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## Polyphenolic Content and Biological Activities of Post-Distillation Waste of Three Sage Species from the Republic of Macedonia

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### Abstract

This research was aimed at investigating polyphenolic content, antioxidant and anti-neurodegenerative activities of post-distillation waste extracts of Macedonian *Salvia amplexicaulis*, *S. jurisicii* and *S. ringens*, for the first time. Total phenolic and flavonoid contents varied in a broad range (3.92-146.49 mg GAE/g and 7.11-67.51 mg QE/g, respectively), with the highest values obtained for *S. amplexicaulis* and *S. ringens* extracts. Certain *S. amplexicaulis* and *S. ringens* extracts neutralized more than 80% of DPPH radicals at the highest concentration, while *S. amplexicaulis* extracts showed up to ≈50% inhibition of β-carotene oxidation in β-carotene/linoleic acid assay. Post-distillation waste extracts inhibited acetylcholinesterase (25.94-38.15%) and tyrosinase (18.84-59.52%), with the strongest inhibition of *S. amplexicaulis* extracts. The obtained results suggest that post-distillation waste of tested species, especially of *S. amplexicaulis*, show antioxidant and anti-neurodegenerative activities and could be considered as potential raw material rich in polyphenols.

**Key words:** *Salvia*, extracts, post-distillation waste, biological activities, polyphenols

## Introduction

Lamiaceae plants are aromatic due to presence of essential oils from which they are usually isolated by hydro-distillation (Bakkali et al., 2008). Post-distillation waste (deodorized extracts) of aromatic plants is proved to be a rich source of polyphenolic components (Dapkevicius et al., 1998; Tepe et al., 2005; Džamić et al., 2013; Gavarić et al., 2015). Polyphenols have shown numerous biological activities including antimicrobial, antioxidant and anti-neurodegenerative activities (Tepe et al., 2005; Orhan et al., 2012; Gavarić et al., 2015) providing health benefits for prevention and treatment of various diseases.

Oxidative stress is generated by accumulation of free radicals in the body, causing development of chronic and degenerative diseases, such as cancer, autoimmune disorders, aging, rheumatoid arthritis, cardiovascular and neurodegenerative diseases. Free radicals affect both the structure and function of neural cells, contributing to a wide range of neurodegenerative disorders, including Alzheimer's disease (AD) and Parkinson's disease (PD) (Jiang et al., 2016). One of the most widely used treatment of AD and PD is the inhibition of acetylcholinesterase and tyrosinase, enzymes involved in pathogenesis (Orhan et al., 2012).

Genus *Salvia* L. (sage) comprises about 1.000 species of which several are commercially cultivated as medicinal, culinary and ornamental plants. In the flora of Europe, this genus is represented by 36 species. *Salvia amplexicaulis* is distributed in Balkan Peninsula, *S. ringens* in Eastern part of Balkan Peninsula, while *S. jurisicii* is endemic species in the central part of the Republic of Macedonia (Hedge, 1972). Dominant components of essential oils of samples collected from the Republic of Macedonia were sesquiterpenes (*S. amplexicaulis* and *S. jurisicii*) and monoterpenes (*S. ringens*) (Alimpić et al., 2015a,b; 2016b). The extracts of all examined plants, especially of *S. amplexicaulis* and *S. ringens*, contained different polyphenolic components, and exhibited antioxidant, antimicrobial, cytotoxic and anti-neurodegenerative activities (Orhan et al., 2012; Alimpić et al., 2014; 2015b; 2016a,b; 2017). Our previous studies proved antioxidant and neurodegenerative enzymes-inhibiting activities of total extracts of examined sage species, while in this study we intended to examine the mentioned activities of residual plant material obtained after essential oil isolation.

The aim of this study was to analyze total phenolic and flavonoid content, as well as antioxidant and anti-neurodegenerative activities of ethanolic, methanolic and aqueous post-distillation waste extracts of Macedonian *S. amplexicaulis*, *S. jurisicii* and *S. ringens*.

