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ORIGINAL ARTICLE

Phytochemical composition and antimicrobial, antioxidant and cytotoxic activities of *Anchusa officinalis* L. extracts

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Abstract
The aim of this study is to examine phytochemical composition and evaluation of antimicrobial, antioxidant and cytotoxic activity of *Anchusa officinalis* plant extracts in different solutions: ethanol, chloroform, petroleum, acetone and ethyl acetate. A comparative analysis has shown that ethanol extract had the highest concentration of phenols (104.03 ± 0.63 mgGA/g), and the highest concentration of flavonoids (30.26 ± 0.40 mgRU/g). The highest concentration of the condensed tannins recorded in chloroform extract (74.65 ± 0.57 mg GA/g). The ethanol extract showed the strongest antioxidant and the best antimicrobial activity as compared to all other tested extracts, while the chloroform and the acetone extracts showed the best cytotoxic activity on cell line of mouse fibroblast carcinoma (L2OB). This is the first report of citotoxic activity of extract *A. officinalis* plant from Balkan region on tumor cell lines. HPLC analysis of *A. officinalis* plant extracts confirmed that the predominant polyphenol components were: rosmarinic acid, chlorogenic acid, naringenin, lutein-glycoside and rutin.

Keywords *Anchusa officinalis* · Biological activity · Phenolics content

Introduction

The *Boraginaceae* family includes approximately 2000 species worldwide, mainly in Europe and Asia (Dresler et al. 2017). Plants of the *Boraginaceae* family are traditionally used in the treatment of fever, asthma, kidney stones, wound healing, as well as in treatment of arthritis, sprains or dislocation of the joints and bone fractures (Al-Snafi 2014). The therapeutic effect of these plants is related to the content of many biologically active compounds, including naphthaquinones, flavonoids, terpenoids and phenols (Sharma et al. 2009). This constituens have a wide range of pharmaceutical activities, including anti-inflammatory, antiviral and anti-bacterial activities (Iqbal et al. 2005).

Anchusa officinalis belongs to the *Boraginaceae* family. Genus *Anchusa* (*Boraginaceae*) is one of the major genera of flowering plants with major diversity center in the southern part of the Balkan Peninsula (Selvi and Bigazzi 2003). The plant is native to the Europe, but absent from the extreme North, much of the West and parts of the Mediterranean region (Jakovljevic et al. 2016). Among the people it is known as blush, duck nest and healing common bugloss. Numerous studies of various species of the *Anchusa* genus indicated their wide application in folk medicine (Khare 2007; Amin 2005). The presence of numerous phytochemicals, alkaloids, tannins, oils, polyphenols, pyrrolizine alkaloids, triterpenoids, in the *Anchusa* genus was confirmed in research by Al-Snafi (2014). HPLC analysis of polyphenol components confirmed the presence of rosmarinic, benzoic acid, rutin and catechin in a methanolic extract of the *Anchusa undulata* subsp. *hybdrida* (Zengin et al. 2015).

Since there has no enough scientific data on phytochemical analysis and biological properties of extracts of *Anchusa officinalis* plant from Balkan region, the data from this study will be the report of potential application of different extracts of *A. offiicinalis* in medical treatment.

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Matherials and methods

Chemicals

Methanol (HPLC, gradient grade), quercetin and formic acid (standards for HPLC) were supplied by Merck KGaA (Darmstadt, Germany). Folin-Ciocalteau's reagent, (DPPH), Muller–Hinton broth (MHB), Muller-Hinton agar (MHA), Sabouraud dextrose broth, Minimum essential medium Eagle (MEM), Fetal bovine serum (FBS), resazurin, ascorbic acid, butylated hydroxytoluene (BHT), ketoconazole, amracin, galic acid, protocatechuic acid, caffeic acid, vanillic acid, chlorogenic acid, syringic acid, ferulic acid, rutin, rosmarinic acid, naringenin, luteolin, kaempferol and apigenin were purchased from Sigma- Aldrich GmbH (Sternheim, Germany).

