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ORIGINAL ARTICLE

Antimicrobial property and antiproliferative activity of *Centaurea babylonica* (L.) L. on human carcinomas and cervical cancer cell lines

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Abstract

Introduction and objective. Since antiquity, *C. babylonica* (L.) L. extracts has been used as a remedy for primary health care in traditional medicine. In this study, a total of seven different crude extracts (acetone, chloroform, hexane, ethylacetate, methanol, ethanol and water) from branches and leaves of *C. babylonica* (L.) L. were prepared to determine antimicrobial and antiproliferative activity against cancer cell lines.

Materials and method. MIC assay was used for antimicrobial activity against gram positive and gram negative bacteria, and one yeast. MTT assay was applied to screen the antiproliferative activity of seven extracts, and to determine dose- and time-dependent effects of the aceton extract on A549, PC-3, MCF-7, and HeLa cell lines.

Results. The aceton extract of *C.babylonica* (L.) L. showed the best antibacterial activity against *Bacillus cereus*, *P. aeruginosa* and *C. albicans* (MIC: 1.6 mg/mL). GC-MS analyses allowed six compounds to be determined; the main constituents of acetone extract from *C. babylonica* (L.) L. were diacetone alcohol (53.47 %), 1-dexadecene (10.19 %) and 1-tetradecene (8.67 %). In addition, seven different solvent extracts at 500 µg/mL caused antiproliferative activity between 84% – 88%, compared to control. Dose-dependent effects of the extracts on A549 cells indicated that chloroform, ethyl acetate, and aceton extract were the most effective extracts with the IC_{50} values of 9, 33, and 36 µg/mL, respectively.

Conclusions. The results clearly demonstrate that *C. babylonica* (L.) L. exhibited a strong antimicrobial effect and antiproliferative activity against cancer cells *in vitro*. Further studies are required to isolate and characterize the active pure compounds responsible for the antimicrobial and antiproliferative activities.

Key words

Centaura babylonica, antimicrobial activity, antiproliferative activity, A-549, PC-3, MCF-7 and HeLa cell lines, chemical composition, GC-MS

INTRODUCTION

Substantial research has been carried out to-date in order to understand microorganisms and their control. Many infectious diseases are mainly treated with antibiotics and herbel remedies; however, indiscriminate use of antibiotics rendered many pathogenic microorganisms resistant to almost all known antibiotics [1]. In addition, serious side-effects caused by some antibiotics limit their use in clinical application. Due to these problems, development of new antimicrobial agents is inevitable. About 250 – 500 thousand plant species are estimated to exist on the planet, but only between 1 – 10 % of them are used as food by humans and animals [2]. Turkish medicinal plants have been shown to be a promising source of potent antimicrobial agents [3].

Cancer is a multistep disease and a major cause of death worldwide. To-date, many chemotherapeutic anticancer agents have been developed, but because of their toxicity and lack of selectivity against cancer cells, development of a new therapeutic option is a priority for many pharmaceutical companies and research centres. Phytochemicals from a variety of plant species have potential in the treatment and prevention of cancer [4]. In fact, plants have a long history of being used for many health benefits by all cultures. Traditional therapy mainly involves the use of plant extracts or their active compounds [5]. In order to overcome the toxic side-effects of chemotherapeutic drugs, using terrestrial plants is a practical approach for developing new anticancer agents that are both effective and safe.

The genus *Centaurea* belongs to the *Asteraceae* family and it is the third largest genus in terms of species number in Turkey. There are 217 species (146 endemics), 36 subspecies (22 endemics), 28 varieties (16 endemics), a total of 255 taxa and with an endemism ratio of 66.8% [6, 7]. According to phytogeographical distribution, *C. babylonica* (L.) L. is a Mediterranean element and distributed in Turkey, Lebanon, and Syria [8]. In Anatolia, the *Centaurea* L. species are called zerdali dikeni, Timur dikeni and peygamber çiçegi [9]. In traditional medicine, they are used for fever, menstrual disorders, vaginal candidiasis, the treatment of liver, kidney

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and ulcer diseases, as antidiarrheal, stomachic, tonic, appetitive, antidiabetic, antipyretic, as well as a diuretic and expectorant [10]. The genus *Centaurea* L. has also been the subject of many antimicrobial and antioxidant activity studies [11–21], antifungal [22, 23], anti-colon cancer [24] and cytotoxic activities [24, 25]. Additionally, the flower heads of *C. cyanus* are commonly used in European traditional medicine for the treatment of minor ocular inflammation, and *in vivo* anti-inflammatory and immunological activities of *C. cyanus* have been reported [26]. However, to the best of the authors' knowledge, there is no study in the literature related to antimicrobial or antiproliferative effects of *C. babylonica* (L.) L (from Adıyaman province in Turkey) crude extracts obtained with seven different solvents.

OBJECTIVE

The aim of this study is to evaluate the antimicrobial activity of seven different extracts (acetone, chloroform, hexane, ethylacetate, methanol, ethanol and water) of *C. babylonica* (L.) L against both clinical and food borne microorganisms; to identify the chemical composition of the most effective extract; and to assess the antiproliferative potential of the extracts against four different human cancer cell lines.

