

## Evaluation of Anticholinesterase Potential of Leaf Extracts of *Plectranthus mollis*

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### Abstract

The present study is targeted to investigate *in-vitro* anticholinesterase potential of ethanol and water extracts prepared from the leaves of *Plectranthus mollis* belonging to family Lamiaceae using Ellman's colorimetric assay. The ethanol and water extracts of the leaf were prepared by soxhlet extraction method. The results of the study indicate that both the ethanol and water extracts of the plant possess mild to moderate cholinesterase inhibitory activity. The higher amount of flavanoids and phenolic compounds may correspond to their activity.

**Keywords:** *Plectranthus mollis*; Alzheimer's; Anticholinesterase; Ethanol; Water Extracts

### Introduction

Alzheimer's is the fourth driving reasons for death and the hazard factor increases with age. It isn't simply considered as the disease of more established people, having age over 65; even the beginning happens during the age between 40 - 50 and the endurance rate fluctuates, it even relies upon the other wellbeing conditions. It is a dynamic neurological issue, wherein there is an accumulation of amyloid plaques and bewilderment of microtubules, prompting memory misfortune, strange conduct and even intellectual decay. The oxidative stress, inflammation and diminished acetylcholine are accounted for to be the major causative elements.

Alzheimer's disease includes the harming of the acetylcholine producing cells in the basal forebrain thereby reducing the synthesis of acetylcholine. Focusing on cholinesterase is one of the procedure to build ACh levels and delay the progression of the disease [1].

Oxidative damage was considered as one of the significant system engaged with the pathogenesis of Alzheimer's illness, which brings about the chemical change of the biomolecules prompting neuronal demise [2]. It has been represented that plants having vitamins C and E, flavonoids, polyphenols like gallic acid have notable cell reinforcement exercises [3].

In our earlier studies, we have reported antioxidant potential and its inhibitory effect on nitric oxide and protein denaturation [4,5]. In the present study an effort has been made to investigate the possible benefits of two leaf extracts of *Plectranthus mollis* (*P. mollis*) in the treatment of Alzheimer's disease by evaluating its anticholinesterase activity.

### Materials and Methods

#### Chemicals

Acetyl thiocholine iodide stored at 2 - 8°C, Inhibitor (reference standard) Neostigmine stored at 2 - 8°C, Ellman's reagent (4.95 mg DTNB (Dithiobis nitro benzoate) dissolved in 50 ml of 0.25 mM phosphate buffer pH 7.7) stored at room temperature. All other chemicals used in the study are of analytical grade.

#### Anticholinesterase inhibition assay

The AChE inhibition assay was carried out according to the Ellman method [6-9] with some modifications, In this assay, the enzyme hydrolyzes acetylthiocholine resulting in the formation of thiocholine which reacts with Ellmans reagent (DTNB) to produce 2-nitro-5-

mercaptobenzoate which can be detected at 412 nm. Acetylcholinesterase activity of the blood sample was determined as per the kit protocol (Sigma Aldrich, MAK-119). Human blood sample was diluted to appropriately to get the activity within the linearity range of the kit. Human blood samples were treated with control buffer, extracts (100 - 600 µg/ml and Neostigmine (Positive control, 0.003 - 0.125 µg/ml) in separate tubes. The treated blood samples were reacted with assay reagent consisting of Acetylthiocholine iodide and Ellman's reagent (4.95 mg DTNB dissolved in 50 ml phosphate buffer pH 7.7 (0.25 mM). Absorbance was measured at 2 minutes intervals for 10 minutes and enzyme activity is calculated based on the calibrator provided with the kit. The enzyme activity was calculated by the formula:

$$\text{AChE activity (units/L)} = (\text{A412})_{\text{final}} - (\text{A412})_{\text{initial}} / (\text{A412})_{\text{calibrator}} - (\text{A412})_{\text{blank}} \times n \times 200$$

where 200 = equivalent activity (units/L) of the calibrator when assay is read at 2 minutes and 10 minutes, n = dilution factor (n = 2 for whole blood), (A412) calibrator = Absorbance of the calibrator at 10 minutes, (A412) blank = Absorbance of the blank at 10 minutes. Inhibitory activity of the extracts and positive control was calculated by comparing the activity of control buffer. The relative activity of the sample can be determined by comparing the IC<sub>50</sub> value of extracts with standard. Higher the IC<sub>50</sub> value, lower will be the relative activity in comparison to standard and vice versa.

