

Full Length Research Paper

***In vitro* antimicrobial and antiviral activities of the essential oil and various extracts of *Salvia cedronella* Boiss**

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The essential oil and various extracts obtained from *Salvia cedronella* Boiss have been evaluated for their possible *in vitro* antimicrobial and antiviral capacities. The GC-EIMS analysis of the essential oil was resulted in detection of 92 components representing 96.1% of the oil. Major components were 1,8-cineole, α -pinene, caryophyllene oxide and sabinene. In the case of antimicrobial activity, hexane and dichloromethane extracts did not show any effects. Methanolic extract and essential oil exhibited various degrees of activity against the tested microorganisms. The antiviral potential of the plant samples was screened in 2 model systems namely; reproduction of 2 influenza viruses in MDCK cells and of 2 herpes simplex viruses in MDBK cells. The methanol extract showed a good anti-influenza virus effect, the growth of both A/Weybridge and A/Aichi was reduced significantly. This extract also exhibited anti-herpetic activity. Amount of the total phenolics was very high in methanol extract. It was followed by dichloromethane. This extract has also been found to be rich in flavonoids. A positive correlation was observed between biological activity potential and amount of phenolic compounds of the extracts.

Key words: *Salvia cedronella*, antimicrobial, antiviral, essential oil, extract.

INTRODUCTION

The genus *Salvia*, with about 900 species throughout the world, as one of the most widespread members of the Lamiaceae family, produces many useful secondary metabolites including terpenes and phenolics and their derivatives that have been in the center of pharmacopoeias of many countries (Tepe et al., 2007). Actually, the name of this genus means "safe" or "well" as a latin word "*salvus*". Healing reputation of *Salvia* species can be traced back to Roman times and a well-known *Salvia* species, *S. officinalis* (sage), is credited for this (Könnemann, 1999).

Numerous studies have been conducted by several research groups concerning several biological activities

of some *Salvia* species, such as *S. officinalis* L. and *S. miltiorrhiza* Bunge. (Chinese Dansheng), proving their potential use in the treatment of coronary heart disease, cerebrovascular disease, hepatitis, hepatocirrhois, chronic renal failure, dysmenorrhea and neuroasthenic insomnia (Li, 1998; Lu and Foo, 2002).

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries. Plants still continue to be almost the exclusive source of drugs for the majority of the world's population. In western medicine substances derived from higher plants constitute ca. 25% of prescribed medicines and 74% of the 121 bioactive plant-derived compounds currently in worldwide use were identified via research based on leads from ethno medicine (Anyinam, 1995). Higher plants have been shown to be a potential

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source for the new antimicrobial agents. The screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases. A number of reports concerning the antibacterial screening of plant extracts of medicinal plants have appeared in the literature, but the vast majority has not been adequately evaluated (Sokmen et al., 1999). This is also particularly valid for the Turkish flora which has one of the most extensive floras in continental Europe with more than 9000 flowering plant species (Davis, 1965 - 1984). Owing to its strategic position, the accumulation of the knowledge of traditional medicine from the west and the east enabled this region to have a rich tradition in terms of the uses of medicinal plants (Gozler, 1993).

Phenolic compounds isolated from this genus have revealed as the excellent antimicrobial agents. The presence of phenolic acids, mainly caffeic acid and its metabolites and flavonoids in many *Salvia* species were discussed in details. Especially, rosmarinic acid (a-O-caffeoyl-3,4-dihydroxyphenyllactic acid) is of great interest as it has biological activities (Tepe, 2007). Antioxidant activities of the many members of the genus *Salvia* were reported elsewhere. Additionally, previous reports concerning the biological activities of *Salvia* species native to the Turkish flora confirm that this genus has great potential, especially in antioxidant systems, for the food and cosmetic industries (Tepe et al., 2004, 2005a, 2005b, 2006, 2007). Apart from phenolic constituents, determination of terpenes in the essential oils of *Salvia* species and their possible pharmacological activities are also of great interest (Lu and Foo, 2002).