Plant material and extracts preparation

The plant *Anchusa officinalis* was collected in the period May–June 2013 in the flowering stage of development on Brđanska gorge near Gornji Milanovac in Serbia. The determination of the plant was conducted at the Faculty of Science in Kragujevac, Department for Botany. The plant material is defatted with petroleum ether (40 °C). The above-ground part of the plant is grinded by cylindrical crusher and extracted with solvents series in Soxhlet apparatus. After that solutions were evaporated on a rotary-evaporator at a temperature of 40 °C.

Determination of the total phenolic content

Total phenol content in the plants is determined by the Folin-Ciocalteu method (Singleton et al. 1999). Series of extract was diluted to a concentration of 1 mg/ml, and 0.5 ml of the extract was mixed with 2.5 ml of Folin-Ciocalteu reagent and 2 ml of $NaHCO_3$ (7.5%). After 15 min of standing at 45 °C absorbance was measured at 765 nm compared to a blank control. Total phenol content is expressed in milligram-equivalents of gallic acid per gram of extract (mg GA / g of extract).

Determination of flavonoid content

The total quantity of flavonoid is carried out by the method Brighente et al. (2007). A volume of 0.5 ml of extract solution was mixed with 0.5 ml of 2% aluminum chloride ($AlCl_3$), and after 1 h standing at room temperature absorbance were measured at 415 nm on a spectrophotometer compared to a blank control sample. The content of total flavonoids is expressed in milligram-equivalents per gram of the routine extract (RU mg / g of extract).

Determination of tannin content

To determine fused tannins was used method by Vermerris and Nicholson (2006). The 2 ml of the extract was mixed with a given amount of floroglucinols, and then with 1 ml of a 2:5 HCl/H_2O solution and 1 ml of a solution of formaldehyde. After incubation overnight at room temperature, are determined in the non-precipitated phenols in the supernatant liquidusing a Folin-Ciocalteu's methods. The concentration of tannin-fused was calculated as the concentration of the residue of total phenols and non-precipitated phenolsand expressed in equivalents of gallic acid.

HPLC data analysis

Determination of polyphenol components in the extracts were performed on a HPLC instrument Agilent 1200 Series (Agilent Technologies, USA) with UV-Vis DAD for the detection of multi wavelengths. After injecting 5 μl of sample, the separation was performed in an Agilent-Eclipse XDB C-18 4.6·150 mm column. The column was thermostated at 25 °C. Two solvents were used for the gradient elution: A - (H_2O + 2% HCOOH) and B - (80% ACN + 2% HCOOH+ H_2O). All identifications of individual compounds were based on the retention times of the original standards, where available, and spectral data (Boskovic et al. 2017).

Determination of the total antioxidant activity

Determination of the total antioxidant capacity was carried out according to the method of phosphomolybdic by Prieto et al. (1999).

DPPH free radical scavenging activity of the plant extracts was determined using DPPH photometric method according to Takao et al. (1994). Determination of inhibition of lipid peroxidation was carried by ammonium thiocyanate method (Hsu et al. 2008). Determining scavenging hydroxyl radical was performed by applying a standard method (Hinneburg et al. 2006).

Screening for the antimicrobial activity

Antimicrobial activity of the plant extracts was tested by microdilution method (CLSI 2012). The tested microorganisms included different bacterial strains: *Bacillus spieizenii* ATCC 6633, *Citrobacter freundi* ATCC 43864, *Enterococcus faecalis* ATCC 29212, *E. faecium* ATCC 6057, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *L. monocytogenes* ATCC 19112, *Listeria inocuu* ATCC 33090, *L. ivanovii* ATCC 19119, *Proteus mirabilis* ATCC 14153, *P. vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* ATCC 13076,