MATERIALS AND METHOD

Collection of plant material. Samples of *C.babylonica* (L.) L. were collected on 2 July 2016 from near Gölbaşı-Sürgü road, on serpentine soil in the Gölbaşı district of Adıyaman province in Turkey. The plants were collected and identified by Dr. Hasan Yildirim at the Department of Biology/Botany, Faculty of Science at Ege University, Izmir, Turkey. A voucher specimen has been deposited in the Herbarium of Ege University in Izmir, Turkey (EGE-42441).

Preparation of leaf and branch extracts. The whole plant (leaf and branches) above ground was collected, without flowers, dried on newspapers to a constant weight in a sunless environment, and milled to a fine powder using a porcelain miller. The powdered leaf (60 g) was soaked in 1,500 mL of DMSO to prepare the extract, and in 150 mL of absolute ethanol (96°, Fluka Chemical), and in 1,500 ml of absolute methanol (97°, Fluka Chemical), acetone (96°, Fluka Chemical), chloroform (96°, Fluka Chemical), hexane (96°, Fluka Chemical), and ethylacetate (96°, Fluka Chemical), to prepare the acetone, chloroform, hexane, ethylacetate, ethanolic and methanolic extracts. The suspension was stirred at 200 rpm at room temperature for four days, after which it was filtered through a Whatman No. 1 filter paper. The residue was reextracted with 1,500 mL of the solvent, as described. The combined extracts were then evaporated to dryness at 40 °C, re-dissolved in the corresponding solvent to obtain extracts (8mg/mL) and stored at 4 °C prior to use [18, 27, 28].

Microorganisms and culture conditions. Antimicrobial studies were carried out against 16 bacteria strains, three Gram-positive bacteria strains, including *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* CCM 99, three Gram-negative bacteria strains, including *Escherichia coli* ATCC 29998, *Salmonella typhimurium* CCM 3819, *Pseudomonas aeruginosa* ATCC 27853 and yeast *Candida albicans* ATCC 10259. The species of bacteria were grown overnight at 37 °C in Mueller-Hinton Broth (Merck). *C. albicans* was grown for 48 h at 30 °C in Sabouraud Dextrose Broth (Oxoid).

Antimicrobial assays – determination of minimum inhibitory concentration (MIC). The MIC was evaluated on plant extracts as antimicrobial activity. The MIC was taken as the lowest concentration that inhibited growth after incubation. The microdilution assay was performed as described in the CLSI standards with some modifications [29, 30]. This test was performed at final concentrations of each extract (51.2, 25.6, 12.8, 6.4,3.2,1.6, 0.8 and 0.4 mg of extract / mL). Sterile 96-well microtiter plates were used in this assay. Mueller-Hinton Broth for bacteria or Sabouraud Dextrose Broth for *C. albicans* was added to give final concentrations. 50 µL of broth containing bacterial suspension (5×10^6 cfu/mL) or yeast (5×10^5 cfu/mL) was then added to each well. Each column of wells contained a single antimicrobial extract in progressive dilutions, and inoculated with a single microorganism. Each plate had a set of both growth and sterility control. Plates were sealed with clean film to ensure that microorganisms did not become dehydrated. The plates were prepared and placed in an incubator set at 37 °C for 18–24 h and at 30 °C for 48 h, respectively, for bacteria and *C. albicans*.

Bacterial growth was assessed by adding 10 µL of 0.2% 2,3,5-triphenyl tetrazolium chloride (TTC) solution to each well of the microtitre plate. The plates containing TTC were incubated for one h at 37 °C for reaction. The colour change was then assessed visually. Any colour changes from purple to pink showed the growth of microorganism. MIC concentration did not exhibit reduction of TTC into formazan; therefore, the MIC was defined as the lowest inhibitory concentration of the antimicrobial agent contained in the microtiter well in which the absence of visual colour change (colourless) was first observed. Acetone, chloroform, hexane, ethylacetate, methanol and ethanol were used as negative control. Erythromycin was used as a standard antibiotic for the bacteria for positive control; nystatin was used for *C. albicans*. They were tested between 0.78 – 400 mg/mL (prepared from 1 mg/mL stock) concentrations.

GC-MS analysis. The steam-distilled components were analysed by GC-MS. A HP 6890 gas chromatograph equipped with a HP-PTV; a 0.32 m X 0.60 m HPInnowax capillary column (0.5 µm coating) was employed for the GC analysis. GC-MS analysis was performed on a HP-5973 mass selective detector coupled with a 6890 gas chromatograph, equipped with a HP 6890 gas chromatograph. The column temperature was programmed from an initial temperature of 60°C to a final temperature of 250 °C at 15 °C/min. The carrier gas was helium (14.1 mL/min). Identification of the individual components was performed by comparison of mass spectra with literature data, and by a comparison of their retention time (Rt) relative to a C8-C32 n-alkanes mixture [31]. A computerized search was carried out using the Wiley 7n.l GC-MS library and ARGEFAR GC-MS library created with authentic samples.

Cell lines and cell culture. Human lung carcinoma (A549), human prostate carcinoma (PC-3), human breast adenocarcinoma (MCF-7) and human cervical cancer (HeLa)

cell lines were procured originally from the American Type Culture Collection (ATCC). All cell lines were maintained by serial sub-culturing in RPMI 1640 medium (Biochrom, Germany), supplemented with 10% foetal bovine serum (FBS, Biochrom, Germany), penicillin (100U/mL) and strepromycin sulphate (100 mg/mL) (Biochrom, Germany). Cells were incubated at 37 °C in 5% CO_2, 95% air in a humified incubator.

