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The *in vitro* antioxidative and cytotoxic effects of selected *Salvia* species water extracts

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Summary

The current paper presents antioxidant and cytotoxic activities and total phenolic and flavonoid content of the selected species of genus *Salvia* (Lamiaceae) growing wild in Macedonia (*S. jurisicii* Košanin, *S. amplexicaulis* Lam., *S. ringens* Sibth. & Sm.) and Libya (*S. fruticosa* Mill. and *S. lanigera* Poir.). Crude water extracts, obtained from aerial parts, were yielded from 6.50 to 14.32%. Total phenolic content was the highest in water extracts of *S. amplexicaulis* and *S. ringens* (226.30 and 189.01 mg GAE/g, respectively), while the flavonoids were the most abundant in *S. jurisicii* extract (32.36 mg QE/g). Antioxidant activities of extracts were measured using DPPH, ABTS and FRAP assays. *S. amplexicaulis* and *S. ringens* extracts showed the strongest antioxidant activity, measured using DPPH (14.21 and 23.44 µg/mL, respectively) and ABTS assays (2.91 and 2.42 mg AAE/g, respectively). In FRAP assay, *S. amplexicaulis* and *S. fruticosa* extracts exhibited strongest activity (1406.73 and 1191.51 µmol Fe(II)/g). Water extract of *S. amplexicaulis* and *S. ringens* performed the strongest cytotoxic activity against K562 cells (151.07 and 173.06 µg/mL, respectively). Based on these findings, it can be concluded that *S. amplexicaulis* and *S. ringens* water extracts could be considered as possible source of antioxidant and cytotoxic agents.

Introduction

The genus *Salvia* (Lamiaceae) represents an enormous and cosmopolitan assemblage of nearly 1000 species worldwide of which 36 were found in Europe (HEDGE, 1972) and 10 in Libya (JAFRI and EL-GADI, 1985). The representatives of *Salvia* genus are widely cultivated and used in flavoring and folk medicines.

Sage and rosemary from the Lamiaceae family were shown to have similar patterns of phenolic compounds and the antioxidant activity had been attributed mainly to carnosic, rosmarinic acid and their isomers. Additional classes of active compounds include terpenoids, flavonoids and other phenolic acids (LU and FOO, 2001). Majority of these compounds, excluding terpenoids, are water-soluble and present in aqueous extract obtained using common techniques of extraction (TIWARI et al., 2011).

The genus *Salvia* was the research topic of numerous chemical, medicinal and pharmacological studies. Species of *Salvia* showed diverse biological activities of plant material and/or isolated essential oil/extracts due to the presence of large number of different compounds. The antioxidant (COULADIS et al., 2003; JANICSÁK et al., 2010; ORHAN et al., 2013; ALIMPIĆ et al., 2014), cytotoxic (FIORE et al., 2006; KAMATOU et al., 2005; JANICSÁK et al., 2007; ABU-DAHAB et al., 2012), antimicrobial (KAMATOU et al., 2005; HAWAS and EL-ASNARI, 2006), antiinflammatory (KAMATOU et al., 2005;

2010), antimalarial (KAMATOU et al., 2005), anticholinesterase (ŠENOL et al., 2010, ORHAN et al., 2012; 2013), etc. effects were reported.

S. jurisicii Košanin, perennial herb inhabiting arid habitats, is an endemic species in the central part of Republic of Macedonia (HEDGE, 1972). It was previously investigated for antioxidant activity (JANICSÁK et al., 2010).

S. amplexicaulis Lam. is a perennial plant, distributed on Balkan Peninsula (HEDGE, 1972). It was investigated for vasodepressor (ULUBELEN, 2003), antioxidant, and neurobiological activity (ORHAN et al., 2012) of extracts.

S. ringens Sibth. & Sm. is a hardy herbaceous perennial plant, distributed in South and Eastern parts of Balkan peninsula (HEDGE, 1972). Total acetone extract and some isolated compounds showed cytotoxic activity against HeLa cells (JANICSÁK et al., 2007), while ethanol extracts performed strong antioxidant activity (COULADIS et al., 2003).

S. lanigera Poir. is perennial herb with thick woody rootstock distributed in North Africa, from northern Egypt and Arabia, to the south of Turkey and Iran (JAFRI and EL-GADI, 1985). There are a few reports on phenolic composition, antimicrobial and cytotoxic activity of *S. lanigera* extracts (HAWAS and EL-ASNARI, 2006; SHAHEEN et al., 2011).