S. typhimurium ATCC 14028, *Staphylococcus aureus* ATCC 25923, *S. saprophiticus* ATCC 15035 and yeasts: *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231. Extract solutions were serially diluted in MHB and Sabouraud dextrose broth in a line hole of the microplate with a 96 holes, followed by addition of the bacterial culture to an approximate concentration of 1.5×10^8 CFU / ml and yeast spore suspension of 3×10^4 CFU / ml. The microplates were incubated for 24 h at 37 °C, and thereafter minimum inhibitory concentration (MIC) was determined. The MIC was then determined and corresponds to the lowest concentration substance which produces no bacterial growth. Commercial antibiotics amracin and ketoconasole were used as a positive control. Upon expiration of 24 h (for bacteria) and 72 h (for fungal growth) at a temperature of 37 °C, the MIC values were notices visually after the addition of resazurin (0.05% solution 0,02 ml) as an indicator, the color of the solution changed from blue in purple. The contents of all wells in which was no visible microbial growth was transferred to a new Petridish with a suitable solid carrier (MHA) and after incubation at 37 °C for a period of 24 h the counting of the grown colonies was carried out. Minimum bactericidal concentration (MBC) was defined as the concentration of active substance which kills 99.9% of the bacterial cells.

In vitro cytotoxic activity by MTT assay

Examination of cytotoxic activity was carried out by the MTT assay in human cell lines Hep2 - human larynx carcinoma (medium MEM Eagle supplemented with 5% FBS), human cell lines RD – rhabdomyosarcom (medium MEM Eagle supplemented with 10% FBS) and mouse cell lines L2OB- tumor fibroblast (medium MEM Eagle supplemented with 10% FBS). All cell lines are adherent. The cell suspensions with a density of 10^4 cells were seeded into microtiter 96-well plates and allowed to incubate at 37 °C and 5% CO_2 in an incubator. After completing the 24 h incubation the medium was replaced with 100 ml of medium containing various concentrations of the extract (from 25 to 1000 μg/ml) and control cells added to fresh medium without the extract. After 48 h treatment cells viability was determined by MTT cytotoxicity assay 3-(4, 5-dimethylthiazol-2-yl) -2,5 diphenyl tetrazolium bromide assay (Mosmann 1983). Upon completion of incubation of the cells with extracts MTT was added (to a final concentration of 5 mg/ml PBS) to each well and the plate was incubated for 2–4 h at 37 °C. Colored formazan crystals generated were dissolved with 150 ml DMSO. The absorbance was measured at 570 nm on a microplate Reader. The percentage of viable cells was determined as the ratio of the absorbance of the treated cells and control cells multiplied by 100. The results were obtained from three independent experiments.

Statistical analysis

Statistical analysis of the results was performed by analysis of variance of monofactorial experiment. Multiple comparison of means test was determined by using the least significant differences (LSD). The results of antioxidant activity are presented as means ± standard deviations (mean ± SD) of three analytical determinations.

Results

The results of total phenolic content of the tested plant extracts of *A. officinalis* using Folin-Ciocalteu's method and expressed as gallic acid equivalents are presented in Table 1. The obtained values were in the range from 87.11 ± 0.54 to 104.03 ± 0.63 mgGA/g. The phenolic contents in the ethanol (104.03 ± 0.63 mgGA/g) and cloroform extracts (100.26 ± 0.40 GA/g) were higher than in the other three tested extracts. The content of flavonoids was determined using spectrophotometric method with $AlCl_3$ and expressed in terms of rutin equivalents. The ethanol extract had the highest value of flavonoids (30.26 ± 0.40 mgRU/g), followed by acetone extract (29.91 ± 0.44 mgRU/g). The results are shown in Table 1. The highest content of tannins achieved in the chloroform (74.65 ± 0.57 mg GA/g) and acetone extract (74.53 ± 0.46 mg GA/g).

