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Turk J. Pharm. Sci. 6 (2), 125-134, 2009

Original article

ANTIVIRAL, ANTIBACTERIAL, AND ANTIFUNGAL ACTIVITIES OF *CENTAUREA TCHIHATCHEFFII* EXTRACTS

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Abstract

In the present study, nine extracts of Centaurea tchihatcheffii were screened for their in-vitro antiviral, antibacterial and antifungal activities. Antibacterial and antifungal activities were evaluated against both standard and the isolated strains of Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis as well as Candida albicans, Candida parapsilosis by broth microdilution method. Susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS). Furthermore, Herpes simplex virus Type-1 (HSV-1) and Parainfluenza-3 virus (PI-3 virus, were employed for antiviral assessment of the extracts by using Madin-Darby Bovine Kidney and Vero cell lines. Ampicilline, gentamicin, ofloxacin, levofloxacin, ketoconazole, fluconazole, acyclovir and oseltamivir were used as the control agents. As our knowledge, this is the first report on these C. tchihatcheffii extracts that were evaluated for their antimicrobial activities. According to the data obtained, all the extracts appear to have antibacterial activity tested against standard Gram-negative and Gram-positive bacteria ranging from minimum inhibitory concentrations of 2 to 16 µg/mL, besides they have have antibacterial activity at 32->128 µg/mL concentrations to isolated strains. Notable activity was observed with the extracts against C. albicans and C. parapsilosis fungi at 4 µg/mL and 8 µg/mL concentrations, respectively.The data showed that water-chloroform interpahse (U_{-1}), chloroform (U_{-2}), and ethyl acetate (U_{-3}), have antiviral activity against both DNA (HSV-1) and RNA (PI-3) viruses.

Key words: *Antiviral, Antibacterial, Antifungal activity, Centaurea tchihatcheffii*

Centaurea tchihatcheffii Ekstrelerinin Antiviral, Antibakteriyel ve Antifungal Aktiviteleri

Bu çalışmada dokuz Centaurea tchihatcheffii ekstreleri in-vitro antiviral, antibakteriyel ve antifungal aktiviteleri yönünden tarandı. Antibakteriyel ve antifungal aktiviteler standart ve izole suşlardan Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacter baumanni, Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis ile Candida albicans ve Candida parapsilosis karşı sıvı mikrodilüsyon yöntemiyle değerlendirildi. Duyarlılık testi Clinical and Laboratory Standards Institute (CLSI; önceki adıyla NCCLS)'e göre yapıldı. Buna ek olarak, ekstrelerin antiviral aktivitelerinin değerlendirmesinde Madin-Darby Bovine Kidney ve Vero hücre kültürü Herpes simplex virus Type-1 (HSV-1) ve Parainfluenza-3 virus (PI-3) kullanılmıştır. Ampisilin, gentamisin, ofloksasin, levofloksasin, ketokonazol, fluknazol, asiklovir ve oseltamivir kontrol ajan olarak kullanılmıştır. Bilgimiz ölçüsünde, bu araştırma C. tchihatcheffii ekstrelerinde yapılan ilk antimikrobiyal aktivite çalışmasıdır. Elde ettiğimiz verilere göre tüm ekstreler test edilen Gram-negatif ve Gram-pozitif bakterilere 2 -16 µg/mL minumum konsantrasyon aralığında antibakteriyel etkili iken 32->128 µg/mL konsantrasyon aralığında izole suşlara aktif bulunmuştur. Ekstreler, C. albicans ve C. parapsilosis funguslarına karşı sırasıyla 4 µg/mL and 8 µg/mL konsantrasyonlarında etkili bulunmuştur. Antiviral aktivite sonuçlarına göre su-kloroform interfaz ekstresi (U_{-1}), kloroform (U_{-2}), etil asetat (U_{-3}), hem DNA (HSV-1) ve hem de RNA (PI-3) viruslarına kartşı aktif bulunmuştur.

