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Original Research Article

In-vitro studies on acetylcholinesterase and butyrylcholinesterase inhibitory potentials of aerial parts of *Vernonia oligocephala* (Asteraceae)

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Abstract

Purpose: *To evaluate the cholinesterases (acetylcholinestrase and butyrylcholinestrase) enzyme inhibitory potential of aerial parts Vernonia oligocephala (V. oligocephala) as a potential remedy for the management of neurodegenerative disorders.*
Methods: *Crude methanol extract of V. oligocephala leaves and its fractions including n-hexane, dichloromethane (DCM), ethyl acetate and n-butanol were investigated for their inhibitory potentials against cholinesterases enzymes employing standard ELISA microtiter plate reader assay.*
Results: *All the extracts were moderately active against both the tested enzymes. The ethyl acetate and n-hexane fractions showed highest inhibition against acetylcholinesterase enzyme with IC_{50} values of 128.81±0.44 µg/mL and 200.51±0.22 µg/mL, respectively. In case of butrylcholinesterase, ethyl acetate (IC_{50}; 145.71±0.19µg/mL) and DCM (IC_{50}; 269.31±0.45 µg/mL) fractions were the most active. All other fractions including crude methanol extract exhibited least inhibition. Overall, ethyl acetate fraction was the most active against both enzymes.*
Conclusion: *Results indicate the promising potential of V.oligocephala as source of new potential compounds for management of Alzheimer's disease. However, further studies to isolate and identify the potential bioactive components are needed.*

Keywords: *Vernonia oligocephala*, Acetylcholinesterase, Butyrylcholinesterase, Alzheimer's disease

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INTRODUCTION

Alzheimer's disease is linked with the loss of cholinergic neurons in different parts of the brain and it is the common cause of dementia in older people [1]. Cortical cholinergic projections arising from the basal forebrain play critical role in the memory component of both Alzheimer and Parkinson types of dementia [2]. Alzheimer's disease originally posit a connection between disturbances in cerebral cholinergic neuro-transmission and some of the cognitive impairments especially memory [3].Symptoms of the disease can be modified by most of the cholinomimetic drugs [4]. Acetylcholine (ACh) and butyrylcholine (BCh) inhibitors are the approved agents by Food and Drug Administration (FDA) for the treatment of Alzheimer's disease (AD) [5]. The serious side effects of synthetic licensed drugs which are used to treat AD have forced the researchers to investigate safer AChE or BChE inhibitors from natural sources [6]. In traditional practices of medicine, numerous plants and their constituents are reported to enhance cognitive functions and to alleviate other symptoms of AD [7]. The discovery of AChE or BChE enzymes inhibitors from the medicinal plants have attracted the attention of the scientists and open a new way in research field [8].

The genus *Vernonia* is one of the major genus of family Asteraceae consisting of about 650 species [9]. The chemical constituents of this family mainly include volatile oils, terpenoids, phenolic compounds and tannins [10]. *V. oligocephala*is one of the important perennial herb of this genus traditionally used in treating cough asthma, colics, inflammation, ophthalmology, pruritus, and to prevent gonorrhea, tetanus and urinary tract infections [11]. The purpose of this study was to evaluate the possible inhibitory effects of the extracts against AChE or BChE.

EXPERIMENTAL

Chemicals and Standards

All enzymes were purchased from Sigma-aldrich and used without further purification. These are AChE*Electric eel* (Sigma-Aldrich GmbH, USA), BChE equine serum lyophilized (Sigma-Aldrich GmbH, USA), substrates: acetylthiocholine iodide (Sigma-Aldrich, U.K.), butyrylthiocholine-iodide (Sigma-Aldrich, Switzerland), DTNB (5,5-dithio-bis-nitrobenzoic acid) (Sigma-Aldrich, Germany) andeserine Sp. (Sigma-Aldrich, France). Chemicals including dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), potassium hydroxide were of extra pure analytical grade from Merck Group (Darmstadt, Germany) were used.

Plant material and extraction

Aerial parts of *V. Oligocephala* were collected from the peripheral areas of Bahawalpur and identified by Dr. Muhammad Arshad Chaudhry (late); Director, Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur and a voucher specimen number was also deposited for future reference.

The shade dried leaves of *V. oligocephala* were macerated in 80% w/v methanol for 7 days with periodic shaking, filtered and then concentrated using a rotary evaporator at 37°C. Concentrated methanol crude extract was further subjected to fractionation with *n*-hexane, dichloromethane (DCM), ethyl acetate and *n*-butanol [thrice with each solvent]. The filtrates of the respective fractions thus obtained were again concentrated by rotary evaporator. The respective concentrates, VOAC (*V. oligocephala* aerial crude methanol extract), VOAD (*V. oligocephala* aerial DCM extract), VOAE (*V. oligocephala* aerial ethyl acetate extract), VOAH (*V. oligocephala* aerial *n*-hexane extract) and VOAB (*V. oligocephala* aerial *n*-butanol extract), were weighed and stored in labelled airtight vials in refrigerator.

