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In vitro acetylcholinesterase and butyrylcholinesterase inhibitory potentials of essential oil of *Artemisia macrocephala*

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Abstract

In this study, we screened the essential oil of *Artemisia macrocephala* for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory potentials. Ellman's assay method was used to investigate the enzyme inhibitory potential of the essential oil. The oil sample showed 87.7 ± 1.2, 77.9 ± 0.6, 74.5 ± 1.9 and 62.5 ± 0.3 percent AChE inhibition at 1000, 500, 250 and 125 µg/mL concentrations respectively with IC_{50} value of 40 µg/mL. Similarly it showed 81.8 ± 0.6, 75.6 ± 1.2, 70.0 ± 0.6 and 64.2 ± 1.4 percent BChE inhibition in 1000, 500, 250 and 125 µg/mL concentrations respectively with IC_{50} value of 30 µg/mL. The results of this study confirm the beneficial applications of the oil sample in the treatment of various neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, ataxia and all other forms of dementia.

Introduction

Being affordable and accessible, medicinal plants have been as primary and essential part of many people's life all over the world. The selection of medicinal plants is a conscious process. This has led to a large number of medicinal plants being used by different cultures of the world (Heinrich et al., 1998). Phytochemicals, as plant components with enhanced distinct activities towards animal biochemistry and metabolism, are being widely explored for their ability to provide health benefits (Sharma et al., 2009).

Plants in nature have been reported to serve as potential sources of AChE and BChE inhibitors, as an alternative treatment for Alzeimer's disease. In traditional practice, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases and different neuropharmacological disorders. Ethnopharmacological approach and bioassay-guided isolation have provided a lead in identifying potential AChE inhibitors from plant sources, including those for memory disorders (Khan and Khatoon, 2008).

Artemisia macrocephala (Synonym: *Artemisia griffithiana* Bioss) belongs to family *Asteraceae,* which is of great medicinal importance. *A. macrocephala* is 20–30 cm tall. It is called "Tarkha" in Pashto language. It is abundantly found in northern areas of Pakistan (Zareh, 2005). Previously, reported constituents from the essential oil of *A. macrocephala* are propionic acid, acetic acid, enanthic acid and isovaleric acid. Its oil also contains camphene, α-pinine, β-pinine, limonene, p-cymene, borneol, 1,8-cineole, and camphor (Dudko et al., 1974). Previously, we reported *A. macrocephala* for preliminary phytochemical screening and its crude extract for antispasmodic activity (Ali et al., 2011). We have also reported antispasmodic activity for the essential oils and different fractions of *A. macrocephala*. Its different fractions were also reported for antioxidant activity (Ali et al., 2013).

In this study, we have screened the essential oil of *A. macrocephala* for AChE and BChE inhibitory potentials to find out its possible beneficial applications in the treatment of various neurodegenerative disorders including Alzheimer's disease, Parkinson's disease,

ataxia and all other forms of dementia.

Methods and Materials

Chemicals and drugs

Enzymes including AChE electric eel (CAS 9000-81-1 Sigma-Aldrich GmbH USA), BChE equine serum Lyophilized (CAS 9001-08-5 Sigma-Aldrich GmbH USA), substrates acetylthiocholine iodide (CAS1866-15-5 Sigma-Aldrich UK), butyrylthiocholine Iodide CAS 2494-56-6 Sigma-Aldrich Switzerland), DTNB 5,5-dithio -bis-nitrobenzoic acid (CAS 69-78-3 Sigma-Aldrich Germany), Galanthamine hydrobromide Lycoris Sp. (CAS 1953-04-4 Sigma-Aldrich France) were used for enzyme inhibition study. For preparation of buffer, di-potassium hydrogen phosphate (K2HPO4), potassium dihydrogen phosphate (KH_2PO4), potassium hydroxide used were of the extra pure analytical grade.