### Statistical evaluation

The IC<sub>50</sub> concentration of extracts and positive control to achieve 50% inhibition in enzyme activity was determined by Graphpad Prism software version 5.0. For compounds showing < 50% inhibition, IC<sub>50</sub> value is not calculated.

### Results and Discussion

In traditional system of practice, many plants have been utilized in the treatment of cognitive disorders and diverse neuropharmacological issues. The historical background of medication has demonstrated that plants containing powerful chemicals have become the new sources to examine for the pharmaceutical ventures [10]. In the present study, we selected the plant *P. mollis* used in traditional practice to treat mental retardation.

*P. mollis* is utilized as a stimulant, vasoconstrictor, febrifuge, cardiovascular depressant and is likewise snakebites just as a general tonic. It is accounted for to show relaxant movement on smooth and skeletal muscles just as cytotoxic and against tumor promoting activities. Antimicrobial and bronchodilatory exercises of the oil are reported. Phytochemical examinations have brought about the separation of unsaturated fats from β-sitosterol from hexane concentrate of the entire plant [11].

Currently no AChE inhibitory activity has been reported from the leaves of *P. mollis*. Physicochemical analysis revealed the occurrence of steroids, terpenoids, phenolic compounds, 6.12% w/w and 12.2% w/w water and alcohol soluble extractives respectively. Phytochemical screening showed the presence of flavonoids (caffeic acid, quercetin and luteolin), triterpenoids and steroids (β-sitosterol, β-amirin) and cinnamic derivatives (chlorogenic acid) in the plant. The total phenol content and total flavonoid content showed strong correlation with total antioxidant capacity [12,13].

The results on the effects of the tested extracts on AChE activity in this study was summarized in table 1. The extracts were analyzed together with Neostigmine, a potent AChE inhibitor, as positive control in order to assess their enzyme inhibition properties. The results were expressed in terms of TNB formation, percentage of inhibition and inhibitory concentration values. It was found that the neostigmine and the plant extracts had dose-dependent inhibitory activity. The ethanol and water extracts showed 9.02 and 4.38% inhibition of acetylcholinesterase at the highest concentration (1.6 mg/ml) used. Both the extracts screened for their anticholinesterase activity exhibited only mild to moderate or weak anticholinesterase activity and not achieved more than 50% inhibition. IC<sub>50</sub> was not calculated due to lower inhibition.

According to the cholinergic hypothesis, the nerve impulse transmission is hindered by AChE through hydrolysis of ACh in neurodegenerative disorder. This implies that the inhibition of AChE is the foremost strategy of treatment to inhibit this neuro dysfunctionality and similar brain disorders [14-16]. Lamiaceae plants contains monoterpenes, sesqui, di-, and triterpenoids, along with some flavonoids and different phenolics having various pharmacological properties including antimicrobial, anti-inflammatory, antioxidant, antiviral, cytotoxic, neuroprotective, and anticholinesterase [17]. Generally, the triterpenes were seen as answerable for acetylcholinesterase (AChE) restraint, while diterpenes were seen as liable for butrylcholinesterase (BChE) hindrance, by and large. All

the types of terpenoids and some phenolics have been reported in species of *Plectranthus*.

Compound	Concentration (µg/ml)	Abs initial	Abs final	Activity (U/L)	% Inhibition	IC <sub>50</sub> value (µg/ml)
Blank	0	0.0403	0.0414			
Calibrator	Cal	0.3216	0.3206			
Contol	0	0.5811	0.7773	281.09	0	
Standard (Neostigmine)	0.003	0.5771	0.7581	259.31	7.75	0.0578
	0.007	0.5544	0.7303	252.01	10.35	
	0.0156	0.5227	0.6794	224.5	20.13	
	0.0312	0.4848	0.6022	168.19	40.16	
	0.0625	0.399	0.4849	123.07	56.22	
	0.125	0.3225	0.3759	76.5	72.78	
Compound	Concentration (µg/ml)	Abs initial	Abs final	Ache Activity (U/L)	% Inhibition	IC <sub>50</sub> value (µg/ml)
<i>P. mollis</i> water	0.1	0.5674	0.7635	280.95	0.05	IC <sub>50</sub> value was not able to calculate due to lower inhibition
	0.2	0.5681	0.7628	278.94	0.76	
	0.4	0.5677	0.7616	277.79	1.17	
	0.8	0.5657	0.7565	273.35	2.75	
	1.6	0.5653	0.7529	268.77	4.38	
<i>P. mollis</i> ethanol	Concentration (µg/ml)	Abs initial	Abs final	Ache Activity (U/L)	% Inhibition	
	0.1	0.5656	0.7611	280.09	0.36	
	0.2	0.5573	0.7504	276.65	1.58	
	0.4	0.5459	0.7361	272.49	3.06	
	0.8	0.5248	0.7116	267.62	4.79	
	1.6	0.5102	0.6887	255.73	9.02	

