Executive Summary of
Final report on
UGC Major Research Project

Project title:

"Acetyl Cholinesterase (AChE) Inhibitors from Indian Spices"

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Submitted by

Dr. Pulok K. Mukherjee, *PhD, FRSC*
Director
School of Natural Product Studies
Dept. of Pharmaceutical Technology
Jadavpur University
Kolkata - 700032
Tele-fax: +91 33 24146046
E.mail: natprodpk@yahoo.com

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1. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR	**:**	Dr. Pulok K. Mukherjee, PhD, FRSC Department of Pharmaceutical Technology Jadavpur University Kolkata 700032, India. Telephone/fax: +91-3324146046
2. NAME AND ADDRESS OF THE INSTITUTION:	**:**	Department of Pharmaceutical Technology Jadavpur University Kolkata 700032, India. Telephone/fax: +91-3324146046
3. UGC APPROVAL NO. AND DATE	**:**	F. No. 33-119/2007 (SR), Dated: 1/4/2008
4. DATE OF IMPLEMENTATION	**:**	1/4/2008
5. TENURE OF THE PROJECT	**:**	1/4/2008 to 31/3/2011
6. TITLE OF THE PROJECT	**:**	"Acetyl cholinesterase (AchE) inhibitors from Indian Spices"

7. Brief objective of the project:

Considering the vast natural entity and the wide variability of the raw materials and the wide complexity of the phyto –constituents, the use of High Throughput Screening (HTS) techniques with de-replication of the extracts can play a lead role for the screening of natural products for a specific disease. This technique can be further correlated with another disorder to alleviate human suffering. Keeping this in view the main objective of this project is to develop HTS screening methodologies for the Alzheimer's diseases through acetyl cholinesterase inhibitory model with known Indian spices like Curcuma longa and Murraya koiengii with reported efficacy. Beside this other spices like Trigonella foenum greaecum (Fenugreek), Coriandrum sativam L. (Dhania), Cuminum cyminum L (Jeera) will be further evaluated for their inhibitory potential against AchE enzyme so as to get a lead for the Alzheimer's disease. Thus, the work is based on the combined approaches of exploitation and exploration of botanicals for the lead finding from Indian medicinal spices.

8. Brief report for the UGC major research project entitled: "Acetyl Cholinesterase (AChE) Inhibitors from Indian Spices" File No 33-119/2007 (SR)

There are only few synthetic medicines with several adverse effects, available for treatment of cognitive dysfunction and memory loss associated with these diseases. A variety of plants has been reported to possess AChE inhibitory activity and so may be relevant to the treatment of neurodegenerative disorders such as AD. Hence, developing

potential AChE inhibitors from botanicals is the need of the day. This project has great impact in promotion and development of Indian food for health benefits particularly in the filed of Alzheimer's diseases (AD). It should be continued further in this context for the promotion and development of natural products. Major works on the present projects are as follows:

In this project we had selected 11 plants which are used as spices for food preparation in Indian population. These plants are *Trigonella foenum greaecum, Murraya koiengii, Illicium verum, Coriandrum sativam, Cuminum cyminum, Carum carvi, Elettaria cardamomum, Cinnamomum tamala, Eugenia caryophyllata, Trachyspermum copticu and Cinnamomum zeylanicum.* These plants have been subjected for hydroalcoholic extraction as well as the volatile oil was also isolated by stem distillation. These volatile oils are characterized by GCMS. The extracts and the volatile oils are standardized with HPLC and HPTLC with the respective marker compound. The extracts and volatile oils together with the marker compounds used for in vitro Pharmacological evaluation for Acetyl Cholinesterase (AChE) and Butaryl Cholinesterase (BChE) inhibition assay. They are also used for in vivo estimation of Brain Acetyl Cholinesterase (AChE) Activity. Most of the above mention spices, their extract, volatile oils and the marker compound found to inhibit Acetyl Cholinesterase. So the present investigation is useful to get a lead for the Alzheimer's disease as well as the beneficiary effect of the spices against the disease

Following Publications were made through this project:

1. N. Satheesh Kumar , Pulok K. Mukherjee , S. Bhadra , B. P. Saha , B. C. Pal. 2009. Acetylcholinesterase inhibitory potential of a carbazole alkaloid, mahanimbine, from Murraya koenigii. Phytotherapy Research 24; 629 – 631.
2. N. SatheeshKumar, P. Mukherjee, S. Bhadra, B. Saha. 2010. Acetylcholinesterase enzyme inhibitory potential of standardized extract of Trigonella foenum graecum L and its constituents. Phytomedicine, 17(3), 292-295.

