CHOLINESTERASE INHIBITION POTENTIAL OF ENDEMIC *SATUREJA SUBSPICATA* L. ESSENTIAL OIL AND EXTRACTS

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ABSTRACT:
Medicinal plants are potential sources of bioactive compounds. One of the medicinal plants used in the traditional medicine of Bosnia and Herzegovina is endemic *Satureja subspicata* L. In this work, we examined the ability of *Satureja subspicata* L. essential oil and hot water and methanol extracts to inhibit the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) using Ellman's method. The ability of *Satureja subspicata* L. essential oil in concentration of 1 mg/mL and 2 mg/mL to inhibit enzymes was moderate: 72.82%, and 76.89% for AChE, and 51.51%, and 27.15% for BChE, respectively. Analyzed hot water and methanol extracts in concentration of 1 mg/mL showed weak ability of cholinesterase inhibition. Extracts were additionally analyzed regarding to ability to protect proteins from oxidation, during 1 h and 24 h incubation periods. After incubation for 1 h hot water extract showed a very good protective effect (10.61%), while the methanolic extract showed prooxidative activity. After incubation for 24 h, both extracts showed prooxidative activity. The obtained results show that the examined essential oil and extracts of *S. subspicata* L. contain compounds with cholinesterase inhibition and antioxidant potential, and therefore can be useful in treatment of Alzheimer's disease.

KEYWORDS: *Satureja subspicata*, essential oil, extracts, cholinesterase inhibition, protein oxidation.

INTRODUCTION

In the central nervous system, the stimulation of nicotine receptors by the action of the neurotransmitter acetylcholine is related to learning and memory processes, while the stimulation of receptors on muscle cells leads to their contractions. Acetylcholine hydrolysis controls the transmission of nerve impulses in the cholinergic synapse of the central and peripheral nervous system. Decomposition of acetylcholine in the synaptic cleft by the action of the enzyme acetylcholinesterase (AChE) establishes the polarization of the postsynaptic membrane and the transmission of impulses stops. Released ACh has a short half-life, since AChE is one of the most efficient enzymes; the rate of hydrolysis for human AChE, *kcat*, is approximately 400 000 min^{-1} [1]. Two types of ChE are currently known; acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). AChE is also called "true cholinesterase", while BChE is also known as "pseudocholinesterase", because it hydrolyzes numerous choline esters and other non-choline esters (butyrylcholine, succinylcholine, acetylcholine, acetylsalicylic acid, cocaine, and heroin). In the normal brain, BChE is present – in relation to total ChE – with a share of 10%, while it is more abundant in the liver, lungs, heart tissue, and blood plasma.

AChE inhibition prevents the hydrolysis of ACh, thus prolonging its activity in the transmission of nerve impulses. This concept is applied in the treatment of diseases characterized by low ACh levels, such as Alzheimer's disease. It is also studied in toxicology due to disease conditions and deaths caused by increased cholinergic stimulation [2].

Alzheimer's dementia (AD) is the most common neurodegenerative disorder and the cause of dementia in elders. It affects about 2% of the population in industrialized countries. In the next fifty years, the number of patients is expected to increase by about three times. Inhibition of the enzyme cholinesterase is one of the strategies in the treatment of Alzheimer's disease and other similar disorders. The use of most of the ChE inhibitors tested so far was followed by side effects, such as fatigue, sleep disorders, cardiorespiratory, gastrointestinal disorders, and low bioavailability. This was the impetus for further research with the aim of finding new ChE inhibitors of natural origin, with greater efficiency and bioavailability and fewer side effects [3]. Since plants are considered potential sources of inhibitors, many of

them, as well as various phytochemicals, are the subject of numerous research [4].

Oxidative stress is related to the etiology and pathogenesis of numerous diseases, so it is considered possible to prevent or delay the occurrence of pathological changes and to reduce the occurrence of diseases by removing the cause of oxidative stress. In a state of oxidative stress, excess free radicals can damage lipids, proteins, carbohydrates, and nucleic acids. Oxidative modification of proteins, both reversible and irreversible, occurs during redox signaling and other cellular processes. It also occurs as a result of oxidative stress. Exposure of proteins to OH• and/or $O_2^{\bullet-}$ leads to their structural modifications. Modified proteins may further undergo spontaneous fragmentation and cross-linking, or show a significant increase in proteolysis. In addition to fragmentation, oxidation of lysine, arginine, proline, and threonine can increase the concentration of carbonyls. Thus, the presence of protein carbonyl groups can be used as an indicator of protein oxidation caused by reactive oxygen species [5], [6].