## Material and Methods

Essential oils of three sage species were isolated by hydro-distillation using Clevenger type apparatus (Alimpić et al., 2015a,b; 2016b). Subsequently, post-distillation waste was air-dried. The extracts were prepared by maceration of post-distillation waste (10 g) using 70% ethanol, 70% methanol and hot distilled water (100 mL) according to the previously described procedure (Alimpić et al., 2014; 2015b; 2016a,b).

Total phenolic and flavonoid contents were measured according to the previously described experimental protocol (Alimpić et al., 2014; 2015b; 2016a,b), and results were recorded using PERKIN ELMER LAMBDA BIO UV/VIS spectrophotometer. Total phenolic and flavonoid content were expressed as gallic acid equivalents per gram of dry extract (mg GAE/g) and quercetin equivalents per gram of dry extract (mg QE/g), respectively.

Antioxidant activity was evaluated by DPPH and  $\beta$ -carotene/linoleic acid assays and results were recorded by PERKIN ELMER LAMBDA BIO UV/VIS spectrophotometer. Commercial antioxidants BHA, BHT and ascorbic acid were employed as positive controls (standards). DPPH assay was performed as described before (Alimpić et al., 2014; 2015b; 2016a,b) with slight modifications. The reaction mixture contained 900  $\mu$ L of methanolic solution of DPPH (40  $\mu$ g/mL) and 100  $\mu$ L of extract/standard solution. Inhibition of DPPH radicals was calculated using equation:  $(A_{sp}-A_{uz}) / (A_{sp}) \times 100\%$ , where  $A_{sp}$  - absorbance of control (without sample) i  $A_{uz}$  - absorbance of reaction mixture (contain extract/standard in different concentration). The  $\beta$ -carotene / linoleic acid assay was performed according to slightly modified experimental procedure of Alimpić et al. (2016a,b). The reaction mixture contained 140  $\mu$ L of sample (extract/standard) and 1000  $\mu$ L of the solution, while control contained methanol instead of sample. The antioxidant activity is calculated using equation:  $[(A_{120}-C_{120})/(C_0-C_{120})] \times 100\%$ , where  $A_{120}$  and  $C_{120}$  are absorbances of samples and control after 120 min incubation, and  $C_0$  is absorbance of control in  $t=0$  min.

Anti-neurodegenerative activity is evaluated spectrophotometrically, using acetylcholinesterase (AChE) and tyrosinase (TYR) inhibition assays, which are performed according to previously published procedures (Alimpić et al., 2016a,b) with slight modifications. Commercial inhibitors galanthamin and kojic acid were used as positive controls. Absorbances were recorded using Tecan Sunrise SN microplate reader equipped by XFluor4 software. The inhibition of AChE was calculated using equation:  $[C-(S-B)/C] \times 100\%$ , where C - control (did not contain sample), S - reaction mixture (contained sample), B - blank (did not contain enzyme).

The inhibition of tyrosinase was calculated using equation:  $[(A-B)-(C-D) / (A-B)] \times 100\%$ , where A - contained buffer and enzyme, B - contained only buffer, C - contained sample, buffer and enzyme, and D - contained buffer and sample.

All experimental measurements were performed in triplicate and presented as average  $\pm$  standard deviation.

## Results and Discussion

The extract yields varied between 3.49% and 12.93%, with the highest values were obtained for ethanolic extracts (Table 1). Total phenolic and flavonoid contents increased in dose-depended manner and ranged from 3.92 to 146.49 mg GAE/g and from 7.11 to 67.51 mg QE/g, respectively. The highest total phenolic and flavonoid contents were recorded in certain *S. amplexicaulis* and *S. ringens* extracts (Table 1).

