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Original article

Cytotoxic and Antibacterial Activities of *Centaurea cadmea* Boiss.

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The cytotoxic activity of the extracts obtained from roots and the aerial parts of Centaurea cadmea Boiss. (Asteraceae) were analyzed by cell proliferation assay using WST-1 reagent against three human cancer cell lines; HeLa, A549 and U20S and one non-cancer cell line; 293HEK. The chloroform extract of the aerial parts of the plant exhibited inhibitory activities against all cell lines (IC50: 14.24-43.10 µg/mL). The antibacterial activity of the extracts were tested against four gram negative (Escherichia coli ATCC 23999, Pseudomonas aeruginosa ATCC 27853, Salmonella typhimurium CCM 5445 and Klebsiella pneumoniae CCM 2318 and four gram positive (Staphylococcus aureus ATCC 6538/P, S. epidermidis ATCC 12228, Enterococcus faecalis ATCC 29212 and Bacillus cereus ATCC 7064) bacteria strains by broth dilution method. The chloroform extract of the aerial parts of the plant showed strong activity on E. faecalis (8 µg/mL) and B. cereus (16 µg/mL).

Key words: *Centaurea cadmea*, Antibacterial activity, Cytotoxic activity.

Centaurea cadmea Boiss.'in Sitotoksik ve Antibakteriyal Aktiviteleri

Centaurea cadmea Boiss. (Asteraceae) kök ve topraküstü kısımlarından elde edilen ekstrelerin sitotoksik aktiviteleri, WST-1 reaktifi kullanılarak üç insan kanser hücre hattı; HeLa, A549 ve U20S ve bir kanser olmayan hücre hattı; 293HEK üzerinde, hücre proliferasyon testi ile analiz edilmiştir. Topraküstü kısımların kloroform ekstresi tüm hücre hatlarında aktif bulunmuştur (IC50: 14.24-43.10 µg/mL). Ekstrelerin antibakteriyel aktivitesi dört gram negatif (Escherichia coli ATCC 23999, Pseudomonas aeruginosa ATCC 27853, Salmonella typhimurium CCM 5445 ve Klebsiella pneumoniae CCM 2318) ve dört gram pozitif (Staphylococcus aureus ATCC 6538/P, S. epidermidis ATCC 12228, Enterococcus faecalis ATCC 29212 ve Bacillus cereus ATCC 7064) bakteri üzerinde broth dilüsyon metodu ile test edilmiştir. Toprak üstü kısımların kloroform ekstresi, E. faecalis (8 µg/mL) ve B. cereus (16 µg/mL) üzerinde güçlü etki göstermiştir.

Anahtar kelimeler: *Centaurea cadmea,* Antibakteriyal aktivite, Sitotoksik aktivite.

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INTRODUCTION

The genus *Centaurea* L. (Asteraceae) is represented by 199 taxons in Turkish flora and 61.1% of them are endemic (1-10). Various species of *Centaurea* are used as herbal remedies for their digestive, tonic, expectorant, antipyretic and antidiarrheal effects in traditional medicine (11). Pharmacological studies on some *Centaurea* species have reported anti-inflammatory, antimicrobial, antipyretic, cytotoxic and immunological activities (12).

Centaurea cadmea Boiss. belonging to section Phalolepis (Cass.) DC. (Asteraceae) with purple florets is an endemic taxon for Anatolia, growing wild in N, W & SW of Turkey (1). Phytochemical studies revealed the presence of a sesquiterpene lactone, ivalin, together with eupatorin, 5-hydroxy-3',4',6,7-tetramethoxyflavone, β-sitosterol and β-sitosterol-3-*O*-β-D-glucopyranoside from the $CHCl_3$ and MeOH extracts of aerial parts of *C. cadmea* (13). Essential oil analysis was also reported for the plant (14).

In vitro anti-inflammatory, antioxidant, antiprotozoal and antifungal activities of the aerial parts of *C. cadmea* extracts have been previously studied (15, 16). Formerly, we have reported the cytotoxic and antibacterial effects of the roots of *C. cadmea* as a poster presentation (17). As a continuation of this study, aerial parts of the plant was planned to be searched for the same activities. So, the present study aims to investigate the cytotoxic and antibacterial activities of extracts obtained from roots and the aerial parts of *C. cadmea*. Cytotoxic activity was performed by cell proliferation assay using WST-1 reagent against three human cancer cell lines; U2OS (human osteocarcinoma), (adenocarcinoma), HeLa (human cervical carcinoma) and one non-cancer cell line; 293HEK (human embryonic kidney). The antibacterial activity of the extracts were tested against four gram negative (*Escherichia coli* ATCC 23999, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* CCM 5445 and *Klebsiella pneumoniae* CCM 2318) and four gram positive (*Staphylococcus aureus* ATCC 6538/P, *S. epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212 and *Bacillus cereus* ATCC 7064) bacteria strains by broth dilution method.