Oxidative modification of proteins is present in diseases and changes associated with the aging process, such as: atherosclerosis, tumor, neurodegenerative diseases, and the aging process itself. Natural antioxidants, individually or in the composition of extracts, can be useful in the therapy of such diseases, which is also the reason for the great interest in researching the antioxidant activity of aromatic, medicinal, and edible plants [7].

Plants from the Lamiaceae family show numerous biological activities; antimicrobial, anti-inflammatory, antioxidant, antiviral, cytotoxic, and neuroprotective activity [8]. Being rich in essential oils, many of them are used as spices and medicinal plants. Lamiaceae (Labiatae) is a family of flowering plants belonging to the order Lamiales with opposite leaves. They are easy to grow, so many plants from this family are cultivated [9]. This family also includes *Satureja* species, which are known as aromatic and medicinal. Many of them are used in the perfume and cosmetic industry, as herbs, and in traditional medicine [10].

Satureja subspicata L. from the Lamiaceae family is a shrub from 8 to 20 cm in height (**Figure 1)**. The plant blooms from August to October. It is found in the higher localities of the Dinaric Karst, on exposed ridges or peaks, from 200 to 800 m above sea level [11]. In the traditional medicine of Bosnia and Herzegovina, the aerial parts of this plant are used in the form of infusions in the treatment of lymphatic system diseases; in the treatment of respiratory system diseases, as well as for improving the complete blood count (CBC). Honey from *S. subspicata* L. is used in the treatment of heart conditions [10].

Figure 1. Medicinal plant *Satureja subspicata* L.

The aim of the paper was to test essential oils, as well as water and methanol extracts in terms of inhibition of the enzymes acetylcholinesterase and butyrylcholinesterase, and to test the extracts in terms of protein protection against oxidation. Inhibition of the cholinesterase enzyme was tested using the Ellman's method, [12] while protein oxidation was tested using the method of measuring the formation of carbonyls [5], [13].

EXPERIMENTAL

PLANT MATERIAL

For the purposes of this work, plant material was used, i.e. aerial parts of the *Satureja subspicata* L. It was collected in the period of full flowering in the area of the Una-Sana Canton (Bosnia and Herzegovina). Confirmation of the identification of plant species was carried out by botanical identification methods, through morphology, by the botanist Mirko Ruščić, Associate professor at the Department of Biology at the Faculty of Science, University of Split. Aerial parts of fresh plants were used for essential oil isolation and preparation of water and methanol extracts.

CHEMICALS

Used reagents and solvents with analytical grade were: Acetylcholinesterase from *Electrophorus electricus* L. – electric eel, type V-S; Butyrylcholinesterase from equine serum; Acetylthiocholine iodide (ATChI); Butyrylthiocholine iodide (BTChI); 5,5-Dithiobis(2-nitrobenzoicacid) DTNB, Ellman's reagent,

guanidine-HCl (Sigma-Aldrich, GmbH, Steinheim, Germany); BHA (Butylated hydroxyanisol); BHT (Butylated hydroxytoluene), BSA (Bovine serum albumin) (Darmstadt, Germany); Ascorbic acid (Sigma-Aldrich, USA); Methanol (J.T. Baker, USA), Ethanol, H_2O_2 (Chemica, Croatia); DNPH, $FeCl_3$ (Acros Organics, New Jersey, USA).

METHOD OF ESSENTIAL OIL EXTRACTION FROM PLANT MATERIAL

Fifty grams of fresh, roughly chopped plant material and 0.5 liters of distilled water heated to boiling temperature were added to the round-bottom flask. With further heating, the volatile components of the essential oil and water vapor from the flask passed through the tube of the apparatus, cooled and condensed in a cooler made according to Allihn. A mixture of organic solvents pentane and diethylether, the so-called "trap", was also in the tube of the apparatus in a ratio of 1 : 3. The "trap" has the task of retaining volatile ingredients, while the water fell to the bottom of a graduated tube. After 150 minutes of hydrodistillation, the mixture of essential oil and organic solvents from the "trap" in the graduated tube was transferred to a dry bottle. Water remains from the essential oil were removed by adding anhydrous sodium sulfate, which was then removed by decantation. Upon drying, the mixture was transferred to a dry, weighed bottle in which the oil was hermetically sealed and stored in a freezer at -20 °C for further analysis.