Although *Salvia* species are abundant in the Turkish flora, as being represented by 88 species and 93 taxa and 45 of which are endemic (Guner, 2000), only biological activities and bioactive properties of a few are available in the literature while most being untapped. As far as our literature survey could ascertain, there is no study on *Salvia cedronella* in the literature. In one case, antioxidant potential and phenolic constituents of this species have only been reported (Yesilyurt et al., 2008). The aim of present study was to evaluate the *in vitro* antimicrobial and antiviral capacities of the essential oil and several extracts of *S. cedronella* (an endemic species in Turkey) and to determine the chemical composition of its essential oil for the first time.

MATERIAL AND METHODS

Plant material and preparation of the extracts

S. cedronella was collected from Burdur in Turkey in July 2007. Extracts of air-dried and ground plant materials were prepared by using solvents of varying polarity and the extraction protocol of each is given below. A portion (100 g) of dried plant material from *S. cedronella* was extracted with hexane (yield; 2.38%, w/w), followed by dichloromethane (yield; 3.08%, w/w) and methanol (13.80%, w/w) in a Soxhlet apparatus (6 h for each solvent) (Tepe

et al., 2005a).

All extracts obtained were lyophilized and kept in the dark at +4°C until use.

Extraction of the essential oil

The air-dried and ground aerial parts of plants collected were submitted to water distillation for 3 h using a clevenger-type apparatus (yield 0.08%, v/w). The essential oil obtained was dried over anhydrous sodium sulfate and after filtration, stored at +4°C until tested and analyzed.

Gas chromatography (GC) /EIMS analysis

GC/EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were: injector and transfer line temperatures 220 and 240°C, respectively; oven temperature programmed from 60 to 240°C at 3°C/min; carrier gas helium at 1 ml/min; injection of 0.2 µl (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons and on computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data (Adams, 1995; Davies, 1990; Jennings and Shibamoto, 1980; Masada, 1976; Stenhagen et al., 1974; Swigar and Silverstein, 1981). Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing.

Assay for total phenolics and flavonoids

Total phenolic constituent of the methanol extracts were determined by employing the methods given in the literature (Chandler and Dodds, 1983; Slinkard and Singleton, 1977) involving Folin-Ciocalteu reagent and gallic acid as standard. 1 ml of extract solution containing 2000 µg extract was added to a volumetric flask. 45 ml distilled water and 1 ml Folin-Ciocalteu reagent was added and flask was shaken vigorously. After 3 min, a 3 ml of Na₂CO₃ (2%) solution was added and the mixture was allowed to stand for 2 h by intermittent shaking. Absorbance was measured at 760 nm. The concentrations of phenolic compounds were calculated according to the following equation that was obtained from the standard gallic acid graph:

$$A = 0.00201 \text{ gallic acid } (\mu\text{g}) - 0.0074 \quad (R^2 : 0.9908)$$

Total flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand et al. (1994). Briefly, 1 ml of 2% aluminum trichloride (AlCl₃) in methanol was mixed with the same volume of the solvent extracts (2000 µg). Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of a 1 ml extract solution with 1 ml methanol without AlCl₃. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

$$\text{Absorbance} = 0.0258 \text{ quercetin } (\mu\text{g}) - 0.0060 \quad (R^2 : 0.9987)$$

Antimicrobial activity

The extracts and essential oil isolated from *S. cedronella* were individually tested against *Bacillus cereus* (ATCC 11778), *B. subtilis*

(ATCC 6633), *Enterobacter aerogenes* (ATCC 13048), *E. faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *P. fluorescens* (ATCC 49838), *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *Listeria monocytogenes* (ATCC 19115), *Proteus mirabilis* (ATCC 25933) and *Candida albicans* (ATCC 90028).

Disc-diffusion, microwell dilution and MIC agar dilution were performed following the methodology given in the previous study (Gulluce et al., 2003). Ofloxacin (10 µg/disc), sulbactam (30 µg) + cefoperazone (75 µg) (105 µg/disc) and netilmicin, (30 µg/disc) were used as positive reference standard antibiotic discs (Oxoid). Amphotericin B was also used as reference antibiotic in micro well dilution (Sigma).