Collection and authentication of plant's materials

Fresh aerial parts of young shoots of *A. macrocephala* were collected in the month of August 2009 from the hills near to Badwan Chowk, Dir Lower, Khyber Pakhtunkhwa, Pakistan. The plant was authenticated by plant taxonomist, Dr. Jehandar Shah, Vice Chancellor, Shaheed Benazir Bhutto University, Dir Upper, Sheringal. A voucher specimen "AM-01-2009" was submitted to the herbarium of Department of Botany, University of Malakand.

Distillation of essential oil

Fresh twigs of *A. macrocephala*, in triplicate, were subjected to hydrodistillation for 6 hours in a Clevenger -type apparatus (Chang et al., 2001). The yellow-colored essential oil with characteristic odor was obtained and stored in airtight containers prior to further analysis.

GC/MS analysis of essential oils

The chemical investigations of the oils were carried out through gas chromatography mass spectrometry (GC/MS). The gas chromatograph (Shimadzu) hyphenated to a mass spectrometer QP 2010 plus (Tokyo, Japan) had automatic sampler and injector (AOC-20S and AOC-20i) respectively. Helium gas was used as a carrier medium. The chromatographic separations were carried out in capillary column (TRB-FFAP; Technokroma) with 30 m length; 0.35 mm i.d.; 0.250 µm thickness and was treated with polyethylene glycol. Other GC-MS conditions include 250°C temperature of ion source (EI), 240°C interface temperature, 100 KPa pressure and 1.8 min cut time for the solvent. The software for GC-MS solutions was used for controlling the system and for getting the data. Compounds were identified by comparing their mass spectra with standard mass spectra.

Anticholinestrase assays

AChE from electric eel and BChE from equine serum were used to investigate the enzyme inhibitory potential of the essential oil using Ellman's assay (Ellman et al., 1961). The essential oil was dissolved in few drops of DMSO and further diluted in phosphate buffer (0.1 M) in different concentrations (125-1000 µg/mL). AChE (518 U/mg) and BChE (7-16 U/mg) were diluted in 0.1M phosphate buffer (pH 8.0) until final concentrations of 0.03 U/mL (AChE) and 0.01 U/mL (BChE) was obtained. Solutions of DTNB (0.2273 mM), ATchI (0.5 mM) and BTchI (0.5 mM) were prepared in distilled water and kept in the eppendorf caps in the refrigerator (8°C). For each assay, enzyme solution of 5 µL was added to the cuvette followed by essential oil (205 µL) and DTNB reagent (5 µL). The solution mixture was maintained at 30°C for 15 min using a water bath with the subsequent addition of substrate solution (5 µL). A double beam spectrophotometer (Thermo Electron Corporation, USA) was used to measure the absorbance at 412 nm. Galanthamine was used as positive control. The absorbance along with the reaction time was taken for four minutes at 30°C. The experiment was performed in triplicate. The percent enzyme activity and enzyme inhibition by control and tested sample were calculated from the rate of absorption with change in time ($V=\Delta Abs/\Delta t$) as follows:

Enzyme inhibition (%) = 100 - %Enzyme activity

Enzyme activity (%) = 100 × V/Vmax

where Vmax is the enzyme activity in the absence of inhibitor drug

Statistical analysis

The essential oil concentrations providing 50% inhibition (IC_{50}) were calculated from the graph of percent inhibition versus extract concentrations in solution, using Microsoft Excel program. Two-way ANOVA followed Bonferroni multiple comparison tests were applied for the comparison of positive control and test groups. P values <0.05 were considered statistically significant. GraphPad Prism was used to draw the graphs. IC_{50} values and mean ± SEM were calculated at 95% confidence intervals.