To measure the biological activity of the essential oil, each essential oil was individually dissolved in ethanol, and its initial mass concentration was determined.

METHODS OF PREPARATION OF WATER AND METHANOL EXTRACTS

Water extracts – infusions

A measured mass of fresh plant material was poured with boiling water and left at room temperature for 24 hours, with occasional stirring (~ 7 mL of deionized water per 1 g of plant material). The extracts were filtered and then evaporated under vacuum at 40 °C (**Figure 2**). Upon drying in a desiccator for 48 h, the extracts were lyophilized, the mass was measured, and the yield was calculated. For the purpose of analyses, the dried extracts were dissolved in water. The samples were stored in glass vials at - 20 °C.

Methanol extracts

A measured mass of fresh plant material was macerated in 70% methanol w/w (~7mL MeOH per 1 g of plant material) at room temperature, with occasional stirring over 48 h. The extracts were filtered and then evaporated under vacuum at 40 °C (**Figure 3**). After drying in a desiccator for 48 h, the extracts were lyophilized, the mass was measured, and the yield was calculated. For the purpose of analyses, the dried extracts were dissolved in 86% ethanol. The samples were stored in glass vials at - 20 °C.

Figure 2. Evaporation of the aqueous extract

Figure 3. Evaporation of the MeOH extract

Method of determining the ability to inhibit the cholinesterase enzyme

The Ellman's method was used to determine the ability to inhibit the enzyme cholinesterase, which is based on the reaction of Ellman's reagent (DTNB) and

thiocholine, resulting in a yellow-colored product [12].

The reaction mixture consisted of 180 μL of phosphate buffer (0.1 M; pH = 8), 10 μL of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), 10 μL of acetylthiocholine iodide (ATChI), 10 μL of acetylcholinesterase (AChE) from electric eel electrophorus, and 10 μL of samples of a certain concentration. Samples of essential oil and methanol extracts were dissolved in 86% ethanol, while water was used to dissolve aqueous extracts. Water and 86% ethanol were used for the control measurement. The total volume of the reaction mixture was 220 μL.

A stock solution of DTNB (6.6 mM) was prepared using phosphate buffer (0.1 M; pH = 7) supplemented with sodium bicarbonate (0.12 M). Stock solutions of AChE (0.66 U/mL) and ATChI (11 mM) were prepared using phosphate buffer (0.1 M; pH = 8).

A multichannel microtiter plate reader "Sunrise" (Tecan GmbH, Austria) was used for measurements, with automatic sample mixing and computerized data recording. Measurements were conducted at 25 °C and a wavelength of 412 nm.

Enzyme activity measurements were carried out under conditions of a constant amount of substrate, lasting 6 minutes, with three repetitions each. The percentage of AChE enzyme inhibition by essential oils or extracts was calculated according to the formula:

When testing the ability to inhibit the enzyme butyrylcholinesterase (BChE) by the action of essential oils or extracts, synthetic butyrylthiocholine iodide (BTChI) and the enzyme butyrylcholinesterase (BChE) from horse serum were used as a substrate. Stock solutions of BTChI (11 mM) and BChE (0.66 U/mL) were prepared using phosphate buffer (0.1 M; pH = 8). The other components of the reaction mixture, as well as the reaction mechanism, were the same as when testing the inhibition of the AChE enzyme. The percentage of inhibition of enzymes AChE and BChE by extracts was calculated using the formula: % inhibition = {[(Ae - Abe) - (Au - Abu)] / (Ae - Abe)} x 100; where: Ae – enzyme solution without sample (inhibitor) absorbance; Abe – blank sample of enzyme solution without substrate absorbance; Au – enzyme solution with sample (inhibitor) absorbance; Abu – absorbance of a blank sample of enzyme solution without a sample (inhibitor).

Method of testing the ability to protect proteins from oxidation

The method is based on monitoring the oxidation of bovine serum albumin (BSA) protein in a metal-catalyzed reaction with or without the presence of the tested extract. The reaction of the resulting aldehyde or keto functional groups with the dinitrophenyl hydrazine (DNPH) reagent produces DNP-hydrazone, with a maximum absorbance at a wavelength of 370 nm [5], [13].