Anahtar kelimeler: *Antiviral, Antibakteriyel, Antifungal, Centaurea tchihatcheffii*

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INTRODUCTION

Centaurea flora is represented with nearly 700 species in the Mediterranean region, of which about 178 species are located in Turkey (1). In addition to beautiful flowers, *Centaurea* genus (Asteraceae) contains medicinal plants that have significant place in traditional medicine. *Centaurea tchihatcheffii* Fisch et. Mey (Mediterranean knapweed) is one of the Mediterranean endemic plants, known for its appealing flowers. A variety of Centaurea species have been widely used single or mixed in Turkish folk medicine for anti-inflammatory, antidiabetic, antidiarrhetic, antirheumatic, antipyretic, hypotensive, digestive, diuretic, and antimicrobial effects (2, 3).

The emergence of organisms resistant to nearly all classes of antimicrobial agents has become a serious public health concern (4, 5). The resistance ratios in the countries that have started to use some antimicrobials clinically have been explained by several factors. The resistance mostly in isolated strains has been explained by the common use of some antimicrobials clinically (5-8).

Traditional healers have long used plants to prevent or cure infectious diseases. A number of these agents seem to have structures and modes of action that distinct from those of the antibiotics in current use. So, it is worthwhile to study plants and plant products for activity against microorganisms. One approach that has been used for the discovery of biological active agents from plants based on the evaluation of traditional medicinal plant extracts (8-14).

In the present study, our objective is to report antibacterial, antifungal and antiviral properties of the water-chloroform interpahse (U_{-1}), chloroform (U_{-2}), ethyl acetate (U_{-3}), water (U_{-4}), n-buthanol (U_{-5}), ethylacetate precipitate (U_{-6}), folia (U_{-7}), flower (U_{-8}) and stem (U_{-9}) extracts of *Centaurea tchihatcheffi* against both standard and isolated strains of Gram-negative bacteria (*Eschericia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*) and Gram-positive bacteria (*Staphylococcus aureus, Enterococcus faecalis*), as well as fungi (*Candida albicans*, *Candida parapsilosis*) by broth microdilution method. In addition, *Herpes simplex* virus Type-1 (*HSV*-1), a DNA virus, and *Parainfluenza-3* virus (*PI*-3), a RNA virus, were utilized by using Madin-Darby Bovine Kidney and Vero cell lines for the antiviral assessment of the extracts.

EXPERIMENTAL

Plant material

Centaurea tchihatcheffii (Asteraceae) was collected from Gölbası district of Ankara in May, 2007. Voucher specimen was located in the Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey (Herbarium Number 2592).

Preparation of extracts

The aerial parts of the plant were airdried. The dry powdered plant materials were extracted with water first and then this extract was fractionated with chloroform, ethylacetate, n-buthanol respectively (The solvents were purchased from d.o.p, HPLC grade, unless otherwise stated). Chloroform-water interface was named as $U_{-7,}$ chloroform fraction named as U_{-2}, ethylacetate upper phase U_{-3}, water U_{-4}, buthanol U_{-5} and ethylacetate precipitate was named as $U_{-6.}$10 g of dried and powdered folia (U_{-7}), flores (U_{-8}) and stem (U_{-9}) of *Centaurea tchihatcheffii* were extracted with ethanol (80%) separately, at room temperature overnight (2x200 ml). Each of combined ethanolic extract was evaporated under reduced pressure. These dried extracts were used for further assays.

Microbiological studies

Preparation of test materials

These several extracts prepared from the aerial parts of *Centaurea tchihatcheffi* were dissolved in ethanol:hexane (1:1) by using 1% Tween 80 solution at a final concentration of 512 μg/mL and sterilized by filtration using 0.22μm Millipore (MA 01730, USA) and used as the stock solutions. Reference antibacterial agents of ampicillin (AMP; Faco), gentamisin (GM; Fako), ofloxacin (OFX; Hoechst Marion Roussel), levofloxacin (LVX; Faco), reference antifungal agents of ketoconazole (KET; Bilim) and fluconazole (FLU; Pfizer), were obtained from their respective manufacturers and dissolved in phosphate buffer solution (ampicillin, pH: 8.0; 0.1 mol/ml), dimethylsulphoxide (ketoconazole), or in water (gentamicin, ofloxacin, levofloxacin, fluconazole). The stock solutions of the agents were prepared in medium according to the Clinical and Laboratory Standards Institute (15).