MTT assay. Antitumoral activity of the plant extracts was determined by colorimetric MTT tetrazolium (Applichem, USA) reaction which reduces yellow MTT to purple formazan by mitochondrial dehydrogenase in living cells. Optical density of reaction reflects the function of mitochondria and cell viability [32]. Exponentially growing cells at $2x10^4$ cells/mL were seeded in triplicate into 96-well plates (Greiner, Germany) in 200 µL of growth medium. Cells were incubated for 24 h before the addition of extracts to allow attachment to the plate. Extracts from six different solvents were dissolved in 10% DMSO and added to the cell culture at the final concentration of 0.5 mg/mL, to be tested against four cancer cell lines. Cells were incubated for 72 h at 37 °C in a 5% CO_2 incubator. In order to determine dose and time responsiveness, cells were treated with the extracts at 0.5 mg/mL, 0.25 mg/mL, 0.12 mg/mL, 0.06 mg/mL, 0.03 mg/mL, and 0.015 mg/mL final concentrations, and incubated for 24, 48, and 72 h. After removing the medium, 100 µL of fresh medium supplemented with 10 µL of PBS containing 5 mg/mL MTT was added to each well. After 4 h incubation, the medium was discarded and formazan purple crystals formed in the cells were dissolved in 100 µl DMSO. Colour intensity was measured by reading the absorbance at 540 nm on an ELISA plate reader (Thermo Scientific Multiscan Spectrum). Untreated cells served as a negative control. Cell viability was expressed as the ratio of absorbance value of treated cells to untreated control cells, and multiplied by 100 to obtain a percentage. The IC_{50} value was calculated from a plot of % cell viability against substance cocentrations using a GraphPad PRISM (GraphPad Software, Inc., San Diego, CA, USA).

Statistical analysis. Data represent the mean ± SD of triplicate samples for each dose. Data were analysed by one-way ANOVA, asfter which Dunnett *post hoc* test was performed to compare the findings among the groups. A difference was considered to have significance at *p<0.05, **p<0.01 and ***p<0.001. Two-way ANOVA was used to compare data from time and dose dependent assays.

RESULTS

Aceton extracts of *C.babylonica* (L.) L. showed the best antibacterial activities against endospor-forming bacteria *B. cereus* (15 mm) and *B. subtilis* (14 mm) (Tab. 1). Similarly, the ethyl acetate extracts had the highest inhibitory zones on these two bacteria. On the contrary, none of the plant extracts had antimicrobial activity on the gram-negative bacterium *P. aeruginosa*. The aceton extracts also displayed the highest antifungal activity against *C. albicans* (14 mm).

The antimicrobial activities of six extracts of *C.babylonica* (L.) L. were also tested *in vitro* by using the microdilution method. According to these results, the ethylacetate extracts of *C.babylonica* (L.) L. leaves and branches showed the best antibacterial activity against *Pseudomonas aeru*ginosa (1.6 mg/mL) (Tab. 2). The chloroform extracts of *C.babylonica* (L.) L. displayed the best antibacterial activity against *S.typhimurium* (0.4 mg/mL); ethanol extracts of *C. babylonica* (L.) L. showed the best antibacterial activity against *B.subtilis* (1.6 mg/mL); acetone extracts of *C. babylonica* (L.) L. showed the best antibacterial activity against *P. aeruginosa* and *B. cereus* (1.6 mg/mL); and the acetone extracts of *C. babylonica* (L.) L. leaves and branches showed the best anticandidal activity (1.6 mg/mL) (Tab. 2).

Table 1. Antimicrobial activity of 6 extracts of *C. babylonica* (L.)L leaves and branches by the disc diffusion method

Microorganisms	Inhibitory zone (mm)*									
	Extracts							Antibiotics		
	EA	E	M	H	A	C	DMSO	Er	Am	N
S.aureus	10	7	-	7	12	9	-	21	11	NT
B.cereus	13	8	7	10	15	11	-	33	31	NT
B.subtilis	13	7	7	14	14	10	-	31	23	NT
E.coli	-	-	7	7	7	7	-	-	-	NT
S.typhimurium	-	9	-	-	-	-	7	7	17	NT
P.aeruginosa	-	-	-	-	-	-	-	17	23	NT
C.albicans	12	11	11	9	14	9	8	NT	NT	24

*Results (mean of 3 replicates) indicate zone of inhibition in mm and include filter paper disc diameter (6 mm).
EA – Ethyl acetate; E – Ethanol; M – Methanol; H – Hegzan; A – Aceton; C – Chloroform; Er – Erithromycin;
Am – Ampicillin; N- Nystatin; DMSO – Dimethyl Sulfoxide; NT – Not tested – No inhibition

Table 2. MIC values of *Centaurea babylonica* leaves and flowers, erithromycin and nystatin against test microorganisms