The reaction mixture contained BSA (4 g/L), $FeCl_3$ (0.05 mM), ascorbic acid (0.1 mM), H_2O_2 (1 mM), and a plant extract with a concentration of 167 mg/L. The total volume of the reaction mixture was 600 μL. Water was used as a blind test for infusions, and 86% ethanol for MeOH extracts. The mixture was incubated for 1 hour, i.e. 24 h at 37 °C.

The amount of carbonyls formed was determined spectrophotometrically. After incubation, 1 mL of DNPH solution (10 mM) was added to the mixture and the solution was incubated for 60 minutes at room temperature. After that, 1 mL of 10% trichloroacetic acid (TCA) solution was added to the sample and the solution was kept on ice for 15 minutes, after which the samples were centrifuged for 40 minutes at 5,000 RPM (*Rotation Per Minute*).

The supernatant was discarded; the precipitate was washed 3 times with 2 mL of a solvent mixture of ethanol/ethyl acetate (1:1 v/v). After the last centrifugation, the precipitates were dissolved in 6M guanidine-HCl in 2 mL of 2 M HCl (pH 2) by vortexing and incubating at 37 °C.

After the sediments were resuspended, they were transferred to cuvettes suitable for measurement in the ultraviolet part of the spectrum, and the absorbance was measured at a wavelength of 370 nm. The spectrophotometer photoLab 6600 UV-VIS was used for the measurement. The results are expressed in % of oxidation inhibition compared to the blind testing, according to formula: % inhibition of oxidation = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$.

Statistical data processing

Measurements were performed in triplicates and the results are expressed as mean value ± standard deviation (SD). Statistical processing was performed using the Microsoft Office Excel 2010 program, upgraded with the statistical program XLStat 2018, Addinosoft.

RESULTS AND DISCUSSION

INHIBITION OF CHOLINESTERASE ENZYME WITH ESSENTIAL OILS AND EXTRACTS OF SELECTED PLANT SPECIES

The ability to inhibit the enzymes cholinesterase (ChE), acetylcholinesterase and butyrylcholinesterase (AChE and BChE) with essential oils and extracts of

the plant *Satureja subspicata* L. was tested by the Ellman's method.

The initial concentration of the extracts was 1 mg/mL, while the essential oils were tested in initial concentrations of 1 and 2 mg/mL. The obtained results were compared with the results for the well-known inhibitor eserine, which was tested at a ten or twenty times lower concentration (0.1 mg/mL). The results of testing the ability of essential oils to inhibit the enzymes AChE and BChE are shown in **Table 1**.

Table 1. Ability to inhibit AChE and BChE enzymes with essential oils and extracts

Sample		**Inhibition %**	
		AChE	BChE
Essential oil	1 mg/mL	72.82 ± 20.71*	51.51 ± 11.71
	2 mg/mL	76.89 ± 13.36	27.15 ± 9.84
Hot water extract	1 mg/mL	32.09 ± 3.66	0.00
Methanol extract	1 mg/mL	8.59 ± 1.15	11.9 ± 0.71
Eserine	0.1 mg/mL	95.92 ± 1.47	79.12 ± 0.00
* Mean value ± standard deviation (n = 3)			

The essential oils of the four tested plants in concentration of 1 and 2 mg/mL (45 and 90 μg/mL in the reaction system) showed inhibition of AChE 72.8% and 76.9%, and BChE 51.5% and 11.7%. The well-known ChE inhibitor eserine, at an initial concentration of 0.1 mg/mL, showed inhibition of AChE of 95.9% and BChE of 79.1%, so compared to it, the essential oil of *S. subspicata* L. showed moderate inhibitory activity.

The inhibition efficiency of the AChE enzyme was 32.1% for the water extract, and 8.6% for the methanol extract. Compared to the previously mentioned results for eserine, the extracts showed weak inhibition of the enzyme acetylcholinesterase. When inhibiting the enzyme BChE, the methanol extract with 11.9% showed a weak ability, while the aqueous extract did not inhibit the enzyme butyrylcholinesterase.

With regard to existing literature data, testing of methanolic and aqueous extracts and essential oil for *S. subspicata* L. in terms of AChE and BChE inhibition is the first of its kind.

As expected, the results for the inhibition ability of essential oils and water and methanol extracts were generally weaker compared to the less specific BChE.

