	207.43±1.22	139.67±0.76	29.70±0.09
CKE	54.21±0.99^{d}	78.48±0.45^{b}	35.45±1.12^{f}	343.31±1.36	166.33±0.88	17.90±0.17
CKM	66.75±1.07^{b}	81.88±0.36^{b}	86.38±0.95^{b}	1213.28±2.54	293.00±1.26	23.89±0.33
CKA	65.04±0.88^{b}	73.38±0.81^{c}	27.51±0.20^{g}	391.69±1.09	263.00±1.07	7.82±0.22
CVW	98.10±0.33^{a}	78.97±0.42^{b}	88.34±0.73^{b}	1085.34±3.07	260.78±0.86	68.36±1.11
CVE	70.15±1.07^{b}	80.10±0.55^{b}	57.24±1.22^{d}	1173.46±2.54	457.44±1.46	50.12±0.55
CVM	98.23±0.27^{a}	98.46±0.09^{a}	93.80±0.13^{a}	1965.75±3.69	834.11±1.72	57.90±0.63
CVA	69.38±0.97^{b}	83.34±0.58^{b}	53.38±0.52^{d}	1241.33±3.01	745.22±1.91	34.01±0.34
CSW	94.81±0.23^{a}	62.84±1.34^{c}	56.48±1.18^{d}	1175.36±1.68	303.00±0.90	106.27±0.19
CSE	50.43±1.34^{d}	66.56±2.12^{c}	47.58±1.55^{e}	513.54±0.87	278.56±0.74	57.53±0.08
CSM	99.94±0.00^{a}	85.14±0.75^{b}	71.96±1.08^{c}	1561.25±1.97	398.49±0.59	97.74±0.55
CSA	60.04±1.02^{c}	49.32±2.95^{d}	21.09±2.02^{g}	395.87±0.34	1084.19±1.36	35.02±0.11
Trolox *Troloks*	96.79±0.52^{a}	94.89±0.74^{a}	97.33±0.81^{a}	-	-	-

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.

Table 2. Flavonoids, phenolic acids, lipid soluble vitamins, phytosterols, fatty acid contents of *C. kurdica*, *C. virgata* and *C. saligna* extracts

Tablo 2. C. kurdica, C. virgata ve C. saligna ekstraktlarının flavonoit, fenolik asit, yağda çözünen vitamin, fitosterol, yağ asidi içerikleri

Flavonoids and Phenolic Acids *Flavonoitler ve Fenolik Asitler* (mg/g)	*C. kurdica*	*C. virgata*	*C. saligna*
Rutin (*Rutin*)	0.80±0.10	nd	1.05±0.05
Myricetin (*Myrisetin*)	nd	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	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 (*Gallik Asit*)	1384.65±2.35	2633.80±2.55	11.40±0.90
Hydroxycinnamic Acid (*Hidroksikinamik Asit*)	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
Vitamin and Sterols- *Vitamin ve Steroller* (mg/g)	*C. kurdica*	*C. virgata*	*C. saligna*
Retinol (*Retinol*)	nd	0.03±0.00	0.05±0.00
α-Tocopherol (*α-Tokoferol*)	0.40±0.05	0.25±0.05	0.05±0.00
δ-Tocopherol (*δ-Tokoferol*)	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.

Table 3. The antimicrobial activities of *C. kurdica* extracts (mm zone)
Tablo 3. C. kurdica ekstraktlarının antimikrobiyal aktiviteleri (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. monocytogenes	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)
Tablo 4. C. virgata ekstraktlarının antimikrobiyal aktiviteleri (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. monocytogenes	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)
Tablo 5. C. saligna ekstraktlarının antimikrobiyal aktiviteleri (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. monocytogenes	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

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. monocytogenes*, *S. aureus* and *B. megaterium* bacteria; ethanol and methanol extracts have an antimicrobial activity on *P. vulgaris*, *E. coli*, *L. monocytogenes*, *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. monocytogenes*, *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

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 IC_{50} values of all the extracts are presented in Table 6 and Figure 1 for the antiproliferative activity.

