ANTIOXIDANT AND ANTI-CHOLINESTERASE INHIBITION PROPERTIES OF *MELISSA OFFICINALIS* SUBSP. *OFFICINALIS* AND *LAVANDULA ANGUSTIFOLIA*

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Abstract

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) inhibitors are promising compounds for clearing up Alzheimer's disease (AD) symptoms. In this study, extracts of Melissa officinalis L. subsp. officinalis and Lavandula angustifolia L. were prepared with different solvents such as methanol, ethanol and distilled water. It was investigated their inhibitory effects on AChE and BChE enzymes. In addition, antioxidant activities and total phenolic contents of plants were determined using the 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging activity method and Folin-Ciocaltau method, respectively. It has been found that the distilled water extract of M. officinalis subsp. officinalis is the most potent inhibitory of both AChE and BChE enzymes among all of the extracts. IC_{50} values of the extracts were calculated as 1.65±0.04 mg/mL and 2.33±0.03 mg/mL for AChE and BuChE, respectively. On the other tests, the antioxidant activities of methanol extracts of M. officinalis subsp. officinalis and Lavandula angustifolia plants were 95%, and the highest total phenolic content was observed as 2.30±0.30 g/100g in the methanol extract of M. officinalis subsp. officinalis.

Keywords: *Acetylcholinesterase, Butyrylcholinesterase, Melissa officinalis* subsp*. officinalis and Lavandula angustifolia, Antioxidant activity*

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive brain disease that is defined by common symptoms such as difficulty in fulfilling the needs of daily life, deterioration in perception, regulation of social behaviors and control of emotional reactions besides memory loss. There are many causes of AD, one of which is called the cholinergic hypothesis, which can be defined as an increase in the activity of cholinesterase enzymes**[1]**. According to this hypothesis, the deficiency of critical neurotransmitters acetylcholine (ACh) and butyrylcholine (BCh) causes impaired neurotransmission and results in cognitive impairment. Therefore, ACh and BCh levels are determining factors for the treatment of the disease**[2,3]**. The cholinesterases inhibition increases the level of ACh and BCh in the brain and can be used for symptomatic treatment of the disease. For the treatment, many researchers have conducted inhibition studies related to these enzymes**[4]**. Some synthetic drugs used as enzyme inhibitors such as tacrine, donepezil and rivastigmine have been reported to cause gastrointestinal disturbances and problems related to bioavailability **[5].** Therefore, the discovery and development of new drugs often focuses on chemical compounds derived from natural sources, i.e. plant extracts **[6].**

Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state where excessive quantities of reactive oxygen and/or nitrogen species (ROS/RNS, e.g., superoxide anion, hydrogen peroxide, hydroxyl radical, peroxynitrite) overcome endogenous antioxidant capacity, leading to oxidation of a variety of biomacromolecules, such as enzymes, proteins, DNA and lipids. Oxidative stress is important in the development of chronic degenerative diseases including coronary heart disease, cancer and aging **[7]**. It is well known that plants have important chemical compounds which can be models for many clinically proven drugs or can be used directly to treat, cure, and prevent diseases as an old-fashioned way of medicinal practice **[8,9].**

It is known that Lamiaceae species have been used in traditional medicine for centuries in the treatment of many diseases, like AD. *M. officinalis* and *L. angustifolia* have been used as relievers and antidepressants in oxidative stress and sleep disorders. In addition, these plants are preferred more reliable and easily accessible than synthetic antibiotics due to having potent antioxidant, antibacterial, antifungal activity, etc**[10-13]**. In this study, extracts of *M. officinalis, L. angustifolia* species were prepared with different solvents (methanol, ethanol, and distilled water). Inhibitory potents on AChE and BChE enzymes, antioxidant activities and total phenolic contents of all extracts were investigated. This study aimed to propose candidate plants for the treatment of AD to be used in the later stages.

2. MATERIALS AND METHODS

2.1. Materials

The plants were collected just before the flowering period and dried in a dark room under room conditions. Dried plant samples were ground into powder with the help of a grinding mill and then stored in glass jars until used in extraction processes. Methanol (CH_3OH), ethanol (C_2H_5OH), DTNB (5.5'-Dithiobis-(2-nitrobenzoic acid)), acetylcholine iodide, butyrylcholine iodide and other chemicals were obtained from SigmaAldrich (St. Louis, Missouri, USA). Spectrophotometric Measurements were made by a 96-well microplate and UV-Visible spectrophotometer.

2.2. Methods

2.2.1. Preparation of plant extracts

A 1 g of each powdered plant was weighed into a tube and 10 mL of solvent was added to it. Separate extracts were prepared for each solvent (MeOH, EtOH, and distilled water). They were mixed in a shaker for 24 h at +4°C. All samples were kept in an ultrasonic bath for 5 min, they were then filtered through Whatman no:1 filters. The extracts were concentrated using a vacuum evaporator and stored at -20°C until used for analysis. Afterward, the samples were adjusted with prepared solvents as stock solutions.