Microorganisms	MIC values (mg/L)							
	Extracts						Antibiotics	
	A	C	H	EA	M	E	Er	N
S. aureus	51.2	51.2	51.2	51.2	51.2	25.6	3.2	NT
B. cereus	1.6	51.2	25.6	25.6	> 51.6	51.2	0.8	NT
P. aeruginosa	1.6	5.12	25.6	1.6	25.6	6.4	0.8	NT
S. typhimurium	51.2	0.4	> 51.2	51.6	25.6	> 51.2	3.2	NT
E. coli	12.8	51.2	> 51.2	> 51.6	> 51.6	25.6	1.6	NT
B. subtilis	6.4	6.4	51.2	51.6	> 51.6	1.6	0.4	NT
C. albicans	1.6	51.2	51.2	3.2	6.4	6.4	NT	0.4

EA – Ethyl acetate; E – Ethanol; M – Methanol; H – Hegzan; A – Aceton; C – Chloroform; H – Hegzan;
EA – Ethyl acetate; E – Ethanol; M – Methanol; Er – Erithromycin; N – Nystatin; DMSO – Dimethyl Sulfoxide; NT – Not tested

The aceton extract of the herb was also evaluated for its chemical composition by GC-MS, which allowed six compounds to be determined; the main constituents of the *C. babylonica* (L.)L**.** leaves and brunches acetone extract were diacetone alcohol (53.47%), 1-dexadece (10.19%) and 1-tetradecene (8.67%) (Tab. 3).

Even though many anti-cancer agents have been isolated from different plant species, there still remain many attempts to be made investigate the anti-cancerous compounds in unexplored plant species. Therefore, the antiproliferative effects of *C. babylonica* (L.) L. extracts against different cancer cell lines were investigated for the first time in this study. The anti-tumour effects of crude extracts obtained from seven different solvents on the viability of A549, PC-3,

Table 3. Volatile components of acetone extracts of *Centaurea babylonica* (L.)L. leaves and brunches extracts (GC-MS analysis)

Component[a]	Area	Retention[b]
Diacetonealcohol	53.47%	19.7
1-Dexadecene	10.19%	24.02
1-Tetradecene	8.67%	28.13
Alpha Octadecene	6.38%	35.94
N tetra cosane	5.07%	47.45
Tetradecamethyl cycloheptasiloxane	2.68%	63.01
Undefined	13.54%	70.12

[a] Components listed in order of elution from HP-1capillary column.
[b] Retention time (as min).

MCF-7 and HeLa cells were investigated by MTT assay. For initial screening of antiproliferative activity, the extracts were used at the high concentration (0.5 mg/mL). Water extract exhibited moderate antiproliferative activity, 58%, and 42%, against MCF-7 and A549 cells, respectively (Fig. 1), whereas 22% and 15% anti-tumour activities were observed against PC-3 and HeLa cells (Fig. 1). Unlike water extract, all six extracts of *C. babylonica* resulted in cytotoxic activity between 72% – 90% against all cancer cell lines tested, indicating significant antiproliferative activity (***p<0.001), compared to the control.

Dose-response curves of A549 cells treated with different concentration of each extract were prepared to determine the IC_{50} value (concentration, µg/ml). Based on the results, chloroform, ethyl acetate, and acetone extracts were the most effective because their IC_{50} values were 9, 33 and 36 µg/mL (Fig. 2). According to the US NCI plant screening program, if the IC_{50} value of a crude extract after treatment of cancer cells for 48 – 72 h is less than 20 µg/mL, it is considered to be an ideal concentration during the preliminary investigation of cytotoxic studies of plant extracts [32]. Therefore, findings obtained in the current study indicate that chloroform, ethyl acetate, and acetone extracts of *C. babylonica* (L.) L. have ideal IC_{50} values for initial examinations of antiproliferative activity against the A549 cell line. These extracts may serve as a significant source for further isolation of individual compounds for cytoxicity of cancer cells.