Microorganisms and inoculum preparation

Antimicrobial activity tests were performed using standard strains: *E. coli* American type culture collection (ATCC) 35218, *P. aeruginosa* ATCC 10145, *P. mirabilis* ATCC 7002, *K. pneumoniae* Culture collection of Refik Saydam Central Hygiene Institute (RSKK) 574, *A. baumannii* RSKK 02026, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *B. subtilis* ATCC 6633, *C. albicans* ATCC 10231 and *C. parapsilosis* ATCC 22019. Antimicrobial activity tests were also carried out using clinical isolates (*A. baumannii* and extended spectrum β-lactamase positive *E. coli*, *K. pneumoniae* and *P. mirabilis*, methicillin-resistant *S. aureus* (MRSA), *E. faecalis* and ceftriaxon-resistant *B. subtilis*) obtained from Department of Microbiology, Faculty of Medicine, Gazi University.

Mueller Hinton Broth (MHB; Difco) and Mueller Hinton Agar (MHA; Oxoid) were used for growing and diluting of the bacterial suspensions. The synthetic medium RPMI-1640 with L-glutamine was buffered to pH: 7 with 3-[N-morpholino]-propansulfonic acid and culture suspensions were prepared as described by Özçelik et al. 2006 (16). The microorganism suspensions used for inoculation were prepared at 10^5cfu (colony forming unit/mL) by diluting fresh cultures at McFarland 0.5 density (10^8cfu/mL). Suspensions of bacteria and fungi were added in each well of the diluted extracts, density of 10^5cfu/mL for fungi, and for bacteria. The fungi suspension was prepared by the spectrophotometric method of inoculum preparation at a final culture suspension of 2.5×10^3cfu/mL (17).

Antibacterial and antifungal tests

The microdilution method was employed for antibacterial and antifungal activity tests. Media were placed into each 96 wells of the microplates. Extract solutions at 512 μg/mL were added into first rows of microplates and two fold dilutions of the compounds (256-0.125 μg/mL) were made by dispensing the solutions to the remaining wells. 10μl culture suspensions were inoculated into whole the wells. The sealed microplates were incubated at 35°C for 24 h and 48 h in humid chamber. The lowest concentration of the extracts that completely inhibit macroscopic growth was determined and minimum inhibitory concentrations (MICs) were reported (18).

Cytotoxicity and antiviral tests

Vero cell line (African green monkey kidney) used in this study was obtained from Department of Virology, Faculty of Veterinary, Ankara University (Ankara-Turkey). The culture of the cells were grown in EMEM (Eagle's Minimal Essential Medium; Seromed; Biochrom; Berlin; Germany) enriched with 10% fetal calf serum (Biochrom, Germany), 100

mg/mL of streptomycin and 100 IU/mL of penicillin in a humidified atmosphere of 5% carbondioxyde (CO_2) at 37°C. The cells were harvested using Trypsin solution (Bibco Life Technologies, UK).

Media (EMEM) were placed into each 96 wells of the microplates (Greiner^R; Essen, Germany). Stock solutions of the extracts were added into first raws of microplates and two-fold dilutions of the extracts (51.2-0.012 µg/mL) were made by dispensing the solutions to the remaining wells. Two-fold dilution of each material was obtained according to Log_2 on the microplates. Acyclovir (Biofarma Co.) and oseltamivir (Roche Co.) were used as the control agents. Strains of *HSV*-1 and *PI*-3 titers were calculated as tissue culture infecting dose ($TCID_{50}$) and inoculated into whole wells. The sealed microplates were incubated in 5% CO_2 at 37°C for 2h to detect the possible antiviral activities of the samples. Following incubation, 50 µl of the cell suspension of 300.000 cells/ mL which were prepared in EMEM together with 5% fetal bovine serum were put in each well and the plates were incubated in 5% CO_2 at 37°C for 48 h. At the end of this period, the cells were evaluated using cell culture microscope by comparison with treated-untreated control cultures and with acyclovir and oseltamivir. Consequently, maximum Cytopathogen Effect (CPE) concentrations as the indicator of antiviral activities of the extracts were determined (16). In order to determine the antiviral activity of the extracts, *Herpes simplex* virus Type-1 (*HSV*-1), as representative of DNA viruses and *Parainfluenza*-3 virus (*PI*-3), as representative of RNA viruses, were used. The test viruses were obtained from Department of Virology, Faculty of Veterinary, Ankara University.

