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ANTIOXIDANT AND CYTOTOXIC POTENTIAL OF SELECTED PLANT SPECIES OF THE BORAGINACEAE FAMILY

SUMMARY

Antioxidant activity is one of the most important properties of plant extracts. Antioxidants from natural sources have been intensively studied in the last few decades. The antioxidant contents of medicinal plants may contribute to the protection of diseases. Bioactive components of plants have a potential role in chemoprevention and inhibition of different phases of the malignant transformation process. Therefore, plant extracts and essential oils are in the focus of research, and in recent decades have been tested on a large number of malignant cell lines. The aim of this study was to examine antioxidant and cytotoxic potential of selected plant species from the Boraginaceae family. Determination of antioxidant activity was performed by ammonium-thiocyanate method. Testing citotoxic activity was performed by MTT test on cancer cell lines: HEP 2c (human larynx carcinoma), RD (human cell line-rhabdomyosarcoma) and L2OB (mouse tumor fibroblast line). The best antioxidant activity showed ethanol, acetone and chloroform extracts of *Anchusa officinalis, Echium vulgare* and *Echium italicum.* The tested extracts showed an inhibitory effect on cancer cells, but chloroform and acetone extracts of all three plant had the most effective effect on L2OB cells. Isolation of individual active components from this plants and their testing for cancer cells would be of great importance for this field of research.

Keywords: antioxidans, cytotoxic potential, plant, extracts, Boraginaceae

INTRODUCTION

The use of plants in the treatment and prevention of various diseases dates back to ancient times. In the last few decades, plants and essential oils have been used studied for their antioxidant properties. Antioxidant agents of natural origin have attracted special interest because of their free radical scavenging abilities (Osawa *et al*., 1990). The antioxidant contents of medicinal plants may contribute

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to the protection they offer from disease (Saeed *et al*., 2012). Also, natural-based antioxidants from a plant origin are seen as a promising approach as they are less toxic and more efective (Mishra *et al*., 2014).

Bioactive components of plants have a potential role in chemoprevention and inhibition of different phases of the malignant transformation process. Cancer continues to be a major health challenge, constituting the second- leading cause of death worldwide, despite intensive research that has revealed much about its biology in last few decades (Tariq *et. al*., 2017). The main carcinogens include smoking, unbalanced diet, hormones, chronic infections, genetic mutations, free radicals and UV radiation. According to research by the World Cancer Research Fund, as many as one-third of cancer causes in economically developed countries are related to overweight or obesity, physical inactivity and poor nutrition (American Cancer Society, 2015).

Plant-based bioactive phytochemicals are capable of inhibiting tumor cytogenesis through various means by inhibition or modification of epigenetic processes which suppresses gene initiation, suppression and progression (Greenwell and Rahman, 2015). Therefore, plant extracts and essential oils are in the focus of research, and in recent decades have been tested on a large number of malignant cell lines.

Research by Dai and Mumper (2010) confirmed that a diet rich in phenolic compounds significantly reduces the risk of developing malignancies. Thus, phenolic extracts of berries (blueberries, blackberries, raspberries, cranberries and strawberries), which contain anthocyanins, camphor, quercetin and esters of coumaric and ellagic acid, have been shown to inhibit the growth of colon cancer cell lines (Seeram *et al*., 2006; Zhang and Demain, 2005). Good cytotoxic potential of the cancer cells showed extracts of wine, black and green tea, citrus fruits, olive oil, apples and legumes (Dai and Mumper, 2010).

The anticancer potential of plant components is based on the ability to reduce free radicals, regulate carcinogen-activating and carcinogen-detoxifying enzymes, as well as the ability to inhibit inflammatory cytokines, then on the ability to lead to changes in the regulation of growth factors and target cell pathways. proliferation and apoptosis, as well as angiogenesis, invasion and metastasis of malignant cells (Surh, 2003; Amin *et al*., 2009; Mehta *et al*., 2010; Neergheen *et al*., 2010).