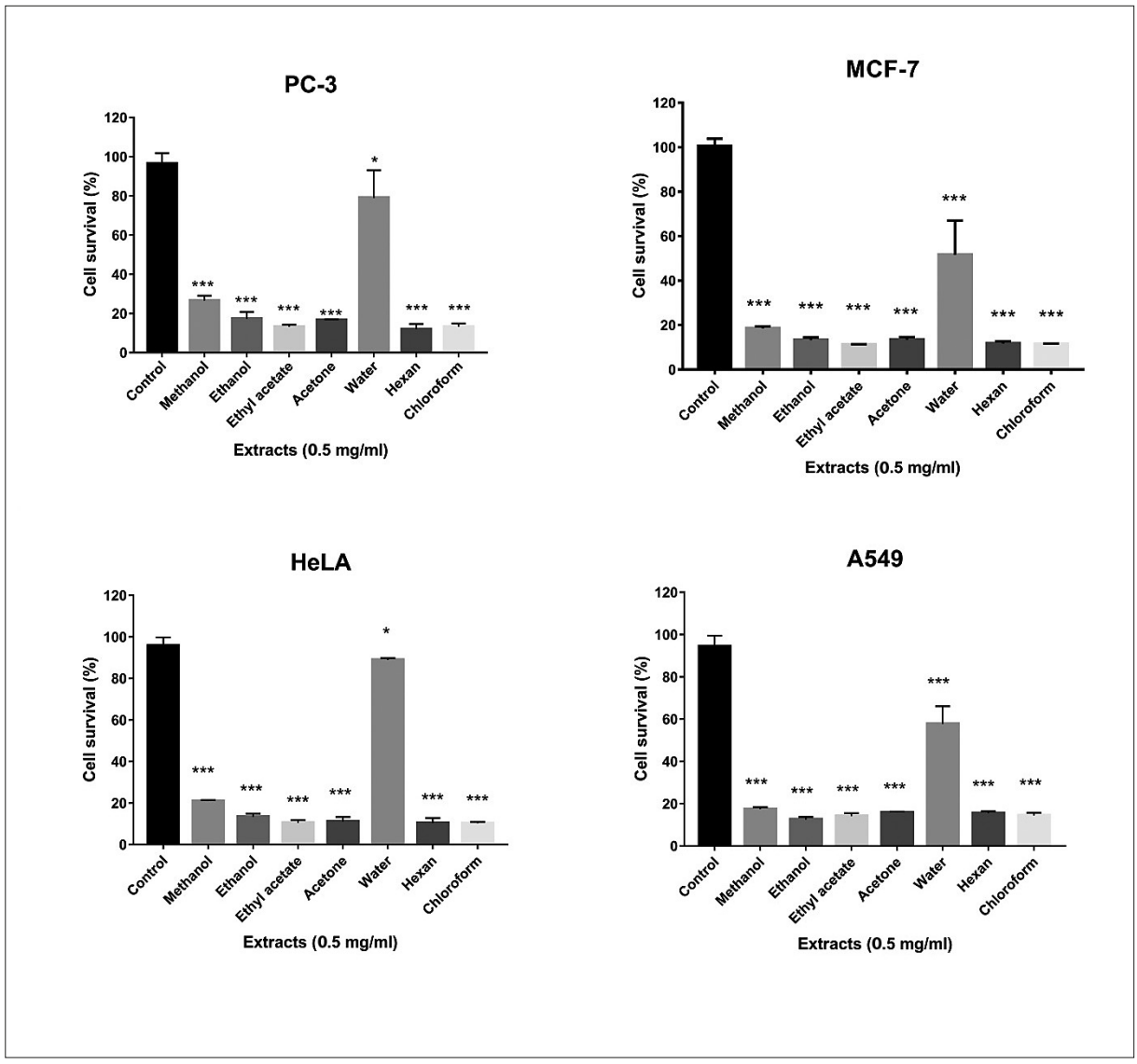

Figure 1. Antiproliferative effects of the different extracts (0.5 mg/ml) on different cancer cell lines. Cells were incubated for 72 hr in the absence or presence of the extracts and cell survival determined using MTT assay.
The data represent mean ± SE of three replicates.
*P<0.05; **P<0.01; ***P<0.001.