S. fruticosa Mill. (*S. triloba* L.) is subshrub distributed in Canary Islands and North Africa. It is sometimes used for flavouring tea and cultivated as an ornamental plant (JAFRI and EL-GADI, 1985). Jordanian *S. fruticosa* ethanol extract performed significant cytotoxic activity (ABU-DAHAB et al., 2012).

The objective of this study was to investigate antioxidative and cytotoxic activity as well as phenolic/flavonoid contents in water extracts of selected Macedonian and Libyan *Salvia* species.

Material and methods

Plant material

Aerial parts of the *Salvia* species were collected in flowering period from their natural populations at localities in Macedonia and Libya and were identified by Prof. P.D. Marin and Prof. V. Matevski. Plant material was dried and kept in shadow at room temperature for further processing. Total sample for each species consisted of at least 20-50 individuals (about 500 g of dry material, depending of species). Voucher specimens were deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade. Collection data and voucher numbers of investigated *Salvia* species are given in Tab. 1.

Preparation of water extracts

Extracts were prepared of whole aerial plant parts using classic maceration procedure. Dry plant material (5 g), randomly taken

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Tab. 1: Collection data and voucher numbers of examined *Salvia* species

<i>Salvia</i> species	Collection data			Voucher No.
	Locality	Date	Geographical data	
<i>S. jurisicii</i> Košanin	Štip (Macedonia)	11.7.2011	N 41°50.324' E 22°08.289' Alt. 420 m	BEOU; 16674
<i>S. amplexicaulis</i> Lam.	Pletvar (Macedonia)	12.7.2011	N 41°22.169' E 21°39.180' Alt. 1010 m	BEOU; 16673
<i>S. ringens</i> Sibth. & Sm.	Krivolak (Macedonia)	2.7.2012	N 41°53.33' E 21°41.08' Alt. 229 m	BEOU; 16675
<i>S. lanigera</i> Poir.	Zintan (Libya)	22.3.2010	N 31°55.50' E 12°14.54' Alt. 694 m	BEOU; 16880
<i>S. fruticosa</i> Mill.	Biadda (Libya)	10.3.2010	N 32°45.59' E 21°44.30' Alt. 624 m	BEOU; 16702

from whole collected sample of each species, was grounded in small pieces (2-6 mm) and extracted by 50 ml of boiling distilled water (10% w/v). Extraction was performed during 24 h at room temperature. The mixture was exposed to ultrasound 1 h before and after 24 h-maceration. Subsequently, extracts were filtered through a paper filter (Whatman No.1) and evaporated under reduced pressure by the rotary evaporator (Buchi rotavapor R-114). The obtained crude extracts (Tab. 2) were stored in the fridge at +4 °C for further experiments.

Tab. 2: The yield, total phenolic and flavonoid content of *Salvia* water extracts. Values are presented as mean ± standard deviation.

Water extracts	Extract yield ^a	Total phenolic content ^b	Total flavonoid content ^c
<i>S. jurisicii</i>	9.50	81.03 ± 0.216	32.36 ± 0.731
<i>S. amplexicaulis</i>	14.32	226.30 ± 1.179	17.87 ± 0.089
<i>S. ringens</i>	6.50	189.01 ± 1.699	22.64 ± 0.898
<i>S. lanigera</i>	7.32	58.47 ± 0.200	17.18 ± 0.544
<i>S. fruticosa</i>	7.62	67.68 ± 0.001	21.73 ± 0.163

^a % of weight of dry plant material

^b mg GAE/g dry extract

^c mg QE/g dry extract

Evaluation of antioxidant activity

Antioxidant activity was evaluated using three spectrophotometric assays: DPPH, ABTS, and FRAP. Stock solutions of dry extracts were prepared in the distilled water in concentration of 1000 µg/mL (w/v).

DPPH assay

For evaluation of antioxidant activity of extracts, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method (BLOIS, 1958) with slight modifications was used. Stock extract solution was diluted with methanolic solution of DPPH (40 µg/mL) to adjust the final volume of reaction mixture (2000 µL) of the test tube. Methanol was used as a blank, while methanol with DPPH solution was used

as a control. BHA, BHT and ascorbic acid were used as positive controls (standards). Each blank, samples and standards' absorbances were measured in triplicate. Absorbance of the reaction mixture was measured after 30 min in the dark at room temperature at 517 nm using the JENWAY 6305UV/Vis spectrophotometer. The decrease of absorption of DPPH radical at 517 nm was calculated using equation:

$$\text{Inhibition of DPPH radical (\%)} = [(A_c - A_s) / A_c] * 100\%$$

where A_c is the absorbance of control (without test sample) and A_s is the absorbance of the test samples at different concentrations. IC₅₀ values (µg/mL) (concentrations of the test samples and standard antioxidants providing 50% inhibition of DPPH radicals) were calculated from DPPH absorption curve at 517 nm.