An integral approach was used to evaluation the antioxidant potential of the plant *Anchusa officinalis*: total antioxidant potential, inhibition of lipid peroxidation, hydroxyl radical ($OH^{\bullet}$) scavenging activity and DPPH free radical scavenging activity. Table 2 shows obtained results antioxidant activities of extracts *A. officinalis* plant. These results implied that ethanoliclic extract have the highest ($OH^{\bullet}$) radical scavenging abilities (53.53 ± 0.91) followed by chloroform, acetone and petroleum extracts. The ethanolic extract of *A. officinalis* had the highest total antioxidant capacity

Table 1 Total phenolics (mgGA/g), flavonoids (mgRU/g) and tannins (mgGA/g) content in the various extracts of plant *Anchusa officinalis*

Extracts	Phenolics (mgGA/g)	Flavonoids (mgRU/g)	Tannins (mgGA/g)
Chloroform	100.26 ± 0.40b	28.24 ± 0.33b	74.65 ± 0.57a
Ethyl acetate	91.79 ± 0.17c	29.49 ± 0.48a	73.26 ± 0.58a
Ethanol	104.03 ± 0.63a	30.26 ± 0.40a	70.67 ± 0.51b
Acetone	99.35 ± 0.43b	29.91 ± 0.44a	74.53 ± 0.46a
Petroleum	87.11 ± 0.54d	29.05 ± 0.28ab	70.41 ± 0.54b

*Statistically significant difference between the level of 0.05% is significant if it is next obtained concentration values (compared to extract employed) is a different letter. If they are the same letters that difference is not significant. If there are two letters, the significance is seen again after that is there at least one letter that is the same or different (insignificant or significant difference)

Table 2 The total antioxidant capacity (μgAA/g), inhibition of lipid peroxidation (μg/g), hydroxyl radical ($OH^{\bullet}$) scavenging activity (μg) and DPPH free radical scavenging activity in the various extracts of plant *Anchusa officinalis*

Extracts	Total antioxidant capacity	Inhibition of lipid peroxidation	Hydroxyl radical $OH^{\bullet}$ scavenging activity	DPPH free radical scavenging activity
Chloroform	88.26 ± 0.98	37.39 ± 1.26	56 ± 0.98	57.04 ± 1.08
Ethyl acetate	87.09 ± 0.95	40.28 ± 1.23	54.33 ± 0.77	59.03 ± 0.98
Ethanol	90.26 ± 0.99	35.45 ± 1.34	53.53 ± 0.91	55.57 ± 0.45
Acetone	87.13 ± 0.99	37.51 ± 1.11	55.13 ± 0.57	57.41 ± 0.76
Petroleum	78.05 ± 1.45	41.32 ± 1.08	62.14 ± 0.77	63.81 ± 0.86

Average antioxidans activity (±SD) of three analytical determinations

(90.26 ± 0.99 μg AA/g) and the lipid peroxidation inhibition (35.45 ± 1.34 IC_{50} μg /ml). Also, the highest antioxidant activity in DPPH test (55.57 ± 0.45 μg /ml) recorded in ethanolic extract. The lowest antioxidative capacity is recorded in petroleum extract.

HPLC analysis of examined extracts of the *A. officinalis* plant showed the presence of the following components: *p*-hydroxybenzoic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, sinapinic acid, rutin, lutein glycoside, apigenin glycoside, rosmarinic acid, quercetin, lutein, naringenin and kaempferol, whereas the most dominant were: rosmarinic, chlorogenic acid, naringenin, lutein-glycoside and rutin (Table 3). Petroleum ether extract of *A. officinalis* had a maximum value (Σ 335.185 mg/g) of the identified compounds: rosmarinic acid (244.300 mg/g), chlorogenic acid (30.925 mg/g), naringenin (14.018 mg/g), lutein-glycoside (13.038 mg/g) and a rutin (11.116 mg/g).