The tested essential oil showed a moderate inhibitory potential on AChE and a weaker inhibitory potential on BChE. Such results may be due to the fact that among the main components of the examined essential oil, of the known cholinesterase inhibitors published so far, only α-pinene (10.2%) and β-caryophyllene (14%) was found [14], while the others are absent or found in low concentrations. The chemical composition of the examined essential oil and extracts of the plant *S. subspicata* L. was presented in a previously published study by Bektašević et al. (2017) [14].

Numerous plant extracts and essential oils, as well as their components, are studied in terms of ChE inhibitory activity [2], [15], [16]. In terms of testing the ability to inhibit ChE, most of them refer to the research of monoterpenes [17]. Among the monoterpenes, 1,8-cineole and α-pinene are the most effective in inhibiting AChE. Besides them, δ-2-carene (2-carene), δ-3-carene (3-carene) and myrtenal [15], [17] as well as geraniol, 3-carene, α-caryophyllene and limonene show their ability to inhibit AChE [4]. Carvone also showed good AChE inhibitory activity [2].

In various studies, the monoterpenes α-pinene, 1,8-cineole, linalool, terpinen-4-ol, linalyl acetate, thymol, γ-terpinene, and the phenylpropanoid eugenol showed good to moderate BChE inhibitory potential [17].

The most frequently investigated sesquiterpenes in terms of AChE inhibition are β-caryophyllene and α-humulene. At the same time, β-caryophyllene had a good inhibition ability, in contrast to α-humulene (α-caryophyllene), which showed a weak ability to inhibit AChE. In several studies, β-caryophyllene also showed good to moderate BChE inhibitory potential [17].

The tested water and methanol extracts of the selected plant species showed a weak inhibition of the AChE enzyme. The methanol extract showed a weak ability to inhibit the BChE enzyme, while the aqueous extract did not show inhibitory activity. In a previous study, Bektašević et al. (2017) [14] found that the following ChE inhibitors are present in the mentioned water and methanol extracts, respectively: naringenin 0.2% and 0.4%, eriodictyol 0.2% and 7.7%, ellagic acid 0.3% and 1%, rosmarinic acid 45% and 49%, kaempferol 0.1 and 1.5%, and luteolin 0.3% and 0.4%. The inhibitors apigenin and diosmetin were present in smaller amounts. The higher content of the mentioned inhibitors in the methanol extract explains the better

ChE inhibitory activity of the methanol extract compared to the water extract obtained in this work.

Several *in vivo* studies have been conducted examining the effect of polyphenol-rich extracts on AChE activity, which have shown inhibition of hippocampal AChE activity and greater cognitive ability, which may be a result of the presence of apigenin, kaempferol, and luteolin [16]. Natural phenolic components that are AChE inhibitors are also chlorogenic acid, rutin, and rosmarinic acid. The results show that rosmarinic acid easily crosses the blood-brain barrier and acts as an AChE inhibitor in the brain [16]. Ellagic acid also inhibits the action of AChE [18].

The ability to inhibit AChE with ethanol extracts of *S. subspicata* plant in initial concentration 0.25; 0.5 and 1 mg/mL was examined in the study by Vladimir-Knežević et al. (2014) and results were 16, 28 and 52%. The same study tested hydroxycinnamic acids (caffeic, chlorogenic, rosmarinic, ferulic acid), which in concentration of 0.25 and 0.50 mg/mL inhibited AChE activity in values from 30.0% to 48.9%, and from 73.6% to 86.6% respectively. Their effectiveness in inhibition was as follows: caffeic acid > ferulic acid > rosmarinic acid > chlorogenic acid [19].

PROTECTION OF PROTEINS FROM OXIDATION

Aqueous and methanolic extracts of the tested plant in an initial concentration of 1 g/L were tested in terms of protein protection against oxidation. Inhibition of BSA carbonylation (expressed as % of inhibition), in the presence of tested extracts and commercial antioxidants, is shown in **Table 2**. The incubation period, at 37 ºC with metal-induced oxidation, was 1 and 24 h.