Results

The detailed GC/MS report of oils of *A. macrocephala* is given in Table I. The data show that α-thujone, 3-thujanone, and cineol were present in major quantity having 56.2, 11.7 and 10.8% respectively. While 1-terpinen-4-ol, alcanfor, sabinene and o-cymene were present at 5.5, 3.9, 3.8 and 2.4% respectively. Apart from these components, other are alpha-phellandrene, alpha-

Table I

GC/MS report for the analysis of essential oils of *A. macrocephala*

SL. No.	PCSIR ID No.	Name of constituents	Retention time	Area	Concentration%
1	1	alpha-Phellandrene	8.6	13042	0.09
2	2	alpha-Pinene	8.9	17076	0.12
3	3	Camphene	9.6	51948	0.37
4	4	Sabinene	10.6	533038	3.84
5	5	beta-Pinene	10.8	13678	0.1
6	6	beta-Myrcene	11.4	15785	0.11
7	9	(+)-4-Carene	12.6	75125	0.54
8	10	o-Cymene	13.0	330695	2.38
9	13	Cineole	13.3	1494375	10.76
10	16	3-Carene	14.6	177870	1.28
11	18	Terpinolene	15.9	23296	0.17
12	20	beta -Linalool	16.8	11748	0.08
13	21	3-Thujanone	17.0	1628020	11.73
14	22	alpha-Thujone	17.5	7807434	56.24
15	23	Alcanfor	18.5	546646	3.94
16	24	1-Terpinen-4-ol	19.6	758210	5.46
17	26	p-Menth-1-en-8-ol	20.0	54360	0.39
18	28	n-Octyl acetate	20.4	1079	0.01
19	31	p-Anisaldehyde	21.3	3546	0.03
20	32	alpha-Citral	21.4	121889	0.88
21	35	Bornyl acetate	20.1	28392	0.2
22	39	p-Menth-1-en-8-ol, acetate	23.2	15475	0.11
23	42	Caryophyllene	24.6	46636	0.34
24	46	beta-Farnesene	25.0	8569	0.06
25	49	Germacrene D	25.5	60629	0.44
26	51	gamma-Elemene	25.7	30959	0.22
27	56	trans-Nerolidol	26.6	4575	0.03
28	63	(Z,E)-Farnesol	28.6	1546	0.01
29	72	1S-alpha-Pinene	32.6	2597	0.02
30	73	p-Cimene	32.9	3441	0.02
31	74	2-Furanmethanol	32.5	2182	0.02

pinene, 3-carene, n-octyl acetate, trans-nerolidol and beta-farnesene etc.

The oil sample showed 87.7 ± 1.2, 77.9 ± 0.6, 74.5 ± 1.9 and 62.5 ± 0.3 percent acetylcholinesterase inhibition at 1000, 500, 250 and 125 µg/mL concentrations respectively as compared to the standard galanthamine (Figure 1**)**. The IC_{50} value for the essential oil sample and galanthamine were 40 and <0.1 µg/mL respectively.

The essential oil sample of *A. macrocephala* showed from good to moderate percent butyrylcholinesterase inhibition in concentration dependent manner. It showed 81.8 ± 0.6, 75.6 ± 1.2, 70.0 ± 0.6 and 64.2 ± 1.4 percent butyrylcholinesterase inhibition in 1000, 500, 250 and 125 µg/mL concentrations respectively as compared to that of galanthamine (Figure 2). The IC_{50} value for the essential oil sample and galanthamine were 30 and <0.1 µg/mL respectively.

Discussion

Alzheimer's disease is the most common cause of dementia. It causes the loss of intellectual and social abilities and thus serves enough to interfere with daily functioning (Loizzo et al., 2008). Alzheimer's disease patients show a progressive loss of cholinergic synapses in the brain regions performing higher mental func-