EXPERIMENTAL

Plant material

Centaurea cadmea Boiss. was collected from Denizli, Evrantepe, 1512 m, in June 2004 (37° 41' 18.6''N; 29° 00' 07'E) and identified by Prof. Dr. Ozcan Secmen, from Section of Botany, Department of Biology, Faculty of Science, Ege University, Izmir, Turkey and a voucher specimens were deposited in the Herbarium of Ege University, Faculty of Pharmacy, Izmir, Turkey (IZEF 5670).

Extraction and isolation

Dried and powdered roots (200 g) were extracted sequentially with chloroform and methanol and the aerial parts (600 g) were extracted also sequentially with *n*-hexane, chloroform and methanol (3x10 mL/g, for each), sonicated at room temperature for 24h, and then filtered. All solvents were analytical grade and obtained from Sigma Aldrich. The combined extracts were evaporated under reduced pressure to dryness at 40 ^{0}C.

Cytotoxic activity

The cytotoxic activity was analyzed by cell proliferation assay. U2OS (human osteocarcinoma), A549 (human lung adenocarcinoma), HeLa (human cervical carcinoma) and non-tumoral 293HEK (human embryonic kidney) cell lines were cultured in DMEM supplemented with L-glutamine (2 mmol/L), 100 U/mL penicillin, 100 µg/mL streptomycin and 10% fetal bovine serum. In order to perform cytotoxicity assay, 5.000 cells were seed in 96 well dishes and 24 hours later samples were added in various concentrations (500, 250, 100, 50 mg/mL). Forty-eight hours after drug exposure, cell viability was measured using WST-1 cell proliferation reagent (Roche) according to the manufacturer instructions (18). All measurements were performed in triplets.

Antibacterial activity

Antibacterial acitivity tests of *C. cadmea* extracts were evaluated by using Minimum Inhibitory Concentration (MIC) measurements (19, 20). The MIC values were determined for eight bacterial strains [*Escherichia coli* (ATCC 23999), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella thyphimurium* (CCM 5445), *Klebsiella pneumoniae* (CCM 2318), *Staphylococcus aureus* (ATCC 6538-P), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus cereus* (ATCC 7064), and *Enterococcus faecalis* (ATCC 29212)].

Those strains were inoculated on Mueller-Hinton broth (Difco) and incubated at 37 ± 0.1°C for 24 h. The inoculated strains were prepared from 24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity and diluted 1:100 (v/v) in Mueller-Hinton broth. Dilution series of the compounds were prepared in test tubes and transferred to the broth in 96-well micro titer plates. Final concentrations were 256 to 0.25 µg/mL in the medium. The last well contained 100 mL of nutrient broth without compounds and 10 mL of the inoculums on each strip was used as a negative control and Gentamycin (Sigma Aldrich) was used as a positive control. All plates were covered with a sterile plate sealer and incubated at 37°C for 24 h.

After incubation, MIC values were detected by adding 50 mL of 0.5% triphenyl tetrazolium chloride (TTC, Fluca) aqueous solution and they were defined as the lowest concentration that inhibited visible growth as indicated by the TTC reduction. In the presence of bacterial growth by reduction reactions, TTC changed the color of microorganisms from colorless to red. This provided clearly defined and easily readable endpoints. All of the assays were performed in triplicate.

RESULTS AND DISCUSSION

Yields of *n*-hexane, $CHCl_3$ and MeOH extracts obtained from roots and the aerial parts of *C. cadmea* is shown in Table 1. Extracts then were tested for their potential cytotoxic and antibacterial activities.

The cytotoxic activity results are presented in Table 2. The *n*-hexane and chloroform extracts of the aerial parts of the plant exhibited inhibitory activities against all cell lines. The strongest activity was observed on HeLa (IC_{50}: 14.24 µg/mL) by chloroform extract of aerial parts. But it is also active against non-cancerous cell line 293 HEK, which is used for detecting selectivity. Hexane extract had weak activity on U2OS and showed moderate effect on the other cell lines (43.05 to 79.03 µg/mL). MeOH extract of aerial parts and the extracts of roots were not exhibited cytotoxic activity.