The maximum non-toxic concentrations (MNTCs) of each samples were determined by the method described previously by Özçelik et al. 2005 (19) based on cellular morphologic alteration. Several concentrations of each sample were placed in contact with confluent cell monolayers and incubated in 5% CO_2 at 37°C for 48 h. After the incubation period, drug concentrations that are not toxic to viable cells were evaluated as nontoxic and also they were compared with nontreated cells for confirmation. The rows that cause damage in all cells were evaluated as toxic in the present concentration. In addition, maximum drug concentrations that did not affect the cells were evaluated as non-toxic concentration. MNTCs were determined by comparing treated and controlling untreated cultures (19).

Control test

In order to exclude any antimicrobial, antifungal and antiviral influence of ethanol:hexane (1:1) and 1% Tween 80 solution , this dissolving solvent also was screened under identical conditions. The final concentration of ethanol:hexane (1:1) + 1% Tween 80 solution , which are known as ineffective on microorganisms, and pure microorganisms, as well as pure media were used as control wells. No effect of dissolving solvent was recorded to take into consideration.

RESULTS AND DISCUSSION

In order to evaluate the antibacterial, antifungal, and antiviral activities of 3 different botanical parts and six different extracts from aerial part containing different phytochemical compounds of *C. tchihatcheffii* were searched against human pathogens. Nine different extracts of *C. tchihatcheffii* against five standard and isolated Gram-negative bacteria, *E. coli*, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, *A. baumannii*, in addition to two Gram-positive bacteria *E. faecalis* and *S. aureus* were tested. Besides, standard strains of *C. albicans*, and *C. parapsilosis* were employed for evaluation of antifungal activity. The minimum inhibitory concentrations (MICs) were determined for the extracts as well as for the reference compounds

(Ampicilline, gentamicin, ofloxacin, ketoconazole, fluconazole) under identical conditions to compare their activities (Table 1).

The results of antibacterial and antifungal evaluation of the fractionated extracts from the aerial part, water-chloroform interpahse (U_{-1}), chloroform (U_{-2}), ethyl acetate (U_{-3}), water (U_{-4}), n-buthanol (U_{-5}), ethylacetate precipitate (U_{-6}), and ethanolic extracts of folia (U_{-7}), flower (U_{-8}) and stem (U_{-9}) obtained from *Centaurea tchihatcheffii* are presented in Table 1. According to the data obtained, all the extracts appear to have antibacterial activity against Gram-negative and Gram--positive bacteria ranging from minimum inhibitory concentrations of 2 -16 μg/mL, besides 32-128 μg/mL concentrations to isolated strains.

As shown in Table 1, the antibacterial activity on Gram-negative bacteria displayed at concentrations ranging 2-8 μg/mL, while isolated strains had remarkable antibacterial activity against isolated strains at 64->128 μg/mL concentrations, which is close to the effective concentrations of the reference ampicillin. On the other hand, the effects of the extracts were seemed to be less active than ofloxacin and levofloxacin at the same concentrations.

Moreover, all of the extracts screened have exerted more inhibitory effect on control strains than the isolates. Some of the extracts; chloroform (U_{-2}), and folia (U_{-7}) seemed to be more effective than their counterparts at 2 -4 μg/mL concentration ranges against Gram-negative (*E. coli*, *P. aeruginosa, A. baumannii*) and Gram-positive (*E. faecalis*, *S. aureus*) standard strains (Table 1). As for effects against isolated strans, folia (U_{-7}) seemed to be more effective than their counterparts at 32 -64 μg/mL concentration ranges (Table 1).

Notable activity was observed with the extracts against *C. albicans* and *C. parapsilosis* fungi at 4 μg/mL and 8 μg/mL concentrations, respectively.

As shown in Table 2, the data obtained from antiviral activity screening showed that water-chloroform interphase (U_{-1}), chloroform (U_{-2}), ethyl acetate (U_{-3}), had antiviral activity against DNA (*HSV*-1) and RNA (*PI*-3) viruses (Table 2). Also, water (U_{-4}), n-buthanol (U_{-5}), ethylacetate precipitate (U_{-6}) and folia (U_{-7}) were active against DNA viruses at 8 μg/ml concentrations. Flower (U_{-8}; 8-2 μg/mL; concentration ranges), and stem ($U_{-9;}$ 16-4 μg/mL; concentration ranges) were found to be active against DNA viruses. Besides, flower and stem (U_{-8}, U_{-9}) were found to be active against RNA viruses at 2 μg/mL and 1 μg/mL concentration, respectively. The rest of the extracts showed no activity against RNA viruses (*PI*-3) (Table 2.).