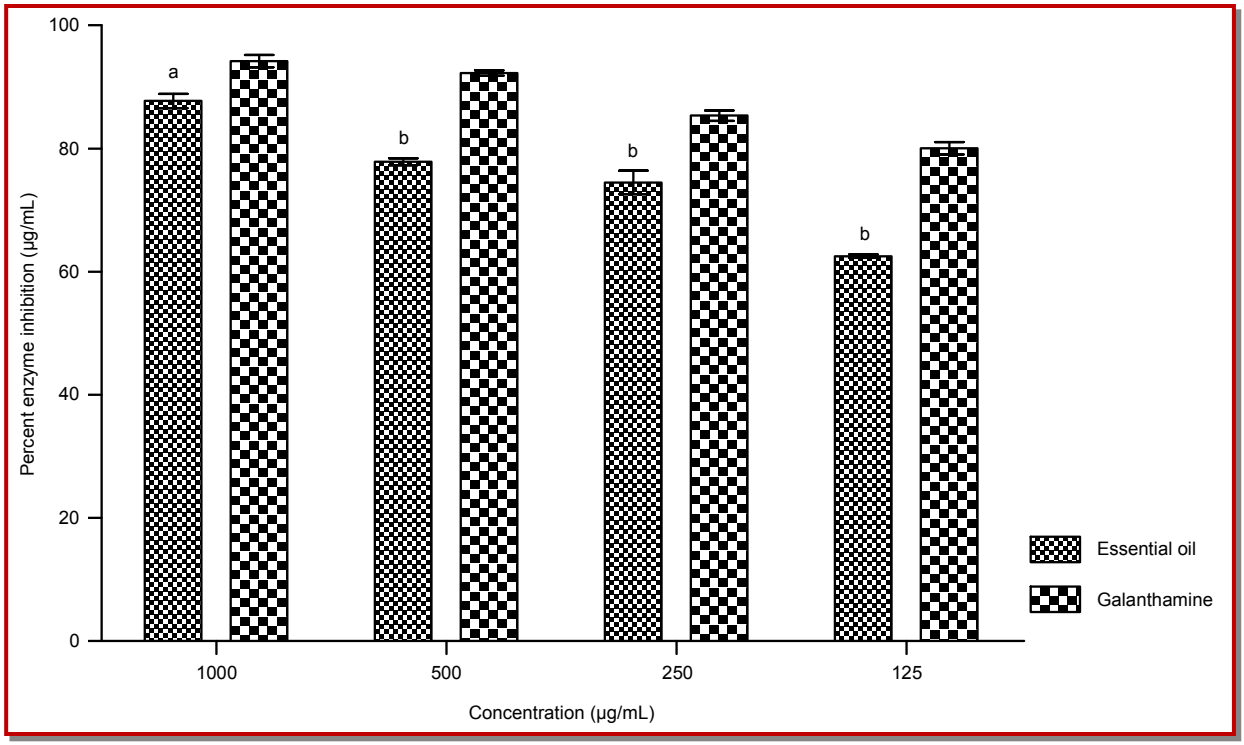

Figure 1: Acetylcholinesterase inhibitory potentials of essential oil of *A. macrocephala*
Values significantly different, [a]p<0.01; [b]p<0.001 in comparison to positive control group (galanthamine)