**Table 1:** Acetylcholinesterase inhibitory activity of ethanolic extract and standard.

Flavanoids seem to be rare in *Plectranthus*. From *P. mollis* leaves, previous study reported the isolation of vernolic acid and cyclopropenoid fatty acids [18]. Also, the herbs which have anti-inflammatory, antioxidant, cognitive-enhancing, neuroprotective [19] and antiaging effects that may be used in the treatment of AD. The anti-oxidant, free radical scavenging activity and anti-inflammatory activity were also reported in this plant in our earlier study [4,5].

Caffeic acid and its derivatives are the most widely occurring chemicals in the Lamiaceae family and of specific consideration as chemotaxonomic markers. Chlorogenic acid was found to be the universal constituent occurring within this family. whereas rosmarinic acid is restricted to the subfamily Nepetoideae [21] But leaves of *Salvia officinalis* (*S. officinalis*) belonging to the same subfamily contains Rosmarinic acid and the chemistry is poorly known for *Plectranthus* [22]. By and large, *Plectranthus* species are fundamental oil-rich (> 0.5% volatile oil on a dry wt premise) in concurrence with the general circumstance that Nepetoideae are oil rich, while the Lamioideae are oil poor. The major common phenolic compounds identified in the extracts of *Salvia* and *Plectranthus* are chlorogenic acid, rosmarinic acid, carnosic acid and salvianolic acid. It seems to be similar to the pattern of diterpenoids of *Salvia*, but no clerodane diterpenoids were found in *Plectranthus* [23]. Moreover, the most popular traditional plant in our region is *S. officinalis*, therefore our aim was to compare the anticholinesterase activity of the other Lamiaceae member, *P. mollis* which is widely available. Several species in the *Plectranthus* have been discovered to exhibit anticholinesterase activity.

In the case of AChE inhibition of *S. officinalis* leaves ethanolic extract, previous study conducted, suggested that it showed the lowest inhibition amongst other plants studied. Other research on *Salvia* plant family has indicated that only chloroform and petroleum ether

extracts have shown significant inhibitory on AChE while alcoholic and water extracts did not show inhibition efficiency [24]. The report was found to be similar to the present work where *P. mollis* also indicated no activity or minimum efficacy, but to the best of our knowledge this is the first report which tried to demonstrate the inhibition of *P. mollis* leaf extract against *invitro* AChE assay. It was also reported that the essential oils obtained from species of *Salvia* inhibit AChE and BChE in a time dependent manner [25].

Caffeic acid present in the leaves of *P. mollis* is suspected to be responsible for inhibiting acetylcholinesterase. One of the powerful *Lamiaceae* family phytochemical Rosmarinic acid have good neuroprotective properties. Plants of *Plectranthus*, Rosemary, Sage and Lemon balm are very good sources of Rosmarinic acid and many other phenolics. Ursolic, oleanolic acid or betulinic acid which are present in *Plectranthus* family are also very good active constituents for treatment of Alzheimer's disease. Diterpenoids are the most common metabolites in *Plectranthus* including highly modified abietanoids and some phyllocladanes and ent-kaurenes [23]. The moderate inhibitory activity of *P. mollis* observed in the present study may not result from acetylcholinesterase hindrance, however by some different components, as stated in other reports. However, the noted activity of these tested extracts which contains flavanoids, hydroxycinnamic acids and terpenic components may be responsible for possessing anti-AChE properties. Also, their significant antioxidant properties likely depend on the phenolic compounds present. These findings suggest that these extracts that containing polyphenols also could affect different targets, including AChE activity and oxidative stress.

In neurodegenerative disorder like Alzheimer's, since the acetylcholine level is being targeted for the symptomatic relief and when the selected plant also possess anticholinesterase activity, it may be useful in such disorders to some extent.

## Conclusion

In conclusion, the present study revealed that *P. mollis* leaves possess moderate anti-AChE activity. In the anti-AChE assay, the ethanolic extract of *P. mollis* showed the better activity in comparison to water extract. The results indicate that the low inhibitory potential of *P. mollis* may not result from acetylcholinesterase inhibition, however by some other mechanisms as mentioned in other reports. Or it is imperative to consider that the extracts used are crude and which when purified, is expected to give higher inhibitory activity and better therapeutic efficacy.