ABTS assay

ABTS assay is performed according to procedure of MILLER et al. (1993) with some modifications. Fresh ABTS⁺ solution was prepared 12-16 hours before use by dissolving ABTS in 5 ml of 2.46 mmol potassium-persulfate to obtain concentration of 7 mmol/L and stored in the dark at room temperature. The ABTS⁺ solution was dissolved by distilled water to achieve an absorbance of working solution 0.700 ± 0.020 at 734 nm. 50 µL of test samples (1 mg/mL) and/or standards (0.1 mg/mL) were mixed with 2 mL of diluted ABTS⁺ solution and incubated for 30 min at 30 °C. Absorbance was recorded at 734 nm using JENWAY 6305UV/Vis spectrophotometer. Distilled water was used as blank. BHA and BHT dissolved in methanol were used as standards. ABTS activity was calculated from ascorbic acid calibration curve (0-2 mg/L) and expressed as ascorbic acid equivalents per gram of dry extract (mg AAE/g).

Ferric-reducing ability of plasma (FRAP) assay

The FRAP assay was performed according to BENZIE and STRAIN (1996) procedure with slight modifications. FRAP reagent was prepared freshly to contain sodium acetate buffer (300 mmol/L, pH 3.6), 10 mmol/L TPTZ in 40 mmol/L HCl and FeCl₃·6H₂O solution (20 mmol/L), i.e. in proportion 10:1:1(v/v/v), respectively. Working FRAP solution was warmed to 37 °C prior to use. 100 µL of test sample (500 µg/mL) were added to 3 mL of working FRAP reagent and absorbance was recorded at 593 nm after 4 min using the

JENWAY 6305UV/Vis spectrophotometer. Blank was prepared to contain distilled water instead of extract. Ascorbic acid, BHA and BHT dissolved in methanol in concentration 0.1 mg/mL were used as standards. The same procedure was repeated for standard solution of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (200-1600 $\mu\text{mol/L}$) in order to construct calibration curve. FRAP values of sample was calculated from standard curve equation and expressed as $\mu\text{mol Fe (II)/g}$ dry extract).

Determination of total phenolic content

The total phenolic content was measured using spectrophotometric method (SINGLETON and ROSSI, 1965). The reaction mixture was prepared by mixing 0.2 mL of extract solution in concentration of 1 mg/mL, 1 mL of 10% Folin-Ciocalteu reagent and 0.8 mL of 7.5% Na_2CO_3 . Blank was prepared to contain distilled water instead of extract. Absorbance was recorded at 740 nm after 2 h incubation at room temperature using JENWAY 6305UV/Vis spectrophotometer. Phenolic content in samples was calculated from standard curve equation and expressed as gallic acid equivalents (mg GAE/g dry extract).

Determination of flavonoid concentration

Flavonoid concentrations of samples were measured spectrophotometrically according to procedure of PARK et al. (1997). The reaction mixture was prepared by mixing 1 mL of extract solution in concentration 1 mg/mL, 4.1 mL of 80% ethanol, 0.1 mL of 10% $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ and 0.1 mL 1M CH_3COOK . Blank was prepared to contain 96% ethanol instead of extract. After 40 min of incubation at room temperature, absorbance was measured at 415 nm using JENWAY 6305UV/Vis spectrophotometer. Concentration of flavonoids in samples (mg/ml) was calculated from standard curve equation and expressed as quercetin equivalents (mg QE/g dry extract).

Cytotoxic assay – MTT assay

To assess cytotoxic effect of sage water solutions, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed (DRAKULIĆ et al., 2012). It is a colorimetric assay which detects conversion of yellow tetrazolium salt into purple formazon. The conversion is catalyzed by cellular enzymes and its rate represents measure of cells viability. K562 cells are human immortalised myelogenous leukemia line. K562 cells are of the erythroleukemia type. The line is derived from a CML patient in blast crisis. K562 cells were maintained in essential minimal medium (MEM) supplemented with 10% FCS. Cells were treated in 96 well plates for 48 h with 50, 100, 150, 200, 300 and 400 $\mu\text{g/mL}$ sage water extracts in MEM. After addition of MTT solution (0.5 mg/mL), plate was incubated for additional 3 h. Acidified isopropanol was added to dissolve tetrazolium salts. Absorbance was measured at 620 nm.