Antiviral activity

Compounds

The samples were treated with DMSO, DCM or hexane accordingly and diluted in bi-distilled sterile water to 10% solutions. For the experiments 2-fold dilutions were made in cell culture medium *ex tempore*. Rimantadine hydrochloride (Rimantadine) was obtained from Hoffman - La Roche Inc., Nutley, NJ, USA; (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) from Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany.

Cells and media

Madin-Darby canine kidney (MDCK) and Madin-Darby bovine kidney (MDBK) cells were passaged in Dulbecco's eagle medium (GibcoBRL, Scotland, UK), supplemented with 5% fetal calf serum (FCS) (BioWhittaker Europe, Germany and antibiotics; cell cultures were cultivated at 37°C in the presence of 5% CO₂ till the formation of confluent monolayers. In the antiviral experiments 0.5% FCS was added.

Viruses

The chicken influenza virus A/ch/Germany/34, str. Weibridge (H7N7) and the human influenza virus A/Aichi/2/68 (H3N2) (A/Aichi) were grown in MDCK cells in the presence of 2 µg/ml trypsin (Sigma); the infectious titre was 10^{5.7} - 10⁷ TCID₅₀/ml (50% tissue culture infectious doses/ml), the hemagglutination titre - 1024. Herpes simplex virus type 1 (HSV-1), strain DA and herpes simplex virus type 2 (HSV-2), strain Bja were grown in MDBK cells; the infectious titre was 10¹⁶ - 10¹⁸ TCID₅₀/ml. The strains were from the collection of the institute of microbiology, Bulgarian Academy of sciences, Sofia. The virus stocks were stored at 70°C.

Cellular toxicity

Confluent MDCK and MDBK cell monolayers in 96-well plastic plates were incubated with 4-fold dilutions of the samples in growth medium and were observed microscopically for changes in cell morphology and viability at 24, 48, 72 h of incubation. The cytopathic effect (CPE) was scored under an inverted microscope (score 0 = 0% CPE, score 1 = 0 - 25% CPE, score 2 = 25 - 50% CPE, score 3 = 50 - 75% CPE, score 4 = 75 - 100% CPE). The dilution, causing 50% CPE (TC₅₀) with respect to cell control (intact cells) was estimated.

Antiviral assays

The methods used are described in Serkedjieva and Hay (1998). In

short:

A. Cytopathogenic effect (CPE) reduction assay

The antiviral effect was studied in multicycle experiments of viral growth. The virus-induced cytopathogenic effect (CPE) was used as a measure of viral replication. Quadruplicate confluent monolayers in 96-well plates were overlaid with 2x drug-containing medium (0.1 ml) and an equal volume of virus suspension (100 TCID₅₀/ml). The virus-induced CPE was scored after 48 - 72 h of incubation at 37°C as described above. The concentration reducing CPE by 50% (EC₅₀) with respect to virus control (virus infected, non-treated cells) was estimated from graphic plots.

The selectivity index (SI) was determined from the ratio TC₅₀/EC₅₀. SI ≥ 4 was considered to stand for a significant selective inhibition.

B. 50% end point titration technique (EPTT)

It was performed according to Vanden Berghe et al. (1986). Quadruplicate monolayers in 96-well microtitre plates were infected with 0.05 ml serial 10-fold dilutions of the virus suspension and then serial 2-fold dilutions of the preparation in a tissue culture medium (0.05 ml) were added. The cultures were incubated at 37°C and examined microscopically daily for CPE. CPE was scored as described above. The antiviral activity was determined by the difference between the virus titres of control and treated viruses (δ log₁₀TCID₅₀/ml). The significance of the difference was estimated by student's t-test. The 90% effective concentration (EC₉₀) was determined from graphic plots as the dose that caused reduction of 1 log TCID₉₀/ml of viral infectious titre.

The selective anti-influenza drug Rimantadine hydrochloride and the antiherpetic drug BVDU were used as positive controls.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The GC-EIMS analysis of the essential oil of *S. cedronella* was resulted in detection of 92 components representing 96.1% of the oil (Table 1). Major components of the oil were 1,8-cineole (13.3%), α-pinene (10.1%), caryophyllene oxide (9.8%) and sabinene (7.3%).