The Boraginaceae family includes approximately 2000 species worldwide, mainly in Europe and Asia (Dresler *et al*., 2017). Plants from the family Boraginaceae are traditionally used in the treatment of fever, asthma, kidney stones, as diuretics and for wound healing (Al-Snafi, 2014). Numerous studies have confirmed anticancer, antioxidant and antimicrobial potential of plant extracts from the family Boraginaceae (Bošković, 2018; Khurm *et al*., 2016; Erdogan *et al*., 2020; Paun *et al*., 2020). Further research and finding new bioactive components with antioxidant and anticancer potential and minimally

harmful effects on healthy untransformed cells is one of the challenges of science today.

Therefore, the aim of this study was to examine the antioxidant and cytotoxic potential of selected plant species from the Boraginaceae family.

MATERIAL AND METHODS

Plant material. Plants of *Anchusa officinalis* L., *Echium vulgare* L. and *Echium italicum* L. were harvested in the period May-June 2013 in flowering phenophase in area of Brđanska gorge near Gornji Milanovac in Serbia. The plant material was ground and degreased with petroleum and then extracted with a series of solvents (chloroform, ethyl-acetate, ethanol, acetone, petroleum) in a Soxlet apparatus. After cooling, they were evaporated on a rotary vacuum evaporator at a temperature of 40°C.

Determination of inhibition lipid peroxidation by ammonium thiocyanate method. The method is based on initiating lipid autooxidation at elevated temperature (Hsu *et al*., 2008). A series of solutions of extracts, ascorbic acid standards and butyl hydroxytoluene with a concentration of 1000 μg/ml in methanol is prepared. The linoleic acid emulsion is prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 emulsion agent and 50 ml of 0.2 M phosphate buffer to pH=7. Homogenization is carried out by vigorous stirring of the solution. A normal container with a prepared linoleic acid emulsion is coated with aluminum foil and left in the refrigerator.

In the series of eight tubes is weighed 0.5 ml of methanol.To the first tube is added 0.5 ml of the stock solution of the test substrate, ie standard, ascorbic acid and butylated hydroxytoluene. Pipette 0.5 ml of the mixed solution from the first tube and transfer to the second tube. By further successive dilution, batches of all solutions with a concentration of 3,901 - 500 μg/ml are prepared. After dilution, linoleic acid emulsion (2.5 ml, 0.02 M, pH = 7) and phosphate buffer (2.5 ml, 0.2 M and pH=7) were added to the extracts and standar solutions. After mixing, the solutions are incubated in the dark, at a temperature of 37ºC to accelerate the peroxidation process.

During the incubation process, aliquots of 100 μl solution are taken at different time intervals (after 24 h, 48 h, 72 h and 96 h) and the degree of autooxidation is determined by adding 4.7 ml of 75% ethanol solution, 100 μl of 30% ammonium thiocyanate solution and 100 μl of a 0.02 M solution of ferric chloride in 3.5% hydrochloric acid solution. After three minutes, by measuring the absorbance of the solution at 500 nm, the degree of inhibition of linoleic acid peroxidation is determined. Ethanol is taken as a test solution in the control sample. The percentage of inhibition is calculated by the formula:

IC (%) = [(Control sample - Sample) / Control test] x 100.

IC50 values are calculated using the formula already mentioned.

Determination of cytotoxic activity by MTT method. Cytotoxic activity of plant extracts was performed by variable MTT test in vitro. In the experiment were used cancer cells grown on nutrient media: Hep2 (medium: MEM Eagle/5%

FCS) human cell line (human larynx carcinoma), RD (medium: MEM Eagle/10% FCS)- (human cell line-rhabdomyosarcoma) and L2OB (medium: MEM Eagle/10% FCS) - (mouse tumor fibroblast line). Cell suspensions, density 10^4, were seeded in a 96-well microtiter plate and allowed to incubate at 37ºC and 5% CO_2 in a thermostat, and the medium was replaced with 100 µl of medium with different concentrations of extracts of plant extracts (concentrations 25, 50, 100, 250, 500, 750 and 1000 µg/ml). Fresh medium without extract was added to the control cells. After 48 h of treatment, cell viability was determined by the MTT cytotoxicity test based on the stained reaction of the mitochondrial enzyme dehydrogenase from living cells with MTT (Mosmann, 1983). After the incubation, MTT (at a final concentration of 5 mg/ml PBS) was added to each well and the plate was incubated for 2-4 h at 37°C. The colored crystals of the formed formazan were dissolved with 150 µl of DMSO. Absorbance was measured at 570 nm on a Microplate Reader. The percentage of viable cells was determined as the absorbance ratio of treated cells and control cells multiplied by 100. The results were obtained from three independent experiments. According to the American National Cancer Institute (NCI), the criterion for cytotoxic activity of plant extracts is IC_{50}<30 µg/ml (Itharat et al., 2004). Experiments using MTT [3-(4,5-dimethylthiazol-2-ol)-2,5-diphenyltetrazolium bromide] were based on the ability of viable cells to degrade tetrazolium with MTT mitochondrial succinate dehydrogenase to give a blue-colored product formazan.