Statistical analysis

All experimental measurements were carried out in triplicate and are expressed as average of three measurements \pm standard deviation. Pearson's correlation coefficients were calculated between on one hand total phenolics and flavonoids and on the other hand cytotoxic and antioxidant assays and interpreted according to TAYLOR (1990). Calculations and constructing of the charts were performed using the MS Office Excel, 2007.

Results and discussion

The yield of extract, total phenolic and flavonoid content

The yields were expressed as percent of dry extract of dry plant material. The yields of obtained water extract were ranged from

14.32% for *S. amplexicaulis* to 6.50% for *S. ringens* extract (Tab. 2). Considering the uniform extraction procedure applied in this study, variations in extract's yields can be attributed to differences of plant material (species). In some previous studies researchers have observed that type of plant material, choice of extraction solvent and extraction procedure affects composition, activity and possible future use of the obtained extract (TIWARI et al., 2011). ŞENOL et al. (2010) obtained that yield of methanolic extract of 55 *Salvia* taxa varied between 2.88 and 13.41% and our results are in agreement with these findings.

Total phenolic and flavonoid contents of water extracts were evaluated spectrophotometrically and expressed as gallic acid equivalents/g dry extract and flavonoids concentrations as quercetin equivalents/g dry extract. As can be seen in Tab. 2, the highest amount of phenolics was measured in water extracts of *S. amplexicaulis* and *S. ringens* (226.30 and 189.01 mg GAE/g, respectively), while the content of phenolics in the other extracts was lower than 100 mg GAE/g. On the contrary to phenolics, flavonoids were the most abundant in *S. jurisicii* extract (32.36 mg QE/g). When the obtained results are compared to the values for total phenolic content of *Camellia sinensis* green tea (140.11 mg GAE/g) and *Ginkgo biloba* (140.18 mg GAE/g) standardized extract (STANKOVIĆ et al., 2010), it can be seen that *S. amplexicaulis* and *S. ringens* have higher values. Our results are in agreement with the literature data for ethanol extracts of fourteen Turkish *Salvia* species (57.10-218.09 mg GAE/g for total phenols and 8.29-108.78 mg QE/g for flavonoids) obtained by ORHAN et al. (2013). STAGOS et al. (2012) found total phenolic content of 267 and 190 mg GAE/g for methanolic and water extract of *S. fruticosa* from Greece, respectively. As many researchers reported, amount of extracted phenolics and flavonoids depends on selection of extraction solvent.

Evaluation of antioxidant activity

Oxygen sometimes can be fatal for the organisms although eukaryotic organisms cannot exist without it, which is known as oxygen paradox. A free radical is any species capable of independent existence that contains one or more unpaired electrons. Reactive oxygen species (ROS) may damage important cellular molecules such as DNA, proteins and lipids, causing cancer, cardiovascular, neurodegenerative and other diseases (HALLIWELL, 2006).

Antioxidant activities of *Salvia* species water extracts measured using DPPH, ABTS and FRAP assays are presented in Tab. 3. DPPH and ABTS assays were applied to examine scavenging activity of extracts, while FRAP assay measured ability of extracts to reduce Fe (III) to Fe (II) ion. Water extract of *S. amplexicaulis* showed markedly the strongest antioxidant activity in all three assays.

Tab. 3: DPPH, ABTS and FRAP activity of *Salvia* water extracts.

Water extracts	DPPH assay (IC50, $\mu\text{g/ml}$)	ABTS assay (mg AAE/g)	FRAP assay ($\mu\text{mol Fe(II)/g}$)
<i>S. jurisicii</i>	212.24	1.15 \pm 0.062	290.14 \pm 6.880
<i>S. amplexicaulis</i>	14.21	2.91 \pm 0.019	1406.73 \pm 8.055
<i>S. ringens</i>	23.44	2.42 \pm 0.019	615.44 \pm 6.720
<i>S. lanigera</i>	230.87	1.77 \pm 0.085	79.13 \pm 5.255
<i>S. fruticosa</i>	48.11	1.98 \pm 0.005	1191.51 \pm 8.109
Positive controls			
BHT	17.94	2.75 \pm 0.021	445.34 \pm 5.772
BHA	13.37	2.82 \pm 0.011	583.72 \pm 5.255
Ascorbic acid	5.11	/	180.81 \pm 8.607