As far as our literature survey could ascertain, no report is available on the chemical composition of the essential oils of *S. cedronella* in the literature. Therefore, this study could be assumed as the first report on this topic.

Amount of total phenolics and flavonoids

Total phenolic assay was carried out based on the absorbance values of the various extract solutions, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of pyrocatechol equivalents as described above. Data obtained from the total phenolic assay supports the key role of phenolic compounds in free radical scavenging and/or reducing systems. As expected, amount of the total phenolics was very high in

Table 1. Chemical composition of the essential oils of *S. cedronella*.

No	LRI ^a	Compound	Composition (%)	No	LRI	Compound	Composition (%)
1	932	α -thujene ^b	0.5	53	1297	carvacrol ^c	1.9
2	937	α -pinene ^b	10.1	54	1354	α -cubebene ^b	0.1
3	955	camphene ^b	0.1	55	1379	α -copaene ^b	0.5
4	972	sabinene ^c	7.3	56	1389	β -cubebene ^b	0.9
5	976	β -pinene ^c	2.5	57	1390	β -bourbonene ^b	0.3
6	981	1-octen-3-ol ^b	0.2	58	1396	β -elemene ^b	0.2
7	993	3-octanol ^b	tr ^d	59	1415	α -gurjunene ^b	tr
8	997	myrcene ^c	0.1	60	1418	β -caryophyllene ^c	1.8
9	1019	α -terpinene ^c	0.1	61	1439	γ -elemene ^c	0.4
10	1023	<i>p</i> -cymene ^c	1.3	62	1440	aromadendrene ^b	0.5
11	1026	limonene ^b	0.6	63	1451	geranyl acetone ^b	0.1
12	1033	β -phellandrene ^b	tr	64	1459	(E)- β -farnesene ^b	0.1
13	1035	1,8-cineole ^c	13.3	65	1483	γ -muurolene ^b	0.6
14	1039	(Z)- β -ocimene ^b	tr	66	1484	germacrene-D ^c	1.7
15	1047	(E)- β -ocimene ^b	tr	67	1491	(E)- β -ionone ^b	tr
16	1056	γ -terpinene ^c	0.5	68	1493	<i>epi</i> -cubebol ^b	1.3
17	1069	<i>cis</i> -sabinene-hydrate ^b	0.3	69	1497	cadina-1,4-diene ^b	tr
18	1071	<i>trans</i> -linalool oxide ^b	tr	70	1499	α -selinene ^b	0.4
19	1085	terpinolene ^b	0.2	71	1504	α -muurolene ^b	0.5
20	1094	linalool ^c	1.1	72	1509	α -bourbonene ^b	0.4
21	1096	<i>trans</i> -sabinene hydrate ^b	1.2	73	1513	γ -cadinene ^c	2.6
22	1100	(E)-2-hexanal ^b	tr	74	1517	cubebol ^b	3.5
23	1103	<i>n</i> -nonanal ^b	tr	75	1519	1-endo-bourbonanol ^b	0.1
24	1123	α -campholenal ^b	0.7	76	1529	δ -cadinene ^c	1.1
25	1126	chrysanthenone ^b	tr	77	1537	<i>cis</i> -calamene ^b	0.1
26	1137	<i>trans</i> -pinocarveol ^b	1.3	78	1543	α -calacorene ^b	tr
27	1140	<i>cis</i> -verbenol ^b	0.3	79	1563	germacrene-B ^b	0.4
28	1142	<i>trans-p</i> -menth-2-en-1-ol ^b	0.8	80	1566	(Z)-3-hexenyl benzoate ^b	0.3
29	1144	<i>cis</i> -sabinol ^c	0.7	81	1571	ledol ^b	0.4
30	1147	<i>trans</i> -verbenol ^b	1.6	82	1575	spathulenol ^c	1.5
31	1149	camphor ^c	1.3	83	1587	caryophyllene oxide ^c	9.8
32	1155	sabina ketone ^b	0.2	84	1605	β -oploponone ^b	0.1
33	1167	pinocarvone ^b	0.1	85	1609	humulene epoxide II ^b	1.4
34	1169	δ -terpineol ^c	0.8	86	1637	<i>epi</i> - α -cadinol ^b	2.6
35	1171	borneol ^c	0.9	87	1643	<i>epi</i> - α -muurolol ^b	0.3
36	1179	terpinen-4-ol ^c	2.6	88	1649	cubenol ^b	0.4
37	1180	<i>p</i> -cymene-8-ol ^b	tr	89	1654	β -eudesmol ^b	1.5
38	1191	α -terpineol ^c	0.8	90	1655	α -eudesmol ^b	0.3
39	1194	<i>cis</i> -piperitol ^b	tr	91	1881	<i>n</i> -hexadecano ^b ^l	2.6
40	1197	myrtenal ^b	0.4	92	2054	abietatriene ^b	0.1
41	1197	myrtenol ^b	0.3			Total	96.1
42	1205	decanal ^b	tr				
43	1208	verbenone ^b	tr				
44	1213	<i>trans</i> -carveol ^b	0.1				
45	1236	<i>p</i> -mentha-1(7),8-dien-2-ol ^b	tr				
46	1245	carvone ^c	0.4				
47	1255	linalyl acetate ^c	0.3				
48	1261	2-phenylethyl acetate ^b	0.2				
49	1267	(E)-2-decanal ^b	tr				
50	1273	nonanoic acid ^b	0.2				
51	1284	bornyl acetate ^c	0.1				
52	1293	thymol ^c	1.8				