The values of antimicrobial activity of different extracts of *A. officinalis* plant are shown in Table 4. The maximum antibacterial activity (MIC = 3.94 μg/mL) was shown by the ethanol extract against *Proteus vulgaris, Salmonella enteritidis, Enterococcus faecalis, Enterococcus faecium, Salmonella typhimurium* and *Candida albicans* and by the chloroform and acetone extract against *E. faecalis* and *C. albicans* (MIC 7.875 μg/mL). The petroleum ether extract remarkably inhibited the growth of *Aspergillus niger* (MIC 3.947 μg/mL). On the other hand, a lower values of antimicrobial activity (MIC from 31.25 to 125.00 μg/mL) was found in ethyl acetate extract againts the tested microorganisms.

Minimum bactericidal concentrations (MBC) of tested extracts of the *A. officinalis* plant ranged from 7.875 μg/ml to 250 μg/ml. Ethanol extract showed the lowest MBC value against the bacteria *P. vulgaris, S. enteritidis, S. typhimurium, E. faecalis, E. faecium* and fungus *C. albicans* (MBC 7.875 μg/ml).

Results of citotoxic activity of the different extracts of *A. officinalis* (in a concentration from 25 to 1000 μg/ml) in three cell lines (Hep 2, RD and L2OB), using MTT assay shows in Table 5.

Table 3 HPLC data analysis of various extracts of plant *Anchusa officinalis*

Compounds	Petroleum	Chloroform	Acetone	Ethyl acetate	Ethanol
Protocatehuic acid	n.d.	n.d.	n.d.	n.d.	n.d.
p-Hydroxibenz. acid	3.888	0.516	0.845	n.d.	0.069
Caffeic acid	n.d.	n.d.	n.d.	n.d.	n.d.
Vanillic acid	n.d.	n.d.	n.d.	n.d.	n.d.
Chlorogenic acid	30.925	n.d.	4.646	1.499	n.d.
Syringic acid	n.d.	n.d.	n.d.	n.d.	n.d.
p-Coumaric acid	3.599	n.d.	0.008	n.d.	n.d.
Ferulic acid	n.d.	n.d.	0.247	n.d.	0.007
Synapic acid	4.109	0.198	2.057	1.772	0.027
Rutin	11.116	2.509	2.073	12.485	0.029
Luteolin glycoside	13.038	0.102	6.775	1.948	0.059
Apigenin glycoside	3.601	n.d.	1.605	2.581	n.d.
Rosmarinic acid	244.300	0.645	58.961	6.181	1.432
Quercetin	0.817	0.204	0.396	0.918	0.016
Luteolin	2.826	n.d.	n.d.	n.d.	n.d.
Naringenin	14.018	0.142	3.142	n.d.	0.059
Kaempferol	2.948	0.269	0.417	1.009	0.010
Apigenin	n.d.	n.d.	n.d.	n.d.	n.d.
Σ	335.185	4.585	81.172	28.393	1.708

Table 4 Antimicrobial activity of various extracts *Anchusa officinalis* expressed as minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