3. Lead finding for Acetyl cholinesterase Inhibitors from natural origin: Structure activity relationship and scope. Pulok K Mukherjee, N. Satheeshkumar, M. Venkatesh, S. Ponnusankar, 2011. Mini Reviews in Medicinal Chemistry, Volume 11, Number 3, 247-262(16).

Dr. Pulok K. Mukherjee
Principal Investigator
Director,
School of Natural Product Studies,
Department of Pharmaceutical Technology,
Jadavpur, Kolkata - 700032, India.

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SHORT COMMUNICATION

Acetylcholinesterase Inhibitory Potential of a Carbazole Alkaloid, Mahanimbine, from *Murraya koenigii*

N. Satheesh Kumar[1], Pulok K. Mukherjee[1]*, S. Bhadra[1], B. P. Saha.[1] and B. C. Pal[2]

[1]School of Natural Product Studies, Department of Pharmaceutical Technology, Jadavpur University, Kolkata – 700032, India
[2]Medicinal Chemistry Division, Indian Institute of Chemical Biology, Kolkata – 700032, India

In the search for acetylcholinesterase (AChE) inhibitors from Indian medicinal plants, via bioassay-guided isolation, a carbazole alkaloid, mahanimbine [3, 5-dimethyl-3-(4- methylpent-3-enyl)-11H-pyrano [5, 6-a] carbazole], was isolated from the petroleum ether extract of the leaves of *Murraya koenigii*. Inhibition of AChE was evaluated based on Ellman's method using 96-well microplate readers. Mahanimbine inhibited AChE activity in a dose-dependent manner with an IC_{50} value of 0.03 ± 0.09 mg/mL, while galantamine was used as a standard. The AChE inhibitory activity of this carbazole alkaloid has not been reported so far, and this study is the first to reveal this activity in carbazole alkaloid mahanimbine, isolated from *Murraya koenigii*.

Keywords: Murraya koenigii; mahanimbine; acetylcholinesterase inhibition; AChE; spices; carbazole alkaloid; Indian medicinal plant.

INTRODUCTION

Alzheimer's disease (AD) is characterized by a progressive degeneration of brain tissue and is the most common cause of dementia among the elderly throughout the world (Mukherjee *et al*., 2007a). Inhibition of acetylcholinesterase (AChE) in the brain enhances acetylcholine activity, which is one of the main strategies in the clinical management of AD. Natural products have already established themselves as an excellent source for AChE inhibitors, including galantamine, huperzine (Perry, 1986). Several reports on the search for AChE inhibitors from natural resources have been made from our laboratory (Mukherjee *et al*., 2007b; 2007c; 2007d). *Murraya koenigii* (L.) Spreng. (Rutaceae) is well known to Indian kitchens as a spice for its characteristic flavor and aroma. Leaves of this plant have been found to have a marked effect on the central nervous system and have been reported for the treatment of epilepsy (Joseph and Peter, 1985), antioxidant (Yukari *et al*., 2003), immunomodulatory (Shah *et al*., 2008), CNS stimulant (Adeleke *et al*., 2004), anti-amnesic (Vasudevan and Parle, 2009) and anti-inflammatory activities (Ramsewak *et al*., 1999). Mahanimbine has been found to inhibit cell cycle (M-phase) and possess apoptotic effect against cancerous cell line (Ito *et al.*, 2006). The present study dealt with the evaluation of the AChE inhibitory potential of different extracts and mahanimbine (**1**) isolated from the leaves of *Murraya koenigii*.

MATERIALS AND METHODS

Plant materials. *Murraya koenigii* leaves were collected from local fields in Erode, Tamil Nadu, India and authenticated by Dr S. Rajan, Field Botanist, Medicinal Plants Collection Unit, Ooty, Government of India. A voucher specimen of the plant material (SNPS–1037) has been retained in the herbarium of the school of Natural Products Studies, at Jadavpur University, India for future reference.

Chemicals and standards. Acetylthiocholine iodide (ATCI), AChE from bovine erythrocytes, 5, 5-dithiobis [2-nitrobenzoic acid] (DTNB), galantamine (purity ≥ 94%) was obtained from Sigma (Poole, UK). Methanol, petroleum ether (60:80) and all other organic solvents (analytical reagent grade) were purchased from Merck, Mumbai, India.