Table 1. Antimicrobial activity of C. tchihatcheffii extracts and MİC (µg/ mL) values against standard and isolated microorganisms

Extracts	*Microorganisms*																	
	E. coli		***P. aeruginosa***		***P. mirabilis***		***K. pneumonae***		***A. baumannii***		***S. aureus***		***E. faecalis***		***B. subtilis***		***C. albicans*** ATCC, 10231	***C. parapsilosis*** ATCC, 22019
	ATCC 35218	Isolated strain ESβL+	ATCC 10145	Isolated strain	ATCC 7002	Isolated Strain ESβL+	RSKK 574	Isolated Strain ESβL+	RSKK 02026	Isolated strain	ATCC 25923	Isolated strain MRSA	ATCC 29212	Isolated Strain	ATCC 6633	Isolated strain		
U_{-1}	**8**	64	**4**	32	**8**	64	**8**	64	**8**	64	**2**	64	**2**	64	**2**	64	**4**	**8**
U_{-2}	**4**	128	**2**	32	**4**	128	**8**	128	**2**	64	**4**	>128	**8**	128	**8**	16	**4**	**8**
U_{-3}	**8**	64	**4**	32	**8**	64	**8**	64	**8**	64	**2**	64	**2**	64	**2**	64	**4**	**8**
U_{-4}	16	64	**4**	64	**16**	64	**8**	128	**8**	64	**4**	>128	**8**	128	**8**	64	**4**	**8**
U_{-5}	16	64	**4**	64	**16**	64	**8**	128	**8**	64	**4**	>128	**8**	128	**8**	64	**4**	**8**
U_{-6}	16	64	**4**	64	**16**	64	**8**	128	**8**	64	**4**	>128	**8**	128	**8**	64	**4**	**8**
U_{-7}	**4**	32	**4**	32	**4**	32	**4**	64	**8**	64	**2**	64	**2**	64	**2**	64	**4**	**8**
U_{-8}	**8**	64	**4**	32	**8**	64	**8**	64	**8**	64	**2**	64	**2**	64	**2**	64	**4**	**8**
U_{-9}	**8**	64	**4**	32	**8**	64	**8**	64	**8**	64	**2**	64	**2**	64	**2**	64	**4**	**8**
AMP	2	>128	-	-	2	>128	2	>128	2	>128	<0.12	>128	0.5	>128	0.12	0.5	-	-
GM	-	-	0.5	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OFX	0.12	0.5	1	64	<0.12	1	<0.12	0.5	0.12	64	0.25	64	1	32	-	-	-	-
LVX	<0.12	0.5	1	64	<0.12	1	<0.12	1	0.12	64	0.25	128	0.5	32	-	-	-	-
KET	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
FLU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	4

AMP: ampicilline; GM : gentamicine; OFX: ofloxasine; LVX: levofloxacin; KET; ketoconazole; FLU: fluconazole; -: Not done. ATCC: American type culture collection, RSKK: Culture collection of Refik Saydam Central Hygiene Institute, ESβL: extended spectrum β-lactamase enzyme positive, MRSA: methicillin resist *Staphylococcus aureus*, water-chloroform interpahse (U_{-1}), chloroform (U_{-2}), ethyl acetate (U_{-3}), water (U_{-4}), n-buthanol (U_{-5}), ethylacetate precipitate (U_{-6}), folia (U_{-7}), flower (U_{-8}) and stem (U_{-9}).

Table 2. Antiviral activity and Cytotoxicity of the *C. tchihatcheffii* extracts and references as MICs (µg/mL) values.