Microorganisms	Ethanol (μg/ml)	Ethylacetate (μg/ml)	Chloroform (μg/ml)	Petroleum (μg/ml)	Acetone (μg/ml)	Amracin (μg/ml)	Ketoconazol (μg/ml)
Proteus mirabilis	62.5 125.00	62.5 125.00	31.25 62.5	62.5 125.00	125.00 250.00	0.49	0
Escherichia coli	7.875 15.75	125.00 250.00	31.25 62.5	125.00 250.00	31.25 62.5	0.97	0
Klebsiella pneumoniae	7.875 15.75	125.00 250.00	31.25 62.5	125.00 250.00	125.00 250.00	0.49	0
Proteus vulgaris	3.94 7.875	62.5 125.00	31.25 62.5	125.00 250.00	62.5 125.00	0.49	0
Salmonella enteritidis	3.94 7.875	62.5 125.00	31.25 62.5	125.00 250.00	62.5 125.00	0.49	0
Enterobacter aerogenes	7.875 15.75	125.00 250.00	31.25 62.5	125.00 250.00	31.25 62.5	0.97	0
Citrobacter freundii	62.5 125.00	62.5 125.00	31.25 62.5	62.5 125.00	125.00 250.00	0.49	0
Salmonella typhimurium	3.94 7.875	62.5 125.00	31.25 62.5	125.00 250.00	62.5 125.00	0.49	0
Pseudomonas aeruginosa	62.5 125.00	125.00 250.00	31.25 62.5	125.00 250.00	125.00 250.00	0.97	0
Listeria ivanovii	7.875 15.75	125.00 250.00	31.25 62.5	125.00 250.00	31.25 62.5	0.97	0
Listeria inocuu	62.5 125.00	62.5 125.00	31.25 62.5	62.5 125.00	125.00 250.00	0.49	0
Enterococcus faecalis	3.94 7.875	31.25 62.5	7.875 15.75	31.25 62.5	7.875 15.75	0.97	0
Listeria monocytogenes	7.875 15.75	125.00 250.00	31.25 62.5	125.00 250.00	31.25 62.5	0.97	0
Bacillus spieizeni	62.5 125.00	125.00 250.00	31.25 62.5	125.00 250.00	125.00 250.00	0.97	0
Enterococcus faecium	3.94 7.875	62.5 125.00	31.25 62.5	125.00 250.00	62.5 125.00	0.49	0
Staphylococcus aureus	62.5 125.00	125.00 250.00	31.25 62.5	125.00 250.00	125.00 250.00	0.97	0
Staphylococcus saprophiticus	62.5 125.00	125.00 250.00	31.25 62.5	125.00 250.00	125.00 250.00	0.97	0
Aspergillus niger	7.875 15.75	62.5 125.00	31.25 62.5	3.94 7.875	62.5 125.00	0	0.97
Candida albicans	3.94 7.875	31.25 62.5	7.875 15.75	31.25 62.5	7.875 15.75	0	1.95

Table 5 Growt inhibitory effects IC_{50} values (μg/ml) of *Anchusa officinalis* plant extracts

Extract	Hep 2cells IC_{50} (μg/ml)	RD cells IC_{50} (μg/ml)	L2OB cells IC_{50} (μg/ml)
Chloroform	144.67 ± 2.12b	176.53 ± 0.54b	102.28 ± 4.10c
Ethyl acetate	173.39 ± 0.27a	188.89 ± 1.35a	150.34 ± 0.52b
Ethanol	135.44 ± 0.87c	187.49 ± 2.52a	147.17 ± 0.16b
Acetone	137.34 ± 1.30c	176.33 ± 0.25b	105.54 ± 2.42c
Petroleum	176.77 ± 2.26a	120.57 ± 1.32c	159.35 ± 0.74a

*Statistically significant difference between the level of 0.05% is significant if it is next obtained concentration values (compared to extract employed) is a different letter. If they are the same letters that difference is not significant. If there are two letters, the significance is seen again after that is there at least one letter that is the same or different (insignificant, or significant difference)

The chloroform (102.28 ± 4.10 μg/ml) and acetone extract of *A. officinalis* (105.54 ± 2.42 μg/ml) were showed the best cytotoxic activity on L2OB cells line but petroleum ether extract was significantly influenced on RD cells line (120.57 ± 1.32 μg/ml). The ethanol and acetone extracts were significantly influenced on Hep2 cells, and chloroform and acetone extracts were significantly influenced on L2OB cells.

Discussion

Since ancient times, medicinal plants were used in folk medicine, and for many years these plants have been particularly investigated for their total phenol and flavonoid contents and their antioxidant effects (Lopes-Lutz et al. 2010). Therefore, the aim of this study was examination phytochemical composition

and evaluation of antimicrobial, antioxidant and cytotoxic activity of *Anchusa officinalis* plant in different solvents: ethanol, chloroform, petroleum, acetone and ethyl acetate. The total phenolic content, phenols, flavonoids and tannins in extracts of *A. officinalis* could be related to the solvent polarity. In this research comparative analysis has shown that ethanol extract of *A. officinalis* had the highest concentration of phenols; the ethanol and acetone extract had the highest concentration of flavonoid and chloroform and acetone extract had the highest concentration of the condensed tannins.