Tab. 1. Yield of extracts, total phenolic and flavonoid contents of three Macedonian sage species post-distillation waste extracts  
*Принос екстраката, садржај укупних фенола и флавоноида у екстрактима постдестилационих остатака три врсте жалфија из Македоније*

Plant species Билјне врсте	Extracts Екстракти	Yield Принос (%)	Total phenolic content Садржај ук. фенола (mg GAE/g)*			Flavonoid content Садржај флавоноида (mg QE/g)**		
			100	200	500	100	200	500
			$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$
<i>S. amplexicaulis</i>	ethanolic/ етанолни	5.18	16.88 $\pm$ 0.37	33.50 $\pm$ 0.75	49.82 $\pm$ 0.61	27.56 $\pm$ 0.47	47.50 $\pm$ 0.56	67.51 $\pm$ 0.63
	methanolic/ метанолни	4.10	30.72 $\pm$ 0.74	60.46 $\pm$ 1.08	104.44 $\pm$ 1.01	10.41 $\pm$ 0.39	20.38 $\pm$ 0.63	36.81 $\pm$ 0.49
	aqueous/ водени	3.49	34.54 $\pm$ 0.64	65.59 $\pm$ 0.85	107.51 $\pm$ 0.78	15.66 $\pm$ 0.34	24.39 $\pm$ 0.44	41.69 $\pm$ 0.35
<i>S. jurisicii</i>	ethanolic/ етанолни	12.57	3.92 $\pm$ 0.56	14.92 $\pm$ 0.44	20.99 $\pm$ 0.56	7.42 $\pm$ 0.33	12.80 $\pm$ 0.33	26.06 $\pm$ 0.44
	methanolic/ метанолни	7.17	11.20 $\pm$ 0.48	24.81 $\pm$ 0.40	47.08 $\pm$ 0.64	9.47 $\pm$ 0.46	13.11 $\pm$ 0.28	20.81 $\pm$ 0.35
	aqueous/ водени	8.26	10.06 $\pm$ 0.61	14.89 $\pm$ 0.57	25.47 $\pm$ 0.46	7.52 $\pm$ 0.33	12.86 $\pm$ 0.47	19.94 $\pm$ 0.34
<i>S. ringens</i>	ethanolic/ етанолни	12.93	17.24 $\pm$ 0.41	33.30 $\pm$ 0.56	69.47 $\pm$ 1.13	10.38 $\pm$ 0.28	17.24 $\pm$ 0.19	33.64 $\pm$ 0.34
	methanolic/ метанолни	4.64	44.73 $\pm$ 1.11	67.97 $\pm$ 1.47	146.49 $\pm$ 1.35	12.80 $\pm$ 0.28	28.05 $\pm$ 0.28	56.14 $\pm$ 0.23
	aqueous/ водени	6.51	5.71 $\pm$ 0.31	7.84 $\pm$ 0.40	15.51 $\pm$ 0.49	7.11 $\pm$ 0.30	9.51 $\pm$ 0.25	22.18 $\pm$ 0.43

\*mg GAE/g - mg gallic acid equivalents per gram of dry extract;

\*\*mg QE/g - mg quercetin equivalents per gram of dry extract

Regarding the tested concentrations, total phenolic and flavonoid contents in post-distillation waste were comparable with previously obtained results for total extracts of same sage species (Alimpić et al., 2014; 2015b; 2016a,b) and with the results obtained for deodorized extracts of hyssop (Džamić et al., 2013) and thyme (Glavarić et al., 2015).

The analyzed extracts performed dose-dependent antioxidant activity in both applied assays. Certain *S. amplexicaulis* and *S. ringens* extracts neutralized DPPH radical at the lowest applied concentration with efficiency similar to commercial antioxidants BHA and BHT. At the highest concentration, the tested extracts reduced more than 80% of DPPH radicals (Table 2).

Tab. 2. Antioxidant activity of three Macedonian sage species post-distillation waste extracts

*Антиоксидативна активност екстраката постдестилационих остатака три врсте жалфија из Македоније*