^a LRI, Linear Retention Indices (HP - 5 column), ^b Tentative identification, ^c Identification of components based on standard compounds, ^d tr, trace ($\leq 0.1\%$).

Table 2. Amount of total phenolics and flavonoids of *S. cedronella* extracts.

Sample	Phenolic content (μg GAEs/mg extract) ^b	Flavonoid content (μg QEs/mg extract) ^c
Hexane extract	18.37 \pm 1.29	14.89 \pm 0.25
Dichloromethane extract	42.98 \pm 2.72	41.26 \pm 0.84
Methanol extract	106.64 \pm 1.33	16.29 \pm 0.72

^a Values expressed are means \pm S.D. of three parallel measurements

^b GAEs. Gallic acid equivalents.

^c QEs. quercetin equivalents.

methanol extract (106.64 \pm 1.33 μg GAEs / mg extract). It was followed by dichloromethane extract with a value of 42.98 \pm 2.72 μg GAEs / mg (Table 2). It is extremely important to point out that a positive correlation was observed between biological activity potential and amount of phenolic compounds of the extracts. On the other hand, dichloromethane extract has been found to be rich in flavonoids with a value of 41.26 \pm 0.84 μg QEs / mg.

Antioxidative activity potential, total phenolics and flavonoids of this species have previously been reported by Yesilyurt et al. (2008). According to this report, acetone extract of *S. cedronella*, had showed a high content of total phenolics, whereas the flavonoid content was found to be quite poor. The results obtained from this study are highly in agreement with the present study.

Antimicrobial activity

The antimicrobial activities of the essential oils and the extracts having various polarities assayed against a wide range of human, plant-associate and food born microorganisms and their potency were qualitatively and quantitatively assessed by evaluating the presence of inhibition zones, zone diameter and MIC values (Table 3). Hexane and dichloromethane extracts did not show any antimicrobial activity. In the case of methanol extract; *B. cereus*, *B. subtilis*, *E. aerogenes*, *E. faecalis*, *S. aureus*, *S. epidermidis*, *P. mirabilis* and *C. albicans* showed various degrees of sensitivity. The growth inhibitions of these microorganisms ranged from 31.25 $\mu\text{g}/\text{ml}$ to 250.00 $\mu\text{g}/\text{ml}$ with the lowest MIC value against *E. aerogenes* and *E. faecalis*. Among the microorganisms, the most sensitive was *B. subtilis* with a 17.00 mm in diameter of inhibition zone (MIC; 31.25 $\mu\text{g}/\text{ml}$). This is followed by *S. aureus* and *B. cereus* with the same MIC value.