2.2.2. Enzymes preparation

The bovine brain was used as a source of AChE and BChE enzymes. Bovine brain (2 g) was homogenized in 10 mL of Tris buffer (50 mM tris + 300 mM sucrose, pH 7.4) by a homogenizer. The homogenate was centrifuged at 1000 g for 10 min. The supernatant was used as a source of AChE and BChE enzymes.

2.2.3. Inhibition of acetylcholinesterase and butyrylcholinesterase

Inhibition of AChE/BChE activities was measured according to the spectrophotometric method developed by Ellman et al. [14]. Shortly, according to the method, 140 µL of 0.1 mM sodium phosphate buffer (pH 8.0), 20 µL of DTNB, 20 µL of the test solution and 20 µL of AChE solution were added by multichannel automatic pipette in a 96-well microplate and incubated for 15 min at 25 0C. The reaction was then initiated with the addition of 10 µL of acetylthiocholine iodide. The hydrolysis of acetylthiocholine iodide for AChE and butyrylcholine iodide for BChE was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 412 nm utilizing a 96-well microplate reader. The analysis was carried out in triplicate. The findings were evaluated by calculating IC_{50} values.

2.2.4. DPPH Radical scavenging (Antioxidant activity)

The antioxidant capacities of the extracts were determined using the 1,1-diphenyl-2-picrylhydrazil(DPPH) radical scavenging activity method**[15]**. DPPH reagent was prepared in MeOH as 6mM. A 0.25 mL of extract, 2.5 mL of DPPH solution and 2.5 mL of MeOH were mixed in a test tube and incubated for 1 hour in a dark environment. MeOH was used instead of the extract for control measurement. The absorbance of all samples was measured at 517 nm. The radical scavenging activity of the samples was done triplicate, and calculated with the following equation;

$$Antioxidant\ activity\ (\%) = [1-(Absorbance_{sample} / Absorbance_{control})] \times 100$$

2.2.5. Total phenolic content

The total phenolic content of the extracts was spectrophotometrically determined using the Folin-Ciocalteu colorimetric method **[16].** One hundred µL of each extract was diluted to 3 mL of the water, and 0.5 mL of Folin-Ciocalteu reagent was added. After 3 min, 2 mL of the 20% sodium carbonate was added, and the contents were mixed thoroughly. After the incubation at 40 ºC for 40 min, the color was developed and the absorbance was measured at 650 nm in UV-visible spectrophotometer after 60 min using gallic acid as a standard. The result was expressed as a gram of gallic acid per 100 g of dried weight material.

3. RESULTS

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) inhibitors are promising compounds for clearing up AD symptoms, like forgetfulness. In this study, extracts of *M. officinalis* and *L. angustifolia* were prepared with different solvents such as methanol, ethanol and distilled water. It was investigated inhibitory effects of the sample extracts on AChE and BChE enzymes of bovine brain. As seen in Table 1, it has been found that the distilled water extract of *M. officinalis* is the most potent inhibitory of both AChE and BChE enzymes among all of the extracts. IC_{50} values of the extracts were calculated as 1.65 ± 0.04 mg/mL and 2.33 ± 0.03 mg/mL for AChE and BChE, respectively. Followed by distilled water extract of *L angustifolia* showed strong inhibition activity on AChE and BChE enzymes, respectively.

Table 1. Inhibition concentrations (IC_{50}) on AChE and BChE enzymes of extracts obtained using different solvents (mg/mL).

	Extract	**AChE**	**BChE**
		(Mean ± SD)	(Mean ± SD)
M. officinalis	Water	1,65 ± 0,04	2,33 ± 0,03
	MeOH	2,87 ± 0,78	2,61 ± 0,04
	EtOH	3,53 ± 0,40	3,78 ± 0,01
L. angustifolia	Water	2,13 ± 0,73	2,41 ± NS
	MeOH	3,25 ± 0,60	7,44 ± 0,62
	EtOH	3,55 ± 0,46	-

(*NS: Negligible standard deviation value.*)

In addition, antioxidant activities and total phenolic contents of plants were presented in Table 2. According to the results, it can be said that there is a correlation between the antioxidant capacities and their total phenolic content of the extracts. Consequently, *M. officinalis* showed the highest antioxidant activity than *L. angustifolia*. The antioxidant activities of methanol extracts of *M. officinalis* and *L. angustifolia* plants were 95%, and the highest total phenolic content was observed as 2.30 ± 0.30 g/100g in the methanol extract of *M. officinalis* (Table 2).

Table 2. DPPH scavenging (antioxidant) activities and total phenolic contents of the plant extracts

	Extract	Antioxidant activity (%)	Total phenolic content (g/100g)
M. officinalis	Water	93	2,17 ± 0,02
	MeOH	95	2,30 ± 0,30
	EtOH	95	1,67 ± 0,29
L. angustifolia	Water	88	2,17 ± 0,44
	MeOH	95	1,53 ± 0,05
	EtOH	77	0,84 ± 0,04

4. DISCUSSION

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are primary cholinesterase that catalyze the breakdown of acetylcholine, butyrylcholine, and some other choline esters that function as neurotransmitters**[17,18].** It has been determined that due to the high activity of cholinesterases during Alzheimer's disease, the flow of information between neurons cannot be ensured and there are symptoms ending with amnesia. Today, rivastigmine, donepezil and their derivatives are used as cholinesterase inhibitors in the treatment process**[19]**. However, recent studies have shown that these drugs cause gastrointestinal problems such as nausea and vomiting and other nervous system side effects such as hallucinations **[20].** Therefore, there is a need for the discovery of new treatments of struggling with types of disorders.