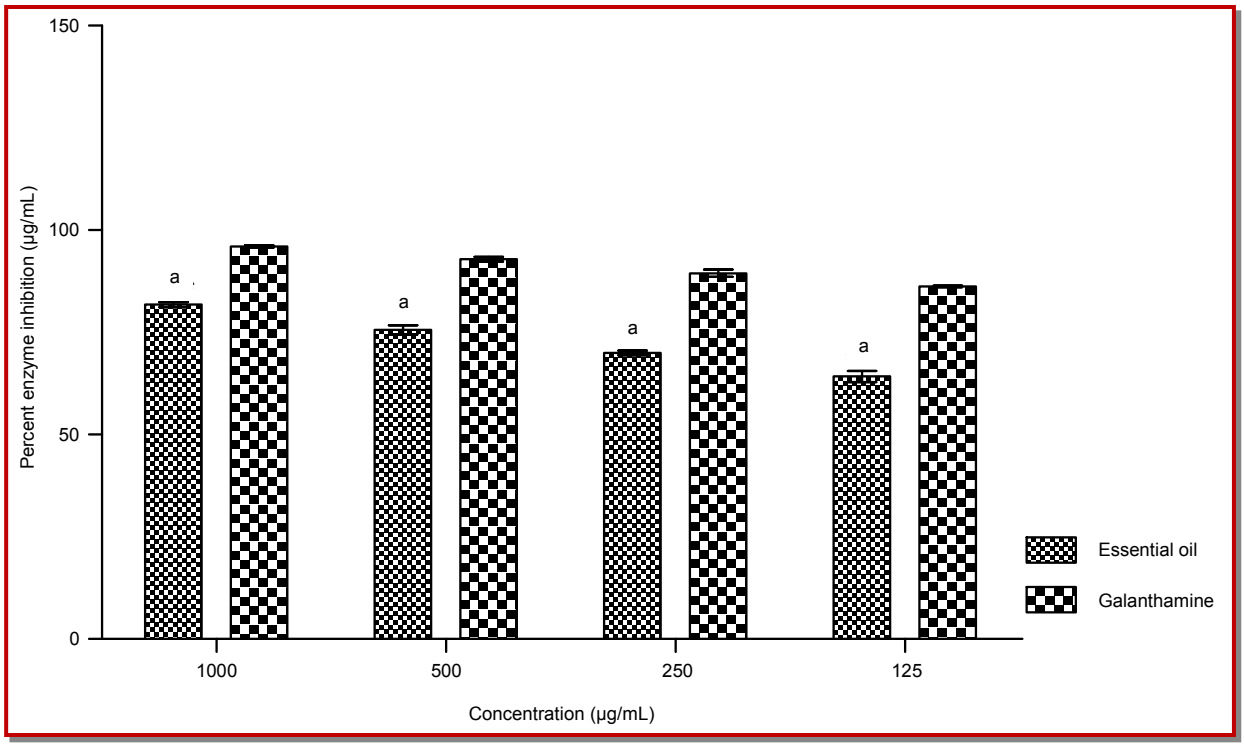

Figure 2: Butyrylcholinesterase inhibitory potentials of essential oils of *A. macrocephala*
Values significantly different, [a]p<0.001 in comparison to positive control group (galanthamine)

tions, mainly the hippocampus and neocortex. In the Alzheimer's disease patients, a decrease in the acetylcholine (ACh), a neurotransmitter, appears to be a critical element in the development of dementia. Hence, Alzheimer's disease and other forms of dementia could be treated by the use of agents that restore the level of

acetylcholine through the inhibition of both forms of cholinesterase: AChE and BChE. Moreover, the inhibition of AChE plays a key role not only enhancing cholinergic transmission in the brain, but also reducing the aggregation of amyloid beta peptide (Aβ) and the formation of the neurotoxic fibrils in Alzheimer's disease (Candy et al., 1983; Sung et al., 2002).

Several treatment strategies have been developed, but AChE and BChE inhibitors have become the most useful alternatives in the treatment of Alzheimer's disease. Drugs as eserine, tacrine, donepezil, rivastigmine, and galanthamine have been approved for the treatment of Alzheimer's disease. However, these drugs are known to have limitations for clinical use due to their short half-lives and antagonistic side effects (Mukherjee et al., 2007). Therefore, the search for new AChE and BChE inhibitors with higher efficacy and safety from alternative sources like natural products is continued.

In search of safe, effective and inexpensive, a number of essential oils, e.g. from some *Salvia* species, *Foeniculum vulgare*, *Acorus calamus*, *Melaleuca alternifolia* (tea tree oil), *Citrus paradisi*, have been so far reported to be effective against Alzheimer's disease. It is very interesting to know that individual constituents of different essential oils from different plants have also been reported with enhanced AChE and BChE inhibition potentials (Orhan et al., 2008).

In this study, we investigated the essential oil of *A. macrocephala* for AChE and BChE inhibition potentials. The oil sample showed good AChE and BChE inhibition in concentration dependent manner. It showed 87.7 ± 1.2 and 81.8 ± 0.6 percent AChE and BChE inhibition respectively at 1,000 µg/mL concentration. The standard galanthamine showed 94.2 ± 1.0 and 96.0 ± 0.3 percent AChE and BChE inhibition respectively at 1,000 µg/mL concentration. The IC_{50} values of the oil sample for AChE and BChE were 40 and 30 µg/mL.

Based on the results of this study it can be concluded that the essential oil of *A. macrocephala* possesses AChE and BChE inhibitory potentials. The study confirms the beneficial applications of the oil sample in the treatment of various neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, ataxia and all other forms of dementia.

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