Statistical processing of the obtained results was performed by analyzing the variance of the two-factorial experiment. Multiple mean comparisons were determined using the least significant difference (LSD) test. A probability value of 0.05 was considered significant. All calculations were performed using a statistical program (SPSS, version 11.0). The results of antioxidant activities are presented as the mean±standard deviation of the three determinations.

RESULTS AND DISCUSSION

The results of antioxidant activity of extracts of *Anchusa officinalis* L., *Echium vulgare* L. and *Echium italicum* L. are shown in Table 1. The results showed that ethanol extract of *Anchusa officinalis* had the highest lipid peroxidation inhibition (35.45±1.34 IC_{50} µg/ml), followed by chloroform (37.39±1.26 IC_{50} µg/ml) and acetone extract (37.51±1.11 IC_{50} µg/ml). Ethanol extract of *Echium vulgare* showed the highest lipid peroxidation inhibition (49.48 ±1.33 IC_{50} µg/ml), followed by acetone (50.50 ±1.10 IC_{50} µg/ml) and chloroform extract (51.34 ± 1.06 IC_{50} µg/ml). The acetone extract of *Echium italicum* showed the strongest ability to inhibit lipid peroxidation (42.54±1.13 IC_{50} µg/ml), followed by chloroform (43.29±1.20 IC_{50} µg/ml) and ethanol extract (44.56±1.29 IC_{50} µg/ml). The results of our research showed that plants from family Boraginaceae have good antioxidant potential. Also, good antioxidant potential of selected plant species from the family Boraginaceae confirmed Gharib and Godarzee (2016), Bošković (2018), Zemmouri *et al.* (2019) and Paun *et al.* (2020) in their study. Accordint to Paun *et al*. (2020) extracts of *A. officinalis* had

the excellent scavenging activity (IC50 = 0.0032 mg/ml), comparable with ascorbic acid as the reference (IC50 =0.0036 mg/ml). Our results indicate that polar solvents, such as acetone and ethanol, had better antioxidant potential than non-polar solvents, which is in line with the results obtained by Barchan *et al*. (2014).

Table 1. Inhibition of lipid peroxidation (IC_{50}) of extracts of plant *Anchusa officinalis, Echium vulgare* L. and *Echium italicum* L.

Extract/ Plant	*Anchusa officinalis*	*Echium vulgare L.*	*Echium italicum L.*
Chloroform	37.39 ± 1.26	51.34±1.06	43.29±1.20
Ethyl acetate	40.28 ± 1.23	55.22±1.27	47.26±1.12
Ethanol	35.45 ± 1.34	49.48±1.33	44.56±1.29
Acetone	37.51 ± 1.11	50.50±1.10	42.54±1.13
Petroleum	41.32 ± 1.08	56.38±1.02	49.36±1.10

The values of inhibition of lipid peroxidation are expressed in μg/ml

The results of cytotoxic activity of extracts of *Anchusa officinalis* L., *Echium vulgare* L. and *Echium italicum* L. on Hep 2c, RD and L2OB tumor cells in vitro are shown in Table 2. The cytotoxic effect of the extracts is expressed as IC_{50} μg/ml (concentration that inhibits 50% of cell growth), and the degree of inhibition depended on the type of plant and the solvent used. The tested plant extracts showed an inhibitory effect on cancer cells. But, chloroform (102.28 μg/ml) and acetone extracts (105.54 μg/ml) of *Anchusa officinalis*, chloroform (77.32 μg/ml) and acetone extracts (80.59μg/ml) of *Echium vulgare* and chloroform (87.30 μg/ml) and acetone extracts (91.56 μg/ml) of *Echium italicum* had the most effective effect on L2OB cells. The results of this study are consistent with the studies of Pehlivan-Caracas *et al*. (2012), which proved that plant extracts of *E. vulgare* cause significant inhibition of bile tumors (82%, 63% and 96%). The cytotoxic potential of plant extracts from the family Boraginaceae on the cancer cell line has been reported by other authors (Bošković, 2018; Poma *et al*. 2018; Erdogan *et al*. 2020). Paun *et al*. (2020) pointed out that *A. officinalis* extract caused moderate cytotoxicity on the on the cell line of mouse fibroblast cells line.