Lamiaceae family has rich terpenes and derivatives content **[21]**. Most studies have shown that terpenes and their derivatives are very effective in inhibiting cholinesterases. In addition, terpene-derivatives attract attention as penetration enhancers to increase drug absorption in the body **[22]**. *M. officinalis and L. angustifolia* were preferred for this study due to their rich phenolic, flavonoid and aromatic compounds that are members of Lamiaceae family. **[10,11,23,24].**

Sanda Vladimir-Knežević et al. **[25]** studied the antioxidant capacities and anticholinesterase activities of 26 plants belonging to the Lamiaceae family in their study. They showed that ethanolic extracts of both plants at a concentration of 1 mg/mL inhibited AChE activity by approximately 50%. One of the differences between our study from this study **[25]** was that there were water and methanolic extracts as well as ethanolic extracts. It was observed that in both plants the water extract was more effective in inhibiting AChE than the ethanolic extract (Table 1). Hajlaoui H. et al. reported that *Origanum majorana* essential oil has an IC_{50} value of 150.33±2.02 µg/mL on AChE **[26]**. On the other hand, Orhan et al. reported that different extracts of *Rosemary officinalis* L. prepared with methanol, petroleum ether, chloroform and ethyl acetate solvents were ineffective on AChE activity with IC_{50} reaching 0.2-0.5 mg/mL. These studies in the literature seem to support our study. In our study, *M. officinalis*, which showed the most potent AChE inhibitory activity, had an IC_{50} value of 1.65±0.04 mg/mL. Some differences between results may vary depending on the plant species, harvest season, drying method, and most importantly, extraction method. Despite all these differences, the results are quite effective and can shed light on further studies.

The antioxidant activity test performed in this study was DPPH test which detects a colour change of the deep purple coloured reagent containing DPPH• (one of the few stable organic nitrogen radicals) into yellow coloured DPPH as a result of the reaction with an antioxidant molecule **[27]**. According to our DPPH test results given in Table 2, it was observed that **%** values were 93-88, 95-95 and 95-77% for water, methanol and ethanol extracts of *M. officinalis* and *L. angustifolia*, respectively. Sanda Vladimir-Knežević et al. **[25]** reported the antioxidant capacities of ethanol extracts of *M. officinalis* and *L. angustifolia* plants as 855.78 ± 6.12 (mg TE/g) and 577.95 ± 13.76 (mg TE/g), respectively.

İt seemed that the highest antioxidant activities were generally found in plant extracts prepared with methanol when compared with these results which were consistent with many other studies. The

amount and rate of polyphenols extracted were mainly dependent on the selection of the right solvent and methanol is known to be more efficient solvent for the extraction of polyphenols with lower molecular weight **[28, 29].** At the same time the antioxidant activity of the plant extracts are known to be significantly affected by extracting solvents because the solubilities of different antioxidant compounds like phenolic components with varying chemical nature depend on the solvent types **[30]**. The use of polar-organic solvents (like methanol or ethanol) were generally recommended for the extraction of plant materials in many studies because it was suggested that they greatly increased the antioxidant efficiency of most plant products **[30-34].**

From these results, it is assumed that the higher level of phenolics in the *M. officinalis* and *L. angustifolia* extracts might have contributed to the observed reducing abilities. Antioxidant activity of plants has been partly ascribed to phenolic compounds **[35]**. This is consistent with the results of several studies. For example Kumar et al.(2010) and Zhang et al.(2015) reported a linear correlation between phenolic content and reducing ability **[36,37].** Most of the antioxidant potential of medicinal plants is due to the redox properties of phenolic compounds, which enable them to act as reducing agents, hydrogen donors and singlet oxygen scavengers **[38].**

However, considering the antioxidant activities and double inhibitory effects of our plants, it can be said that our results are positive. Because it is known that dual inhibition maximizes therapeutic efficacy in the treatment of cognitive dysfunctions **[39].**

5. CONCLUSION

The focus of this study was on two members of the Lamiaceae family, *M. officinalis*, and *L. angustifolia* and their inhibitory activities on AChE and BChE. It is seen that all extracts obtained from both plant species show successful inhibitory effects. In addition, it was determined that the extracts were quite high in antioxidant activity and phenolic contents. Because, antioxidant remedies are noted as an alternative or additional therapy option for AD **[40]**. Actually, a great number of works have studied the positive benefits of antioxidants to reduce or block neuronal death occurring in the pathophysiology of neurodegenerative disorders like AD. In the light of all these results and the negative effects of the previously mentioned drugs, it can be suggested that *M. officinalis* and *L. angustifolia* plants should be considered as potential candidates for AChE and BChE inhibitors in the pharmaceutical industry against Alzheimer's disease or consumed as a dietary supplement for patients in the treatment process.

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