	MDBK Cells			Vero Cells		
	MNTCs (µg/ mL)	CPE inhibitory concentration (µg/ mL)		MNTCs (µg/ mL)	CPE inhibitory concentration (µg/ mL)	
Extracts		***HSV*-1**			***PI*-3**	
		Max.	Min.		Max.	Min.
U_{-1}	8	4	1	16	8	4
U_{-2}	16	8	2	16	8	4
U_{-3}	8	4	2	16	8	4
U_{-4}	16	8	-	32	-	-
U_{-5}	16	8	-	32	-	-
U_{-6}	16	8	-	32	-	-
U_{-7}	16	8	-	32	-	-
U_{-8}	32	8	2	8	2	-
U_{-9}	32	16	4	16	1	-
Acyclovir	16	16	<0.012	-		
Oseltamivir	-	-	-	16	16	<0.012

MNTCs: maximum non-toxic concentrations, CPE: cytopathogenic effect, -: No activity observed. water-chloroform interpahse (U_{-1}), chloroform (U_{-2}), ethyl acetate (U_{-3}), water (U_{-4}), n-buthanol (U_{-5}), ethylacetate precipitate (U_{-6}), ethanol ext. folia (U_{-7}), ethanol ext flower (U_{-8}), and ethanol ext stem (U_{-9})

A number of antimicrobial activity studies with different methods (disc diffusion or microdilution) against Gram-positive and Gram-negative bacteria had been reported that species of the genus *Centaurea* such as *C. chilensis, C. floccosa*, *C. hermannii*, *C. malacitana*, *C. melitensis*, *C. aspera, subsp. aspera*, *C. nicolai*, *C. sonchifolia* (20-26). In one study, dried chloroform extract of *Centaurea floccosa* demonstrated significant activity against Gram-positive bacteria *S. aureus*, *B. subtilis* and *S. epidermidis* than tested Gram-negative bacteria *E. coli* and *P. aeruginosa* with a disc diffusion test (20). It is reported that aerial parts of *Centaurea sonchifolia* demonstrated effect against *S. aureus* (21).

The antibacterial effects of flavonoids which were identified from *Centaurea virgata*, *C. kilea* and *C. inermis* were fount to be active against Gram-negative bacteria such as *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*, and *E.coli*, which are similar with our study.

In a previous study, several derivatives including cynidin, cyclo-tenolide, and sesquiterpen lactones such as costunolide, dehydrocostus, lactone, licnophplide, eremantolide with different structure from 6 *Centaurea* species were revealed for their antifungal activities. Since costunoilde and dehydrocostus had different structures, they displayed significant antifungal activies against *Cunninghemalla echinulata* (14). Differently; in our study antifungal activity was observed agains yeast like fungi (*C. albicans* and *C. parapsilosis*) at 4-8 µg/mL consentration ranges .

In another study, it had been reported that petroleum extract of *Centaurea hermanii* demonstrated antibacterial activity against *S. aureus* and chloroform extract showed significant activity against *C. albicans* and *C. glabrata* (22). Similarly, Sur-Altıner et al. used disc diffusion to investigate antibacterial and antifungal activity of petroleum ether, chloroform and ethanolic extract of *C. hermanii* and they found the extract were active against 6 yeast including *C. albicans* and *C. glabrata* (23). A study on the extract of aerial parts of *Centaurea nicolai* with modified agar diffusion test against fungi such as *Aspergillus niger*, *A. ochraceus*, *Penicillium ochrochloron*, *Trichoderma viride*, and *Cladasporium cladosporoides* was performed by Vajs et

al. In this study the extract was found to be active against whole tested fungi except *T. viride.* (24).

Furthermore, antiviral effects of *C. nigra* were demonstrated by bioassay-guided fractionation method against *HSV*-1, a DNA virus and *poliovirus*-II, a RNA virus (25). In this study, it was particularly observed that water-chloroform interpahse (U_{-1}), chloroform (U_{-2}), ethyl acetate (U_{-3}) ethanol ext flower (U_{-8}), and ethanol ext stem (U_{-9}) were active against both *HSV-1* and *PI-3* viruses.

Although antibacterial, antifungal and antiviral effects of *Centaurea* spp. were investigated in various studies, as our knowledge, it has not been studied before that the activity of these species confronted human pathogens. Further researches about on the isolation and identification of the active principle(s) of these effective extracts have been studying using chromatographical methods is in progress in our laboratory in order to discover new and potent plant originated antibacterial, antifungal and antiviral compound(s) and to determine the relationships between them.

ACKNOWLEDGMENT

The authors wish to thank Dr. T. Karaoğlu for his kind help to conduct antiviral tests.

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Received: 25.12.2008
Accepted: 02.07.2009

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