Plant species Билне врсте	Extracts Екстракти	DPPH assay (%) DPPH test (%)			β-carotene / linoleic acid assay (%) β-karoten / linolna kiselina test (%)		
		100	200	500	100	200	500
		μg/mL	μg/mL	μg/mL	μg/mL	μg/mL	μg/mL
<i>S. amplexicaulis</i>	ethanolic/ етанолни	10.88± 0.43	30.34± 0.84	71.96± 1.31	39.69± 1.62	44.00± 2.34	47.72± 2.94
	methanolic/ метанолни	40.98± 0.66	73.35± 0.66	80.10± 0.43	39.57± 1.98	44.96± 2.70	49.04± 2.55
	aqueous/ водени	43.25± 0.96	74.62± 1.26	80.66± 1.31	15.47± 1.16	20.98± 2.45	27.34± 1.50
	ethanolic/ етанолни	2.82± 0.56	4.37± 0.30	13.58± 0.56	na*	5.40± 0.91	9.83± 1.98
<i>S. jurisicii</i>	methanolic/ метанолни	5.80± 0.84	16.24± 0.43	38.88± 0.43	na	8.87± 1.90	13.31± 1.81
	aqueous/ водени	4.13± 0.60	6.12± 0.36	16.68± 0.73	na	na	na
	ethanolic/ етанолни	17.91± 0.60	42.53± 0.59	80.94± 0.48	na	6.59± 1.26	12.35± 3.10
<i>S. ringens</i>	methanolic/ метанолни	54.85± 0.78	66.56± 0.79	80.46± 0.78	6.24± 1.16	12.23± 1.70	16.19± 2.53
	aqueous/ водени	3.77± 0.99	8.86± 0.66	13.90± 0.50	na	na	4.20± 1.08
	BHA	43.33± 0.87	-	-	57.70± 1.91	64.47± 0.54	87.58± 1.44
BHT	34.31± 0.43	-	-	56.29± 1.44	59.12± 1.44	80.97± 2.84	
Vitamin C	91.65± 0.21	-	-	na	6.92±0. 66	14.15± 0.94	

\*na - not active (нема активности)

Post-distillation waste extracts tested in the present study performed significantly lower DPPH activity compared with previously tested total extracts of the same species (Alimpić et al, 2014; 2015b; 2016a,b). This finding is in accordance with the previous reports (Tepe et al., 2005; Gavarić et al., 2015).

In  $\beta$ -carotene/linoleic acid assay, antioxidant activity of tested extracts (4.20-49.04%) was lower compared with synthetic antioxidants BHA and BHT, but higher in comparison with vitamin C (Table 2). Post-distillation waste extracts of *S. amplexicaulis* performed stronger inhibition of  $\beta$ -carotene oxidation than total extracts of this plant (Alimpić et al., 2016b). On the contrary, deodorized methanolic-aqueous extract of *S. tomentosa* showed weaker inhibition comparing to total extract of this plant obtained by the same extraction procedure (Tepe et al., 2005).

Tab. 3. Anti-neurodegenerative activity of three Macedonian sage species post-distillation waste extracts

Анти-неуродегенеративна активност екстраката постдестилационих остатака три врсте жалфија из Македоније

Plant species Билне врсте	Extracts Екстракти	Acetylcholinesterase inhibition (%) Инхибиција ацетилхолинестеразе (%)			Tyrosinase inhibition (%) Инхибиција тирозиназе (%)		
		100 $\mu\text{g/mL}$	200 $\mu\text{g/mL}$	500 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$	200 $\mu\text{g/mL}$	500 $\mu\text{g/mL}$
<i>S. amplexicaulis</i>	ethanolic/ етанолни	31.75± 1.48	33.17± 1.10	36.89±0. 98	33.20± 2.20	36.19± 1.48	41.38± 1.37
	methanolic/ метанолни	32.94± 1.28	31.76± 1.44	36.53± 1.60	32.90± 1.41	37.39± 1.25	59.52± 1.35
	aqueous/ водени	31.07± 1.54	31.33± 1.13	38.15± 0.95	40.38± 1.95	42.47± 1.35	47.96± 1.30
	ethanolic/ етанолни	25.94± 0.84	26.90± 1.18	27.82± 1.20	27.32± 1.79	21.44± 0.75	23.33± 1.21
	methanolic/ метанолни	27.69± 0.82	28.39± 1.44	29.49± 1.30	30.91± 1.96	25.72± 1.95	26.32± 1.65
	aqueous/ водени	28.03± 1.59	28.26± 0.93	29.68± 1.03	18.84± 1.51	21.04± 1.37	24.03± 2.07
<i>S. jurisicii</i>	ethanolic/ етанолни	29.69± 1.15	31.36± 1.23	33.86± 1.59	42.87± 2.66	43.17± 1.37	38.19± 1.90
	methanolic/ метанолни	30.92± 1.11	32.22± 1.48	33.78± 1.64	34.20± 2.34	44.97± 1.50	31.21± 2.34
	aqueous/ водени	28.93± 1.76	31.74± 1.19	30.20± 1.21	45.56± 2.09	37.79± 0.60	30.11± 1.70
Galanthamine		57.11± 1.67	62.59± 0.53	-	-	-	-
	Kojic acid	-	-	-	51.81± 2.55	87.91± 7.91	-