The plants contain a wide variety of antioxidants and investigations for novel natural antioxidants of plant origin are in increasing. In this study ethanolic extract of *A. officinalis* showed the strong antioxidant activity. Previous studies of total antioxidant activity methanolic extract of plants *Anchusa italica* showed similar values as values in our study (Al-Snafi 2014). Also, the aqueous extract and methanolic extract of *Anchusa strigosa* have significant antioxidant potential (Al-Snafi 2014).

HPLC analysis was showed that rosmarinic acid is the most dominant in extracts of *A. officinalis*, which is in line with the results of similar studies of plants from family *Boraginaceae* (Zengin et al. 2015). This acid exhibits a wide range of biological activities, and various studies have shown antioxidant, antimicrobial, antiviral, anti-cancer and anti-allergic properties of rosmarinic acid (Hooker et al. 2001; Huang and Zheng 2006). The rosmarinic acid is an efficient antagonist of lipid peroxidation and this polyphenol is able to insert spontaneously in lipid membranes, with a higher affinity for unsaturated than for saturated lipids (Fadel et al. 2011).

The antimicrobial properties of herbal compounds are valuable resources in medicine. The ethanolic extract of the *A. officinalis* plant exhibited the great antimicrobial activity against Gram (+) and Gram (−) bacteria. This can be related to the biological nature of the active ingredients, whose activity may be increased in the presence of alcohol because of its stronger extractant capacity, by which a larger number of active components with antimicrobial activity is being isolated (Bhattacharjee et al. 2006). Manifested antimicrobial activity is closely related to the total concentration of phenol and with a wide range of biological activities, including anti-thrombotic, cardio-protective and vasodilatory, as well as with a broad anti-oxidative potential of the tested extracts, as evidenced by the results of other authors (García-Lafuente et al. 2009).

Natural phytochemicals derived from plants have found use in the treatment of many diseases, including cancer. In this study, the chloroform and acetone extract of *A. officinalis* plant were showed the best cytotoxic ativity on L2OB cells line. Chloroform and acetone extracts of *A. officinalis* exhibited the most effective cytotoxic activity compared to other tested extracts, which can be related to their strong antioxidant potential, variety of pharmacologically active compounds and their synergistic effects, this is in agreement with other research (Ioku et al. 1995; Chen and Chen 2013).

Conclusion

In the summary, this study indicate that *Anchusa officinalis* have certain high content of phenolic components of various extracts and the evaluation of its antimicrobial, antioxidant and cytotoxic potential, which showed that the extracts of *A. officinalis* are enriched with pharmacologically active compounds. This is the first report of citotoxic activity of extract *A. officinalis* from Balkan region on tumor cell lines and therefore could have a medical significance in the future.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict interest.

References

Al-Snafi AE (2014) The pharmacology of Anchusa italica and Anchusa strigosa. A Review. Int J Pharm Pharm Sci 6(4):7–10

Amin GH (2005) Popular medicinal plants of Iran. Tehran University of Medicinal Sciences, Tehran, pp 38–162

Bhattacharjee I, Chatterjee SK, Chatterjee S, Chandra G (2006) Antibacterial potentiality of Argemone mexicana solvent extracts against some pathogenic bacteria. Mem Inst Oswaldo Cruz 101: 645–648

Boskovic I, Djukic D, Maskovic P, Mandic L (2017) Phytochemical composition and biological activity of *Echium italicum* L. plant extracts. Bulg Chem Commun 49(4):836–845