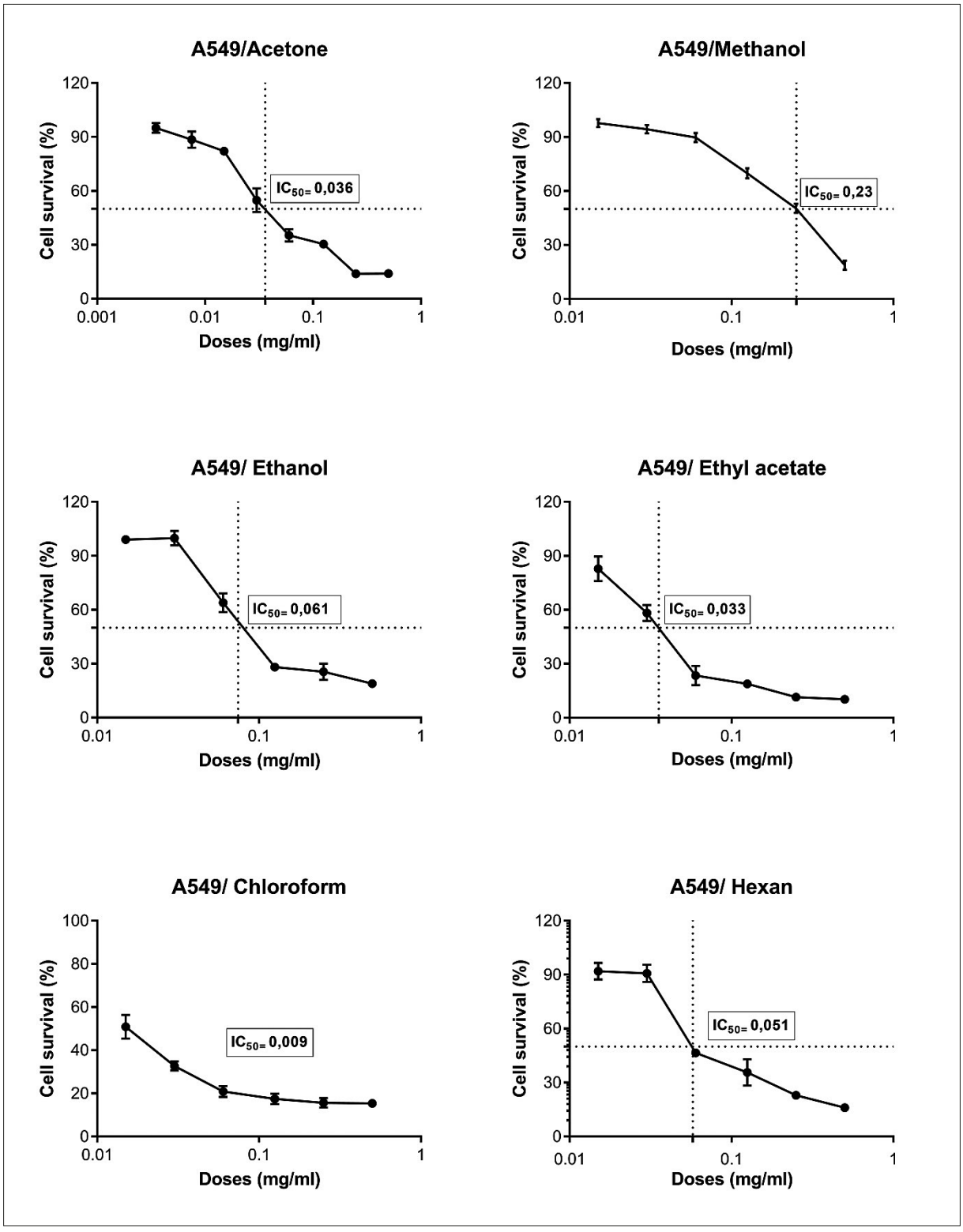

Figure 2. Dose curve and IC_{50} values of extracts on A549 cell line. A549 cells were incubated for 72 hr in the presence of the extracts at 6 different concentrations (0.5, 0.25,0.12, 0.06, 0.031,0.01mg/ml).
Data represent mean ± SE of 3 replicates. LC_{50} value determined using GraphPad PRISM, programme as described previously.

Moreover, time and dose responsiveness of A549, HeLa, PC-3, and MCF-7 cell lines were determined after 24, 48 and 72 h treatment with the acetone extract at different concentrations. Results show that MCF-7 cell line was the most sensitive cell type which demonstrated 50% cell death with the extract at 3 µg/mL, even after 48 hr treatment (Fig. 3). PC-3 cells exhibited 78% cell death after treatment with six µg/ml acetone extract. In addition, the dose response curve of HeLa cells was similar to that of PC-3 cells after 48 and 72 h treatment. However, 50% cell death was obtained with the extract at six µg/mL. In comparison with other cell lines, A549 cells were less sensitive to acetone extract because it