Acetylcholinesterase assay

The AChE inhibition activity was performed a slight modification of the protocol previously reported [12]. Total volume of the reaction mixture was 100 µL. It contained 60 µL Na_2H PO_4 buffer with concentration of 50 mM having pH 7.7. 10 µL test compound (0.5 mM/well) was added, followed by the addition of 10 µL (0.005 unit/well) enzyme. The contents were mixed and pre-read at 405 nm. Then, contents were pre-incubated for 10 min at 37°C. The reaction was initiated by the addition of 10 µL of 0.5 mM/well substrate (acetylthiocholine iodide), followed by the addition of 10 µL DTNB (0.5 mM/well). After 30 min of incubation at 37°C, absorbance was measured at 405 nm using 96-well plate reader (Synergy HT, Biotek, USA). All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM/well) was used as a positive control. Percentage inhibition was calculated as follows:

$$Inhibition \cdot (\%) = \frac{Control - Test}{Control} x \cdot 100 \quad \ldots\ldots \ (1)$$

where Control is the total enzyme activity without inhibitor and Test is the enzyme activity in the presence of test compound.

IC_{50} values (concentration at which there is 50 % enzyme catalyzed reaction) were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

Butyrylcholinesterase assay

The BChE inhibition activity was performed using a slight modification of the method previously reported [12]. Total volume of the reaction mixture was 100 µL containing 60 µL, Na_2HPO_4 buffer, and 50mM having pH 7.7. 10 µL test compound 0.5 mM/well was added followed by the addition of 10 µL (0.5 unit/well) BChE. The contents were mixed and pre-read at 405 nm and then pre-incubated for 10 min at 37°C. The reaction was initiated by the addition of 10 µL of 0.5 mM/well substrate (butyrylthiocholine chloride), followed by the addition of 10 µL DTNB, 0.5 mM/well. After 30 min of incubation at 37°C, absorbance was measured at 405 nm using 96-well plate reader (Synergy HT, Biotek, USA). All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM/well) was used as positive control. Inhibition (%) was calculated using above Eq 1.

Statistical analysis

The results obtained were expressed as mean value and standard deviation.All the tests were performed for three times and the data analysis was carried out in triplicates. To calculate $IC_{50,}$ graph Pad Prism software (Version 6.03) was used.One-way analysis of variance (ANOVA) and Tukey's significant difference post hoc test ($p < 0.05$) were used to calculated differences. SPSS v22.0 software was used to carry out all experimental analysis.

RESULTS

The results of percentage AChE and BChE enzyme inhibition (at the concentration of 0.5 mg/mL) and half maximal concentration (IC_{50}) values of crude methanol extract and different fractions of *V. oligocephala* leaves were calculated. The results of AChE inhibition are presented in Table 1.

The effect of the *V. oligocephala* extract and its fractions on the inhibitory activity against BchE are depicted in Table 2.

Table 1: Percentage enzyme inhibition and IC_{50} values of *V. oligocephala* extract and fractions against acetylcholinesterase

Sample code	AChE inhibition (%)	AChEIC_{50} (µg/mL)
VOAC	52.41±0.39	**>500
VOAD	23.31±0.34	**>500
VOAE	73.79±0.11	128.81±0.44
VOAH	68.01±0.41	200.51±0.22
VOAB	43.01±0.21	**>500
Eserine	91.29±1.17	0.04±0.0001

Values are expressed as means ± SD of three replicates; ** IC_{50} value was higher than 500 µg/mL, AChE= acetylcholinesterase. Eserine was used as positive control.

Table 2: Percentage enzyme inhibition and IC_{50} values of *V. oligocephala* extract and fractions against butyrylcholinesterase

Sample code	BChE inhibition (%)	BChEIC_{50} (µg/mL)
VOAC	29.44±0.55	**>500
VOAD	60.78±0.11	269.31±0.45
VOAE	74.25±0.44	145.71±0.19
VOAH	44.01±0.41	**>500
VOAB	34.63±0.34	**>500
Eserine	82.82±1.09	0.85±0.0001

Values are expressed as means ± S.D. of three replicates; **IC_{50} value was higher than 500 µg/mL, BChE= butyrylcholinesterase. Eserine was used as positive control.

DISCUSSION

This study has demonstrated that the aerial parts of *Vernonia oligocephala* has moderate inhibitory activity against cholinesterases (acetylcholine-strase and butyrylcholinestrase) enzymes with IC_{50}values in the range of 128.81±0.44 - 269.31±0.45 µg/mL. The methanol crude extracts of the plant did not produce any significant inhibition of cholinesterases unlike the *n*-hexane and ethyl acetate extracts.

The aerial part of *V. oligocephala* is known to possess sesquiterpene lactones, triterpene (3β-acetoxyneohop-13(18)-ene) along with β-sitosterol, 3β-hydroxylup-20,29-ene, 3β-hydroxy-olean-12en-28-oic acid and β-sitosterol-3-O-β-D-glucopyranoside [13]. The AChE inhibitory potential of *V. oligocephala* extracts can be attributed to the occurrence of the sesquiterpenes in this plant [14-16]. Similarly, some alkaloids, such as galantamine, physostigmine [17], and other compounds like glycosides, terpenoids, flavonoids [18], lignans[19], coumarins [20], ursolic acid [21] terpenoid, flavonoids and phenolic compounds are known potent cholinesterases inhibitors [22-

24]. Several *venonia* species have been found to contain phenolic and flavonoids like naringenin, apigenin, protocatechuic acid and luteolin-catechin [25]. These compounds could also have contributed to the observed bioactive potential of *V. oligocephala* reported in this study.

CONCLUSION

The leaves of *V. oligocepha* has inhibitory activity against both acetylcholinesterase and butyrylcholinesterase enzymes. Although further studies are required to isolate the compound responsible for the observed bio-activity, the plant could be considered for further studies in the treatment of neurodegenerative disorders.

DECLARATIONS

Conflict of interest

The authors declare that they have no conflict of interest with regard to this work.

Authors' contribution

We declare that this work was performed by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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