Statistical significance of the differences was observed between the plants *Anchusa officinalis* and *Echium vulgare*, *Anchusa officinalis* and *Echium italicum*, as well as between the plants *Echium italicum* and *Echium vulgare* on all cancer cells (factor A). Statistical significance of differences was also observed between ethyl acetate and petroleum extract compared to other tested extracts for Hep 2c cells and L2OB cancer cells (factor B). Chloroform and acetone extracts showed the most effective cytotoxic effect compared to other tested extracts, which can be related to their pronounced antioxidant capacity and

the presence of many pharmacologically active substances, which are in agreement with the results by Boskovic (2018).

Tabela br. 2. Citotoxic activity IC_{50} (µg/ml) tested extracts *Anchusa officinalis* L., *Echium vulgare* L. and *Echium italicum* L. on cancer cell

		Hep 2c	RD	L2OB
Plant	*Ancusa officinalis*	129.51	141.91	111.96
	Echium vulgare	108.69	121.1	91.16
	Echium italicum	117.37	129.76	100.47
Extract	Chloroform	131.34	163.23	88.97
	ethyl-acetate	160.08	175.25	137.67
	Ethanol	122.45	174.18	134.21
	Acetone	124.03	136.35	92.56
	Petroleum	163.79	107.59	146.69
Anchusa officinalis	Chloroform	144.67	176.53	102.28
	ethyl-acetate	173.39	188.59	150.34
	Ethanol	135.44	187.49	147.17
	Acetone	137.34	176.33	105.54
	Petroleum	176.77	120.57	159.35
Echium vulgare	Chloroform	119.65	151.59	77.32
	ethyl-acetate	148.43	163.56	125.30
	Ethanol	110.42	162.54	122.25
	Acetone	112.39	151.37	80.59
	Petroleum	151.81	95.61	134.38
Echium italicum	Chloroform	129.7	161.56	87.30
	ethyl-acetate	158.42	173.61	137.36
	Ethanol	121.48	111	133.20
	Acetone	122.37	162.35	91.56
	Petroleum	162.8	106.59	146.33
LSD 0,05	Factor A	1.676	1.504	2.308
	Factor B	2.37	2.126	3.263
	Factor AxB	4.105	3.683	5.652

Based on the results of antioxidant activity, it is possible to assume that extracts with pronounced antioxidant activity can affect the redox state of cells and thus lead to a decrease in cell proliferation. According to research by Robinson *et al.,* (2017) antioxidant and free radical scavenging activity of the extract may be the reason behind its anti-cancer property.

Finding new anticancer agents that would show a pronounced selective antitumor effect against malignant cells, as well as minimal toxic effect against healthy untransformed cells, and especially against healthy immunocompetent cells, which are involved in immune control of tumor suppression, is extremely important for the development of new drugs in oncology.

CONCLUSIONS

The plant world is an inexhaustible source of pharmacologically active components. Numerous studies in the past few decades have confirmed that these components have remarkable antioxidant potential, and are used in medicine in the prevention and treatment of many diseases, among them cancer. Therefore, the aim of this study was to examine the antioxidant and citotoxic potential of plant from the family Boraginaceae.

Research has indicate that ethanol, acetone and chloroform extracts of *Anchusa officinalis*, *Echium vulgare* and *Echium italicum* showed the best antioxidant activity.

The tested plant extracts showed an inhibitory effect on cancer cells, but chloroform and acetone extracts of the tested plants had the most effective effect on L2OB cells.

This study showed that plant extracts from the Boraginaceae family have cytotoxic potential on cancer cells, and these plants are a source of antioxidants. Isolation of individual active components from plants and their testing for cancer cells would be of great importance for this field of research.

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