DPPH activity of extracts was assessed as good ($IC_{50} < 30 \mu\text{g/ml}$) for *S. amplexicaulis* and *S. ringens*, as moderate $30 < IC_{50} < 80 \mu\text{g/ml}$ for *S. fruticosa* and as poor ($IC_{50} > 80 \mu\text{g/ml}$) for *S. jurisicii* and *S. lanigera* according to KAMATOU et al. (2010). *S. amplexicaulis* water extract showed the best DPPH radical scavenging activity ($14.21 \mu\text{g/ml}$), higher than activity of the synthetic antioxidant BHT ($17.94 \mu\text{g/ml}$) and commercially used green tea ($20.62 \mu\text{g/ml}$) (STANKOVIĆ et al., 2010) and very close to BHA ($13.37 \mu\text{g/ml}$). Besides, water extract of *S. amplexicaulis* performed stronger activity than ethanol and methanol extracts (28.74 and $21.28 \mu\text{g/ml}$, respectively) as previously reported by ALIMPIĆ et al. (2014). *S. fruticosa* water extract showed lower DPPH activity than those measured by STAGOS et al. (2012) for methanolic and water *S. fruticosa* extracts, 22 and $16 \mu\text{g/ml}$, respectively. JANICSÁK et al. (2010) previously found low antioxidant activity of *S. jurisicii* aqueous-methanol extract ($191.2 \mu\text{g/ml}$), as confirmed in this study.

In ABTS assay, *S. amplexicaulis* and *S. ringens* showed the strongest activity ($> 2 \text{ mg AAE/g}$), while other extracts were quite weaker than aforementioned (Tab. 3). Some researchers preferred to express ABTS activity by IC_{50} value (KAMATOU et al., 2010; STAGOS et al., 2012), and more frequently using standard equivalents (Trolox, ascorbic acid, etc.). In this study, the ascorbic acid calibration curve was chosen for presentation of obtained results. LI et al. (2008) measured wide range of ABTS activity of selected Chinese medicinal plants ($0.97\text{--}265.43 \mu\text{mol Trolox/g}$ dry plant material). *S. fruticosa* showed activity of $13 \mu\text{g/ml}$ for methanolic and $29 \mu\text{g/ml}$ for water extract (STAGOS et al., 2012).

S. amplexicaulis followed by *S. fruticosa* water extract showed FRAP activity over $1000 \mu\text{mol Fe(II)/g}$. In contrast, *S. lanigera* water extract performed FRAP activity lower than $100 \mu\text{mol Fe(II)/g}$ (Tab. 3). BENZIE and STRAIN (1996) suggested expressing of results using Fe(II) calibration curve. Some researchers preferred to present results by IC_{50} values or as Trolox equivalents. The lacking of a unique form of presenting results makes comparison of results obtained in various studies rather complicated. LI et al. (2008) measured FRAP ability of methanol extracts of 45 Chinese medicinal plants, and results ranged as $1.23\text{--}453 \mu\text{mol Fe(II)/g}$. Ethyl-acetate extracts of selected Turkish *Salvia* species exhibited quite low FRAP activity, whereas methanol extracts were highly active (ORHAN et al., 2012).

Cytotoxic activity of extracts

Cytotoxic activity of extracts was tested using MTT assay against K562 cell line over 48 h. Obtained results, expressed as IC_{50} , are presented in Fig. 1. All tested water extracts exhibited cytotoxic effect on K562 cells. Among examined *Salvia* species, *S. amplexicaulis* and *S. ringens* showed the strongest antioxidant activity ($IC_{50} <$

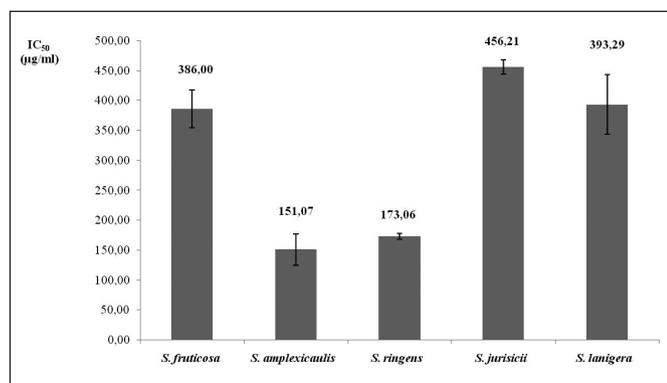


Fig. 1: Cytotoxic activity of water extracts of tested *Salvia* species tested against K562 cell line

$200 \mu\text{g/ml}$), followed by *S. fruticosa* and *S. lanigera* (IC_{50} $200\text{--}400 \mu\text{g/ml}$) and *S. jurisicii* ($> 400 \mu\text{g/ml}$).