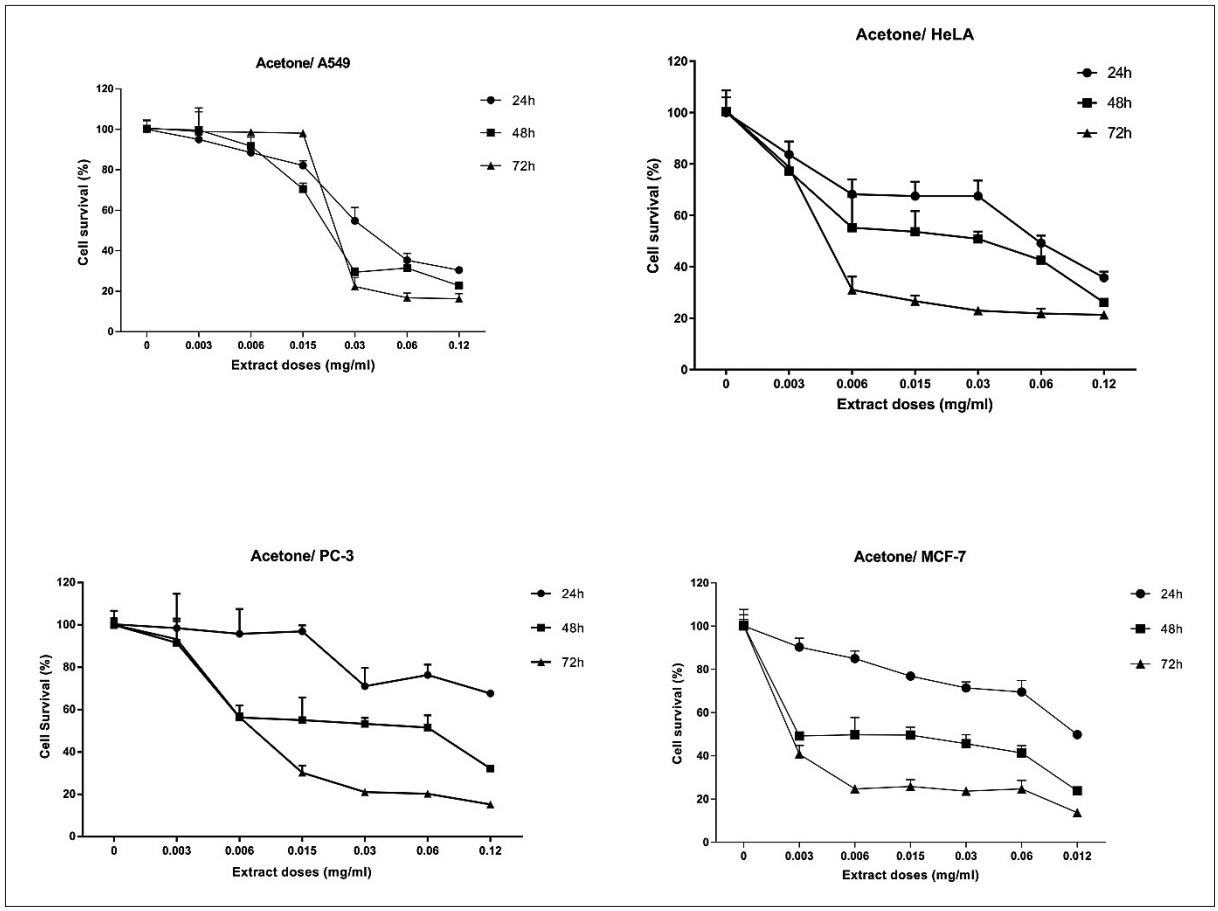

Figure 3. Time and dose effects of acetone extract on A549 cell line. Cell were treated with the extract at 7 different concentrations and incubated for 24, 48 and 72 hr.
Data represent mean ± SE of 3 replicates.

caused 50% cell death at around 30 µg/mL. In other words, acetone extract at less than 15 µg/ml reduced cell viability to less than 30% in HeLa, PC-3 and MCF-7 cells, but not in A549 cells. Taken together, these data indicate that the antiproliferative effects of acetone extract on cancer cell lines were time- and dose-dependent; moderate antiproliferative effects were also observed for acetone extract with IC_{50} in the range of 3 – 6 µg/mL for MCF-7, HeLa, and PC-3 cells.

DISCUSSION

Natural products have been used to treat many human diseases, including bacterial infection, cancer and immune disorders [33]. *Centaurea spesies* have a wide distribution in the Mediterranean region and Turkey is the centre for a variety of these species. Many biological properties were attributed to *C. species* [34]. In the presented study, seven different extracts of *C. babylonica* (L.) L. leaves and branches were evaluated for the first time for their antimicrobial and antiproliferative potetials against cancer cells. The chemical composition of the most effective acetonic extract was also identified.

In the current study, all the extracts exhibited the strongest antimicrobial activity against *B. cereus* and *B. substilis* in the range of inhibition zone between 7 – 15 mm. In addition, ethyl acetate and acetonic extracts were found to be the most effective extracts against *S. aureus*, *B. cereus*, *B. substilis* and *C. Albicans,* compared to other extracts (Tab. 1). Kumarasamy *et al.* (2002) tested *Centaurea scabiosa* L. seeds for antimicrobial activity and found that the extracts were effective only for *Proteus mirabilis* bacterium. On the other hand, they found significant antimicrobial activity of serotonin conjugates from *Centaurea nigra* L. against penicillin-resistant *Escherichia coli* [14]. However, in the current study, little antimicrobial activity against this bacterium using four different extracts was observed (Tab. 1). Moreover, Cansaran *et al.* (2010) examined the antimicrobial activity of various extracts of *Centaura cankiriense* [35]. Both the ethyl acetate and methanol extracts of this species inhibited the growth of 13 bacteria, and the MIC values of the ethyl acetate extracts were determined as 250 mg/mL for *E. coli* and 62.5 mg/mL for *S. aureus*. Unlike the results obtained by Cansaran *et al*., the current study found that ethyl acetate and methanol extracts of *C. babylonica* exhibited MIC values of 51.6 mg/mL for *E. coli* and 51.2 mg/mL for *S. aureus* (Tab. 2). In other words, ethyl acetate and methanol extracts of *C. babylonica* include more effective phytochemical compounds against these bacteria than that of *C. cankiriense*. Furthermore, Ugur *et al.* (2009) reported that the chloroform and ethyl alcohol extracts of *Centaurea cariensis* Boiss. subsp. *niveo-tomentosa* (Hub.-Mor.) Wagenitz exhibited strong antibacterial activities on many resistant bacteria, especially Staphylococcus strains [21].

Some authors focused on the antimicrobial activity of the essential oil of *Centaurea* species, for example, Yayli *et al.*

(2005) investigated the antimicrobial effect of the essential oils from two *Centaurea* species (*Centaura appendicigera,* K. Koch. and *Centaura helenioides,* Boiss & Hausskn.) and demonstrated a moderate antibacterial activity on Gram-positive and Gram-negative bacteria [16]. Similar results were obtained for the essential oils of *Centaura sessilis* Willd. and *Centaura armena* Boiss. The essential oil of *Centauara aladaghensis* Wagenitz had an antibacterial effect against seven human pathogenic microorganisms [36]. However, the essential oils of *Centaura nicaeensis* All. and *Centaura parlatoris* Heldr. showed low activity on 14 selected microorganisms [37].

Although many studioes on the antifungal activity of *Centaurea* taxa can be found in the literature [27, 28, 38], the presented study is the first record of the antimicrobial activity of the aerial parts of extracts of *C. Babylonica,* and in which a moderate antimicrobial activity was observed against the bacteria and yeast tested. Among the extracts, acetonic extract was the most effective extract against *C. albicans* with a 14 mm inhibition zonen whereas hexane and chloroform extracts were less effective extracts, with a nine mm inhibition zone (Tab. 1). Cansaran *et al.* reported that the ethyl acetate extract of *C. cankiriense* showed weak activity profile in *C. albicans* [35]. They reported 12 and 14 mm inhibition zones against *C. albicans* with the plant extracts from flower and stem part of the plant, respectively. Similarly, ethyl acetate extract also showed 12 mm inhibition zone against *C. albicans* in the presented study. In contrast to the reults related with *C. babylonica* and *C. cankiriense*, Yayli *et al.* reported no antifungal activity of two Centaurea species *Centaurea sessilis* and *Centaurea armena* against *C. albicans* and *C. tropicalis* [16].