In a previous report we have evaluated *in vitro* cytotoxic properties of extracts of five *Centaurea* species and the strongest effect has been determined on chloroform extract of *C. polyclada* on KB (human epidermal carcinoma, oral) and BT-549 (breast ductal carcinoma) cell lines (33 and 30 µg/mL, respectively) (21). Most of the studies involved the activities of pure compounds isolated from various extracts of *Centaurea* species. Especially isolated sesquiterpenes and flavonoids were found to be responsible for cytotoxic properties (22-26).

In our earlier study, we have isolated a sesquiterpene lactone, ivalin, from the $CHCl_3$ extract of aerial parts of *C. cadmea* (13). The activity of chloroform extract of aerial parts of the plant may be due to the presence of ivalin that is known as a potent cytotoxic compound on several tumor cell lines (27).

Antimicrobial activities were tested against 8 bacteria strains by using NCCLS method. Results are shown in Table 3. The chloroform extract of the aerial parts of the plant showed strong activity on *E. faecalis* (8 mg/mL) and *B. cereus* (16 mg/mL) with concentrations more or equal to the standart antibiotic gentamycin. MeOH extract also had a strong effect on these strains (16 mg/mL, both). Hexane extract of the aerial parts and MeOH extract of the roots have weak activity against all tested microorganisms (64-256 mg/mL).

Table 1. Yields of various extracts of *C. cadmea*.

	Obtained extracts (g)	Yield of extracts (% of dry weight)
Root $CHCl_3$	1.5	0.75
Root MeOH	5.36	2.68
Aerial parts *n*-hexane	7.8	1.3
Aerial parts $CHCl_3$	23.58	3.93
Aerial parts MeOH	53.51	8.91

Antimicrobial activities were tested against 8 bacteria strains by using NCCLS method. Results are shown in Table 3. The chloroform extract of the aerial parts of the plant showed strong activity on *E. faecalis* (8 mg/mL) and *B. cereus* (16 mg/mL) with concentrations more

Table 2. Cytotoxic activities of *C. cadmea* extracts (IC_{50}, µg/mL).

	U2OS	A549	HeLa	293HEK
Root $CHCl_3$	NA	NA	NT	NA
Root MeOH	>100	NA	NT	NA
Aerial parts *n*-hexane	>100	79.03	50.25	43.05
Aerial parts $CHCl_3$	43.10	35.00	14.24	23.50
Aerial parts MeOH	NA	NA	NA	NA

NT: Not tested, NA: Not active at 500 µg/mL concentration.

or equal to the standart antibiotic gentamycin. MeOH extract also had a strong effect on these strains (16 mg/mL, both). Hexane extract of the aerial parts and MeOH extract of the roots have weak activity against all tested microorganisms (64-256 mg/mL).

There are several reports on antimicrobial activities of different *Centaurea* species from Turkey. Various extracts of six *Centaurea* taxons (*C. pseudoscabiosa* subsp. *glechnii, C. spicata, C. glastifolia, C. salonitana, C. balsamita* and *C. behen*) had been evaluated for

Table 3. Antibacterial activities of *C. cadmea* extracts (µg/mL).

Microorganisms	Root $CHCl_3$	Root MeOH	Aerial parts *n*-hexane	Aerial parts $CHCl_3$	Aerial parts MeOH	Gentamycin
Escherichia coli ATCC 23999	> 256	128	128	128	128	1.0
Staphylococcus aureus ATCC 6538/P	> 256	256	256	128	128	1.0
S. epidermidis ATCC 12228	> 256	256	128	128	128	1.0
Salmonella typhimurium CCM 5445	> 256	64	128	128	128	1.0
Bacillus cereus ATCC 7064	> 256	128	64	16	16	4.0
Klebsiella pneumoniae CCM 2318	> 256	128	128	128	128	4.0
Enterococcus faecalis ATCC 29212	> 256	128	64	8	16	16.0
Pseudomonas aeruginosa ATCC 27853	> 256	64	128	128	128	2.0

their antimicrobial activities by disc difusion method. Ethanol and ethyl acetate extract of *C. glastifolia* have showed strong activity on *Staphylococcus epidermidis* and *Proteus mirabilis* when compared with ciprofloxacin (28). In another report, water extract of *C. helenoides* had shown to have significant effect against *Branhamella catarrhalis*, *Staphylococcus aureus* and *Helicobacter pylori* (29).

E. faecalis can cause life threatening gastrointestinal infections in humans which

has high levels of antibiotic resistance and *B. cereus* is responsible for a minority of foodborne illnesses, causing severe nausea, vomiting and diarrhea (30). *C. cadmea* may be a potential natural source for discovering new anti bacterial agents due to its remarkable activity on these pathogens.

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