Some of the species examined in this study were partially investigated before for their cytotoxic activity. Ethanol extract of *S. fruticosa* collected in Jordan showed lower IC_{50} values ($17.43\text{--}38.91 \mu\text{g/ml}$) against breast cancer cell lines measured by Sulphorhodamine B assay (ABU-DAHAB et al., 2012) than those in our study. Water extract of Libyan *S. lanigera* in this study was less active than several extracts of Egyptian *S. lanigera* (IC_{50} values from 9.83 to over $100 \mu\text{g/ml}$), especially obtained by acetone (SHAHEEN et al., 2011). JANICSÁK et al. (2007) reported on cytotoxic activity of Bulgarian *S. ringens* extract and its isolated components. The literature data on cytotoxic activity of *S. ringens*, *S. jurisicii* and *S. amplexicaulis* were not available. Previous studies have demonstrated that South African and Jordanian *Salvia* species showed IC_{50} values from approximately 20 to above $100 \mu\text{g/ml}$ (KAMATOU et al., 2005) and from 90 to $400 \mu\text{g/ml}$ (FIORE et al., 2006), respectively. Our findings are in accordance with previous reports.

Correlation between cytotoxic and antioxidant activities and total phenolic and flavonoid content

Pearson's correlation coefficients were calculated between total phenolics and flavonoid content of the *Salvia* water extracts and their antioxidant and cytotoxic activities (Tab. 4). In this study, interpretation of correlation coefficients according to TAYLOR (1990) was chosen. On the contrary to ABTS and FRAP activity, cytotoxic and DPPH activity of extracts were negatively correlated to phenolic and positively to flavonoid content because of presenting of results as IC_{50} values (Tab. 4). Correlation between total phenolic and flavonoid contents was negative (data not presented). It was previously reported that water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics were only important as antioxidant compounds (TIWARI et al., 2010). However, obtained results indicated that biological activities of extracts were negatively correlated to flavonoid content in extracts. Previously, BEN FARHAT et al. (2014) reported on negative correlation of some flavonoids from *Salvia officinalis* extracts and antioxidant activity evaluated by DPPH, ABTS and FRAP assays. Our findings are in agreement with various studies which showed that antioxidant activity of rosemary and sage, both belonging to the family Lamiaceae, is mostly manifested by presence of the phenolic acids (rosmarinic and carnosic acids and their derivatives) and then terpenoids, flavonoids and other phenolic acids (LU and FOO, 2001; KAMATOU et al., 2010; ORHAN et al., 2012).

Tab. 4: Linear correlation coefficients (r) of cytotoxic and antioxidant activities versus total phenolic and flavonoid content of *Salvia* water extracts

	Total phenolic content	Flavonoid content
Cytotoxic activity	-0,9514 ^c	0,4886 ^b
DPPH activity	-0,7338 ^c	0,3248 ^a
ABTS activity	0,8394 ^c	-0,6972 ^c
FRAP activity	0,5512 ^b	-0,3169 ^a

According to Taylor (1990):

^a $r \leq 0.35$ weak correlation; ^b $0.36 < r < 0.67$ moderate correlation;

^c $0.68 < r < 1$ strong correlation

Conclusions

Based on these findings, it can be concluded that some of the examined extracts could be taken into consideration as possible anti-

oxidant and cytotoxic agents. The results showed that certain species have optimal ratio of the yield, total phenolic and flavonoid content and performed antioxidant and cytotoxic activities, such as Macedonian *S. amplexicaulis*, *S. ringens* and Libyan *S. fruticosa*. Taking into account non-toxicity of the water as extraction solvent, their application in prevention and treatment of some free radical caused disorders such as cancer, cardiovascular and neurodegenerative diseases could be proposed. Future studies will provide data on qualitative composition of phenolics and other possible biologically active components of the water extracts (research in progress).

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