Brighente IMC, Dias M, Verdi LG, Pizzolatti MG (2007) Antioxidant activity and total phenolic content of some Brazilian species. Pharm Biol 45(2):156–161

Chen AY, Chen YC (2013) A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention. Food Chem 138(4): 2099–2107

CLSI (2012) Methods for dilution antimicrobial susceptibility tests f or Bacteria that grow aerobically; approved St andard—ninth edition. CLSI document M07-A9, Wayne pp. 18

Dresler S, Szymczak G, Wójcik M (2017) Comparison of some secondary metabolite content in the seventeen species of the Boraginaceae family. Pharm Biol 55(1):691–695

Fadel O, Ei Kirat K, Morandat S (2011) The natural antioxidant rosmarinic acid spontaneously penetrates membranes to inhibit lipid peroxidation in situ. BBA Biomembranes 1808(12):2973–2980

García-Lafuente A, Guillamón E, Villares A, Rostagno MA, Martínez JA (2009) Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. Inflamm Res 58(9):537–552

Hinneburg I, Dorman HJD, Hiltunen R (2006) Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem 97: 122–129

Hooker CW, Lott WB, Harrich D (2001) Inhibitors of human immunodeficiency virus type 1 reverse transcriptase target distinct phases of early reverse transcription. J Virol 75(7):3095–3104

Hsu CK, Chiang BH, Chen YS, Yang JH, Liu CL (2008) Improving the antioxidant activity of buckwheat (*Fagopyrum tataricm* Gaertn) sprout with trace element water. Food Chem 108(2):633–641

Huang SS, Zheng RL (2006) Rosmarinic acid inhibits angiogenesis and its mechanism of action *in vitro*. Cancer Lett 239(2):271–280

Ioku K, Tsushida T, Takei Y, Nakatani N, Terao J (1995) Antioxidative activity of quercetin and quercetin monoglucosides in solution and phospholipid bilayers. BBA Biomembranes 1234(1):99–104

Iqbal K, Nawaz SA, Malik A, Riaz N, Mukhtar N, Mohammad P, Choudhary MI (2005) Isolation and lipoxygenase-inhibition studies of phenolic constituents from *Ehretia obtusifolia*. Chem Biodivers 2:104–111

Jakovljevic D, Vasic S, Stankovic M, Topuzovic M, Lj Č (2016) The content of secondary metabolites and in vitro biological activity of *Anchusa officinalis* L. (Boraginaceae). Indian J Tradit Knowl 15(4): 587–593

Khare CP (2007) Indian medicinal plants - an illustrated dictionary. Springer Science and Business Media LLC, New York, p 49

Lopes-Lutz D, Dettmann J, Nimalaratne C, Schieber A (2010) Characterization and quantification of polyphenols in Amazon grape (Pourouma cecropiifolia Martius). Molecules 15:8543–8552

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: Aplication to proliferation and cytotoxicity assays. J Immunnol Methods 65:55–63

Prieto P, Pineda M, Aguilar M (1999) Spectrofotometric quanitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 269(2):337–341

Selvi F, Bigazzi M, (2003) Revision of genus Anchusa (Boraginaceae-Boragineae) in Greece. Bot J Linn Soc 142 (4):431-454

Sharma RA, Singh B, Singh D, Chandrawat P (2009) Ethnomedicinal, pharmacological properties and chemistry of some medicinal plants of Boraginaceae in India. J Med Plant Res 3(13): 1153–1175

Singleton V, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 299:152–178

Takao T, Watanabe N, Yagi I, Sakata K (1994) A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci Biotechnol Biochem 58(10):1780–1783

Vermerris W, Nicholson R (2006) Phenolic compound biochemistry. Springer, USA, p 276

Zengin G, Sarikurkcu C, Aktumsek A (2015) Phenolic composition of *Anchusa undulata* L. subsp. hybrida (ten.) Coutinho from Turkey. J Med Herbs and Ethnomed 1(1):1–3