## Bibliography

1. Hasselmo ME. "The role of acetylcholine in learning and memory". *Current Opinion in Neurobiology* 16.6 (2006): 710-715.
2. Zhao Y, et al. "Investigating the antioxidant and acetyl cholinesterase inhibition activities of *Gossypium herbaceum*". *Molecules* 18.1 (2013): 951-962.
3. Devasagayam TP, et al. "Free radicals and antioxidants in human health: current status and future prospects". *Journal of the Association of Physicians of India* 52 (2004): 794-804.
4. Ramu G and Dhanabal SP. "Antioxidant Studies on Ethanol Extracts from Two Selected Genera of Indian *Lamiaceae*". *Indian Journal of Pharmaceutical Sciences* 77.6 (2015): 780-782.
5. Ramu G and Krishna Mohan G. "Inhibitory effect on nitric oxide and protein denaturation of two traditional plants used in arthritis treatment". *Pharmacologyonline* 3 (2018): 145-149.
6. <http://www.maripoisoncenter.com/ctr/9504cholinesterases.html>
7. <http://www.occuphealth.fi/e/info/anl/195/rama.html>
8. Atta-ur-Rahman and Iqbal Choudhary. "Recent studies on bioactive natural products". *Pure and Applied Chemistry* 71.6 (1999): 1079-1081.
9. Gerhard Vogel and Wolfgang Vogel. "Drug Discovery and Evaluation Pharmacological assays". Springer Verlag, New York (1997): 320-321.
10. Adewusi EA, et al. "Medicinal plants with cholinesterase inhibitory activity: A review". *African Journal of Biotechnology* 9.49 (2010): 8257-8276.

11. Lukhoba CW, et al. "Plectranthus: a review of ethnobotanical uses". *Journal of Ethnopharmacology* 103.1 (2006): 1-24.
12. Ramu G., et al. "Pharmacognostical evaluation on the leaves of *Plectranthus mollis*". *World Journal of Pharmacy and Pharmaceutical Sciences* 4.9 (2015): 1675-1679.
13. Ramu G., et al. "Preliminary phytochemical and antioxidant studies of hydroalcoholic extracts from selected genera of Indian Lamiaceae". *Asian Pacific Journal of Tropical Biomedicine* 2.2 (2012): 685-688.
14. Sharififar F, et al. "Essential oil and methanolic extract of *Zataria multiflora* Bliss with anti cholinesterase effect". *Pakistan Journal of Biological Sciences* 15.1 (2012): 49-53.
15. Bartus RT, et al. "The cholinergic hypothesis of geriatric memory dysfunction". *Science* 217 (1982): 408-417.
16. Lee HP, et al. "Antioxidant approaches for the treatment of Alzheimer's disease". *Expert Review of Neurotherapeutics* 10 (2010): 120-128.
17. Bival Štefan M., et al. "Total hydroxycinnamic acids assay: Prevalidation and application on *Lamiaceae* species". *Food Analytical Methods* 6 (2013): 1-11.
18. Mahmood C., et al. "Vernolic and cyclopropenoid fatty acids in *Plectranthus mollis*, syn. *Plectranthus incanus*, link seed oil: A rich source of oil". *Journal of Chemical Technology and Biotechnology* 45.2 (1989): 143.
19. Dastmalchi K, et al. "Acetylcholinesterase inhibitory guided fractionation of *Melissa officinalis* L". *Bioorganic and Medicinal Chemistry* 17.2 (2009): 867-871.
20. Agata I, et al. "A tetrameric derivative of caffeic acid from *Rabdosia japonica*". *Phytochemistry* 28.9 (1989): 2447.
21. Von Litvienko VI, et al. *Planta Medica* 27 (1975): 372.
22. Rivera Nunez D and Obon de Castro C. "The ethnobotany of old world *Labiatae*". In *Advances in Labiate Sciences*, R.M. Harley and T. Reynolds, editors, Royal Botanic Gardens: Kew (1992): 455.
23. Rodriguez-Hahn L, et al. "The distribution of diterpenoids in *Salvia*". In *Advances in Labiate Sciences* R.M. Harley and T. Reynolds, editors, Royal Botanic Gardens: Kew (1992): 335.
24. Orhan I, et al. "Inhibitory effect of Turkish *Rosmarinus officinalis* L. on acetylcholinesterase and butyryl cholinesterase enzymes". *Food Chemistry* 108.2 (2008): 663-668.
25. Savelev S, et al. "Butyryl and acetyl-cholinesterase inhibitory activities in essential oils of *Salvia* species and their constituents". *Phytotherapy Research* 18.4 (2004): 315-324.

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