Table 6. The IC_{50} 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)

Tablo 6. Antiproliferatif aktivite testi için C. kurdica, C. virgata ve C. saligna ekstraktlarının MCF-7, HCT-116 ve LNCaP kanser hücre serileri üzerinde IC_{50} değerleri (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

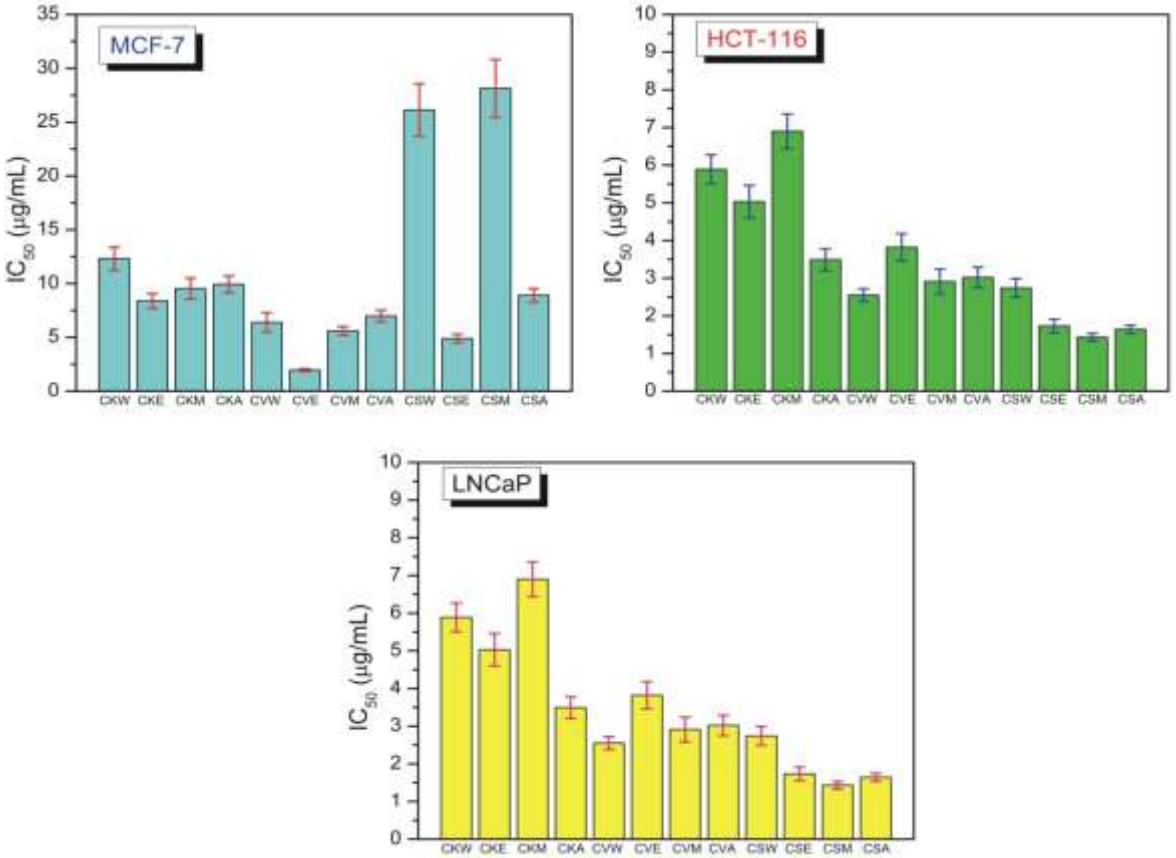

Figure 1. The IC_{50} values of *C. kurdica*, *C. virgata* and *C. saligna* extracts on the MCF-7, HCT-116 and LNCaP cancer cell lines after 24-hour treatment for the antiproliferative activity assay (µg/mL)

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 IC_{50} 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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