As can be seen from the Table 3, the essential oil was found to possess remarkable antimicrobial activity against *B. cereus*, *B. subtilis*, *S. aureus*, *S. epidermidis*, *P. mirabilis* and *C. albicans* strains. Among these strains, the most sensitive microorganism was *B. subtilis* ATCC 6633 with an MIC value of 15.62 $\mu\text{g}/\text{ml}$ followed by *B. cereus* and *S. aureus* with an MIC at 31.25 $\mu\text{g}/\text{ml}$.

As far as our literature survey could, as certain, several

reports are available concerning the antimicrobial activity of the members of *Salvia* genus. Additionally, antimicrobial activities of *S. tomentosa*, *S. cryptantha* and *S. multicaulis* have previously been reported by our research group (Tepe et al., 2004; Tepe et al., 2005). On the other hand, we could not reach any report dealing with the antimicrobial properties of *S. cedronella*.

Antiviral activity

The antiviral potential of the plant samples was screened in 2 model systems. Reproduction of 2 influenza viruses in MDCK cells and of 2 herpes simplex viruses in MDBK cells (Table 4). The MeOH extract of *S. cedronella* showed a good anti-influenza virus effect; the growth of both A/Weybridge and A/Aichi was reduced significantly. This extract also exhibited anti-herpetic activity.

The literature data on the antiviral effects of *Salvia* species are scarce. Tada et al. (1994) isolated antiviral diterpenes from *S. officinalis*. Han and Lee (1999) claimed that the roots of *S. miltiorrhiza* contained antiviral components. Sivropoulou et al. (1997) established that the essential oils of *S. fruticosa* inactivated herpes simplex virus. *S. officinalis* extracts were used as active ingredients in combined herbal preparation with anti-influenza virus effect (Manolova et al., 1995) and for the treatment of herpes labialis (Saller et al., 2001).

Since antiquity, *Salvia* species have been a well known plants and widely used as folk medicines with antibacterial (Ulubelen et al., 2001), antituberculosis (Ulubelen et al., 1994), antiviral, cytotoxic (Topcu et al., 2003; Topcu and Ulubelen, 1999), cardiovascular (Topcu et al., 2003; Ulubelen et al., 2002), liver protective and other properties (Zhou et al., 2005). Phytochemical investigations have shown that *Salvia* species are mainly rich in diterpenoids (Topcu and Ulubelen, 2007; Ulubelen and Topcu, 1992; Ulubelen et al., 1995; Ulubelen et al., 1992) and triterpenoids (Topcu, 2006; Topcu et al., 2004; Topcu et al., 1994) as well as in flavonoids (Topcu et al., 1995) and other phenolic compounds (Lu and Foo, 2002).

Phenolics are non-essential dietary compounds which have been associated with inhibition of atherosclerosis and cancer and the phenolic content of the plant extracts

Table 3. Antimicrobial activity of the extracts and essential oils of *S. cedronella*.

Microorganisms	Samples									
	Essential oil		Hexane extract		Dichloromethane extract		Methanol extract		Antibiotics ^a	
	DD ^b	MIC ^c	DD	MIC	DD	MIC	DD	MIC	DD	MIC
<i>B. cereus</i> ATCC 11778	16.00	31.25	-	-	-	-	13.00	31.25	28.00 (OFX)	62.50
<i>B. subtilis</i> ATCC 6633	20.00	15.62	-	-	-	-	17.00	31.25	28.00 (OFX)	125.00
<i>E. r aerogenes</i> ATCC 13048	-	-	-	-	-	-	8.00	250.00	20.00 (NET)	31.25
<i>E. s faecalis</i> ATCC 29212	-	-	-	-	-	-	8.00	250.00	18.00 (SCF)	31.25
<i>E. coli</i> ATCC 25922	-	-	-	-	-	-	-	-	12.00 (OFX)	125.00
<i>K. pneumonia</i> ATCC 13883	-	-	-	-	-	-	-	-	12.00 (OFX)	125.00
<i>P. s aeruginosa</i> ATCC 27853	-	-	-	-	-	-	-	-	22.00 (NET)	15.62
<i>S. s aureus</i> ATCC 25923	17.00	31.25	-	-	-	-	14.00	31.25	22.00 (SCF)	31.25
<i>S. epidermidis</i> ATCC 12228	10.00	62.50	-	-	-	-	9.00	62.50	12.00 (SCF)	15.62
<i>L. a monocytogenes</i> ATCC 19115	-	-	-	-	-	-	-	-	12.00 (OFX)	125.00
<i>P. fluorescens</i> ATCC 49838	-	-	-	-	-	-	-	-	18.00 (NET)	125.00
<i>P. mirabilis</i> ATCC 25933	8.00	250.00	-	-	-	-	10.00	62.50	12.00 (OFX)	125.00
<i>C. a albicans</i> ATCC 90028	10.00	62.50	-	-	-	-	12.00	31.25	28.00 (Amp B)	31.25