(-) not tested (није тестирано)

Post-distillation waste extracts of analyzed sage species inhibited AChE and TYR ranging from 25.94 to 38.15% and from 18.84 to 59.52%, respectively. At all applied concentrations, the extracts performed lower activity than commercial inhibitors galanthamin and kojic acid. The *S. amplexicaulis* extracts showed the strongest inhibition of both tested enzymes, while *S. jurisicii* extracts were the least effective. The enzymes inhibition level was not apparently dependent on type of extract and applied concentration (Table 3).

Post-distillation waste extracts tested in this study showed similar levels of AChE inhibition, but significantly weaker TYR inhibition comparing to total extracts of tested sage species (Alimpić et al., 2016a,b). Methanolic and ethyl acetate extracts of 16 Turkish *Salvia* species, including *S. amplexicaulis* (Orhan et al., 2012), exhibited lower AChE and TYR inhibition in comparison with the extracts analyzed in the present study. Ethanol extracts of 14 *Salvia* species exhibited AChE inhibition values in range 2.93–27.40% (Orhan et al. 2013). Masuda et al. (2005) reported a broad inhibition range (6.8–60.8%) of tyrosinase inhibition by ethanol extracts of 39 Japanese species.

This is the first report on antioxidant and anti-neurodegenerative activities of *S. amplexicaulis*, *S. jurisicii* and *S. ringens* extracts obtained from post-distillation waste.

## Conclusion

Based on the obtained results, it can be concluded that post-distillation waste of three investigated sage species from Macedonia, particularly *S. amplexicaulis*, showed high polyphenol content, as well as antioxidant and anti-neurodegenerative activities. Therefore, residual plant material after essential oil isolation could be exploited as potential source of bioactive polyphenols.

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## Садржај полифенола и биолошка активност постдестилационих остатака три врсте жалфија из Македоније

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### Сажетак

Ово истраживање је спроведено у циљу анализе садржаја полифенола и антиоксидативне и антинеуродегенеративне активности екстраката постдестилационих остатака *S. amplexicaulis*, *S. jurisicii* и *S. ringens* из Републике Македоније по први пут. Садржаји фенола и флавоноида су варирали између 3,92 и 146,49 mg GAE/g, односно 7,11 и 67,51 mg QE/g, са највишим вриједностима измјереним за екстракте *S. amplexicaulis* и *S. ringens*. Поједини екстракти *S. amplexicaulis* и *S. ringens* су неутралисали више од 80% DPPH радикала на највишој тестираној концентрацији, док су у  $\beta$ -каротен/линолна киселина тесту екстракти *S. amplexicaulis* показали и до 50% инхибиције оксидације  $\beta$ -каротена. Постдестилациони остаци тестираних врста жалфија су инхибирали активност ацетилхолинестеразе (25,94 до 38,15%) и тирозиназе (18,84 до 59,52%), гдје су најснажније дејство испољили екстракти *S. amplexicaulis*. Добијени резултати показују да постдестилациони остаци све три врсте жалфија, а посебно *S. amplexicaulis*, испољавају одређена биолошка дејства и могу бити размотрени као потенцијалне фенолима богате лековите сировине.

*Кључне ријечи:* *Salvia*, екстракти, постдестилациони остаци, биолошке активности, полифеноли

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