Several studies investigated the effects of different *Centaurea* species on cytotoxicity of different cancer cell lines. For example, in the studies of Zater *et al.*, raw chloroform extract of *Centaura diluta* subsp. *algeriensis* were investigated for cytotoxicity, and showed that the viability of A549 cells was reduced with the IC_{50} value of 21 µg/ml [39]. In contrast to the above result, in the current study it was found that the IC_{50} value of nine µg/ml on the same cell line, indicating that chloroform extract of *C. babylonica* (L.) L. is more effective against A549 cells than that of *C. diluta* subsp. *algeriensis*. In addition, crude methanolic and aqueous extracts of three different *Centaurea* species, *C. calcitrapa*, *C. ptosimopappa* and *C. Spicata,* were evaluated for cytotoxic activity against HeLa and Vera cells by Erol-Dayi *et al.* [40]. They observed the highest cytotoxicity against both cell lines with <100 µg/mL IC_{50} value of *C. calcitrapa* methanolic extract. Methanolic extracts of the other two species exhibited cytotoxicity with the IC_{50} value of ≥ 250 µg/mL. However, the IC_{50} value of aqueous extract was higher than 1,000 µg/mL for *C. ptosimopappa* and *C. spicata*. Similarly, in the current study, it was found that aqueous extract exerted the least antiproliferative effect (Fig. 1), whereas methanolic extract of *C. babylonica* (L.) L. was the least effective extract for cytotoxicity of A549 cells with the IC_{50} value of 230 µg/mL, compared to other the extracts (Fig. 2).

One recent study examined the anticancer activity of *C. babylonica* (L.) on Glioma, A549, and MCF-7 cells [27], in which there occurred a cytotoxic effect against these cell lines, but did not explain the IC_{50} value. Sekerler *et al.* investigated the anticancer effect of chloroform extracts of five different *Centaurea* species against HepG2 cells, and found that *C. cuneifolia* Sm. (CCC) was the most effective extract with a 0.002 µg/mL IC50 value [41]. Artun *el al*. also reported the cytotoxic activities of methanolic plant extracts, including *C. nerimaniae* and *C. antiochia*. Two *Centaruea* species displayed cytotoxic activity against HeLa cells with LC_{50} values of 253µg/mL; 427µg/ml and Vero cells with LC_{50} values of 194µg/mL and 1,000µg/ml, respectively [42]. Similarly, the current study indicates that methanolic extract of *C. babylonica* exhibited cytotoxicity with a similar LC_{50} value of 239 µg/mL. However, it was found that chloroform, ethyl acetate, and aceton extracts of *C. babylonica* L. were the most effective extracts with the IC_{50} values of 9, 33, and 36µg/mL on cytotoxicity of cancer cell lines. These findings indicate that plant extracts from the same genus or from the same species exert cytotoxicity at different levels, depending on the type of solvents used for extraction, type of cell lines tested, and chemical profiles of the plants.

It is known that plant extracts or essential oils have a very different mode of action in eukaryotic and bacterial cells. For bacterial cells, they show strong bactericidal properties, while in eukaryotes, they modify apoptosis and differentiation, post-translational modification of cellular proteins and expression of hepatic detoxifying enzymes. Therefore, plant extracts may induce very different effects in prokaryotes and eukaryotes [43]. In the presented study, GC-MS analysis indicated the presence of six different volatile compounds and one unidentified compound in acetone extract. All these substances were first identified for *C. babylonica*. Diacetone alcohol was the main constituent (54%) while tetradecamethyl cycloheptasiloxane was less frequent compound of the extract. Köse *et al.* reported that hexadeconoic acid (28.9 %) and dodecanoic acid (22.8 %) were the main constituents of the oil of *Centaura paphlagonica* [44]. In the study of Belgaty, 1-Hexanol, 2 ethyl (70 %) was the main constituent of ether extracts of *Centaura scoparea* [45]. It was reported that ethanolic extracts exhibited promising anti-tumour activity against HeLa, Hep-G2, HCT-116 and MCF-7 cell lines. Even though sesquiterpene lactone extract of *C. scoporea* did not cause antineoplastic activity against HeLa cells, this extract was more effective than ethanol extracts on the other cell lines. However, acetone extract of *C. babylonica* (L.) L. exerted similar antiproliferative activity against A549, HeLa, PC-3 and MCF-7 cells after treatment with 60 µg /mL extract for 72 hr. In future studies, isolated pure compounds may be required to reveal the most potent substance of acetone extract for cytotoxicity on cancer cell lines.

CONCLUSIONS

The study proved that *C. babylonica* (L.) L. exhibited a strong antimicrobial and antiproliferative activity *in vitro*. These findings suggest that *C. babylonica* (L.) L. have a good potential to be used as the source of natural antimicrobial and antiproliferative substances for a new drug. Further studies are needed to isolate and characterize each active compound responsible for the antimicrobial and/or antiproliferative activities.

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