Table 2. Activity of the extracts regarding the protection of proteins from carbonylation

Sample	Inhibition %	
	1 h	24 h
Hot water extract	10.61 ± 3.67*	- 69.81 ± 4.18
Methanol extract	- 6.06 ± 2.02	- 79.05 ± 3.43
BHT	11.12 ± 4.29	13.54 ± 3.45
BHA	-29.91 ± 6.27	8.04 ± 3.63
* Mean value ± standard deviation (n = 3)		

The obtained results, after incubation for 1 h, show a very good protective activity of the aqueous extract of *S. subspicata* L. (10.61%), compared to the standard antioxidant BHT, which showed an inhibition of carbonylation of 11.12%. The methanolic extract of *S. subspicata* showed prooxidation activity, as did BHA. After incubation for 24 h, all extracts showed prooxidation activity, while BHT and BHA inhibited BSA carbonylation by 13.54% and 8.04%.

Regarding the examination of the protective role of extracts of the investigated plant, in terms of protecting proteins from oxidation, there is no literature data available. The data for plants from the same family (Lamiaceae) from the territory of Bosnia and Herzegovina regarding the inhibition of protein carbonylation, after incubation for 1 h, show a better inhibition ability for the aqueous extract of the plant *Mentha pulegium* (14.6%), and a weaker inhibition ability for the *Mentha pulegium* methanol extract (5.6%). Aqueous and methanolic extracts of *Clinopodium vulgare* showed pro-oxidation activity. After incubation for 24 h, all extracts showed prooxidative activity [20], [21].

The prooxidative activity of the extracts can be explained by the following literature data. Polyphenols from food act as prooxidants catalyzing oxidative damage to DNA, proteins and carbohydrates, despite their role in protecting lipids from oxidation. Dorman and Hiltunen's research (2011) [7] shows the complexity, i.e. the paradox of certain phytochemicals with antioxidant activity, which can have a protective role by protecting some biologically important molecules from oxidative damage, e.g. lipids, but also show prooxidative activity towards other biologically important molecules, e.g. proteins. In the aforementioned study, gallic acid showed prooxidative activity, as did extracts of juniper (*Juniperus communis* L.), basil (*Ocimum basilicum* L.), caraway (*Carum carvi* L.), and laurel (*Laurus nobilis* L.). In Mayo et al.'s research (2003) [5] on the protection of proteins from oxidative damage, trolox and ascorbic acid showed prooxidative activity. Ascorbic acid is known to be a strong reducing agent, and is used to reduce transition metals, such as Fe^{3+} or Cu^{2+}, as well as to generate $^{\bullet}OH$ radicals. The combination of vitamin C and Cu^{2+} causes significant protein damage. In the same study, resveratrol did not show a protective effect [5].

The results obtained in this work related to protein protection from oxidation can be explained by the reaction mechanism. Namely, metal-catalyzed protein oxidation is a process that mainly takes place at specific metal binding sites on the protein, where one or several amino acids are oxidized. In this regard, the amino acid residues of histidine, proline, arginine, and lysine are most subject to oxidation. The reaction takes place as a "closed" process in which iron undergoes a redox cycle from Fe^{2+} to Fe^{3+} in reaction with H_2O_2 and again by protein-dependent reduction to Fe^{2+}. At the same time, the oxygen radical, which occurs as an intermediate product, does not diffuse into the

surrounding medium, because it reacts with the mentioned amino acid residues of the protein [6]. Since potential antioxidants cannot come into contact with free radicals, they are unable to compete with amino acids for them, and thus they lack protective activity.

CONCLUSIONS

The essential oil of *S. subspicata* L. showed a moderate ability to inhibit the enzymes AChE and BChE, compared to the known inhibitor eserine. Inhibition of AChE was more successful than inhibition of the less specific BChE. The components of this essential oil, known for their anticholinesterase effect, are probably responsible for this type of activity: α-pinene and ß-caryophyllene. The tested extracts showed a weak ability to inhibit the enzymes AChE and BChE. The obtained results show that the examined essential oil and extracts of *S. subspicata* L. contain active phenolic and terpene compounds, which show activity in terms of cholinesterase inhibition. They can be of importance in the prevention and therapy of Alzheimer's disease and other neurodegenerative disorders, which implies additional research. The results of testing the protection of proteins from oxidation, after incubation for 1 h, in comparison with the standard antioxidants BHT and BHA, showed a very good protective effect of the aqueous extract, while the methanolic extract of *S. subspicata* showed prooxidative activity. After incubation for 24 h, both aqueous and methanolic extracts showed prooxidative activity, which can be attributed to the protein oxidation reaction mechanism itself.

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