^a OFX: Ofloxacin (10 µg/disc); SCF: sulbactam (30 µg)+cefoperazona (75 µg) (105 µg/disc) and NET: Netilmicin, (30 µg/disc) were used as positive reference standards antibiotic discs (Oxoid); AmpB: Amphotericin B was used as reference antibiotic in micro well dilution (Sigma).

^b DD: Disc Diffusion, Inhibition zone in diameter (mm) around the discs impregnated with 300 µg/disc of methanol extract.

^c MIC: Minimal Inhibitory concentrations as (µg/ml),

Table 4. Antiviral activity of *S. cedronella* extracts on influenza and herpes type viruses.

Samples	Toxicity to MDCK cells and anti-influenza virus effect						
	MDCK		A/Weybridge			A/Aichi	
	TC ₅₀ ^a (mg/ml)	EC ₅₀ ^b (mg/ml)	SI ^c	EC ₉₀ ^d (mg/ml)	EC ₅₀ (mg/ml)	SI	EC ₉₀ (mg/ml)
Hexane extract	0.60	0.25	2.40	-	> TC ₅₀	-	-
Dichloromethane extract	0.04	> TC ₅₀	-	-	> TC ₅₀	-	-
Methanol extract	3.00	0.30	10.00	0.52	0.60	5.00	-
Rimantadine hydrochloride	> 0.03	0.40 µg/ml	> 80.00	-	0.10 µg/ml	>	-
						300.00	

Samples	Toxicity to MDBK cells and anti-herpetic virus effect						
	MDBK		A/Weybridge			A/Aichi	
	TC ₅₀ ^a (mg/ml)	EC ₅₀ ^b (mg/ml)	SI ^c	EC ₉₀ ^d (mg/ml)	EC ₅₀ (mg/ml)	SI	EC ₉₀ (mg/ml)
Hexane extract	0.50	> TC ₅₀	-	-	> TC ₅₀	-	-
Dichloromethane extract	0.75	> TC ₅₀	-	-	> TC ₅₀	-	-
Methanol extract	3.00	0.60	5.00	-	0.50	6.00	0.12
BVDU ^e	> 100.00 µg/ml	1.00 µg/ml	> 100.00	1.00 µg/ml	2.00 µg/ml	> 50.00	2.00 µg/ml

^a 50% toxic concentration, the dose required to cause visible changes in 50% of intact cultures

^b 50% effective concentration, the dose that caused 50% reduction of virus-induced CPE

^c Selectivity index

^d 90% effective concentration, the dose that caused reduction of infectious virus yield 1 lg TCID₅₀

^e (E)-5-(2-bromovinyl)-2'-deoxyuridine

was found to be well correlated with their biological activities (Velioglu et al., 1998). In the course of our search for the antimicrobial and antiviral properties of *S. cedronella* (an endemic species in Turkey) essential oils and extracts having various polarity, remarkable

antimicrobial and antiviral activity was determined against the tested microorganisms and viruses.

For improving the data presented here, further studies are needed for better clarifying the cytotoxicity and other biological properties of the plant species presented here.

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