Instrumentation. The identification and quantification of the isolated compound were carried out using ^{1}H-NMR and ^{13}C-NMR (500 MHz) spectra, which were recorded on a Bruker AM600 FT-NMR spectrometer with tetramethylsilane (TMS) as internal standard and deuterated DMSO-d6. The mass spectrum was obtained on a JMDS-300 instrument. TLC was performed over plates made of silica gel G (Merck, Mumbai, India). Column chromatography was performed on silica gel (200–400 mesh). AChE *in vitro* assay was performed by BioRad microplate reader.

* Corresponding author: Pulok K. Mukherjee, PhD, FRSC, Director, School of Natural Product Studies, Department of Pharmaceutical Technology, Jadavpur University, Kolkata – 700032, India.
E-mail: pknatprod@yahoo.co.in
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Extraction and isolation. Dried leaves of *Murraya koenigii* (1 kg) were extracted with 80% methanol (2 L). The filtrate was evaporated *in vacuo* to get a dark brownish semi solid residue. The resultant methanol extract (30 g) was successively extracted with petroleum ether (6.2 g) and $CHCl_3$ (5.4 g) and subjected for *in vitro* AChE inhibition activity. The petroleum ether fraction showed better inhibition than the methanol and chloroform fractions. The petroleum ether fraction was then subjected to column chromatography (15 cm in length, 4 cm I.D) using gradient elution of petroleum ether and $CHCl_3$. Each fraction collected was tested for the presence of alkaloids by Mayer's test and Dragendroff's test and then assayed for *in vitro* AChE inhibition activity. The most potent fractions F12–23 were pooled together and subjected to preparative TLC with the mobile phase of petroleum ether: $CHCl_3$ (6.5:3.5 v/v) for the isolation of Compound (**1**) (13 mg). Compound (**1**) was identified as mahanimbine by comparing the NMR and Mass spectral data with previously published literature (Roy and Chakraborty 1974; Ramsewak *et al.*, 1999).

Thin layer chromatography (TLC) with bioassay detection for AChE inhibition. The TLC with bioassay detection for AChE inhibition was modified from the study of Rhee *et al.* (2001). Briefly, petroleum ether extract (10 mg/mL) and isolated Compound 1 (1 mg/mL) were chromatographed over silica gel plate F_{254} (Merck, Darmstadt, Germany) by using 50:50 petroleum ether: chloroform solvent system (v/v) and the plates were subjected to AChE inhibition assay based on the method of Ellman *et al.* (1961). The plate was sprayed with DTNB/ATCI reagent (1 mM DTNB and 1 mM ATCI) until the silica was saturated with the solvent, but not so much that the spots ran off. It was allowed to dry for 3–5 min and then 3 U/mL of enzyme solution was sprayed. A yellow background appeared, with white spots for inhibiting compounds becoming visible after 5 min. These were observed and recorded within 15 min because they disappeared in 20–30 min. Another plate (treated as above) was sprayed at the same time for false-positive detection in each case. White spots on the yellow background were recorded and the results were compared with the results of TLC assay. If white inhibitory spots observed in the TLC assay were also seen as white spots in the false-positive activity assay, it was considered as false positive activity. If white spots were seen only in the TLC assay, and not in the false-positive activity assay, it was considered to be true activity.

Enzyme assay. AChE inhibitory activity was measured for methanol, petroleum ether, chloroform extracts and mahanimbine isolated from *Murraya koenigii*. Galantamine was used as the standard AChE inhibitor. AChE activity was measured using a 96-well microplate reader (Ellman *et al.*, 1961; Mukherjee *et al.*, 2007b; 2007c). The enzyme hydrolyzes the substrate acetylthiocholine resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 405 nm. In the 96-well plates, 125 µL of 3 mM DTNB, 25 µL of 15 mM ATCI, 50 µL of buffer and 25 µL of sample dissolved in phosphate buffer were added. The absorbance was measured at 405 nm every 13 s for 65 s. 25 µL of 0.22 U/mL of AChE enzyme was then added and the absorbance was again read every 13 s for 104 s. The absorbance was read using a BioRad microplate reader at 405 nm. Absorbance was plotted against time and enzyme activity was calculated from the slope of the line so obtained and expressed as a percentage compared to an assay using a buffer without any inhibitor.

RESULT AND DISCUSSION

Bioassay guided isolation of the petroleum ether extract of *Murraya koenigii* leaves led to the separation of several fractions (10 mL each). Based on *in vitro* AChE inhibitory activity and the presence of alkaloids (positive Mayer's test and Dragendroff's test) the fractions F12–F23 were found to be most active. Further purification of fraction (F12–F23) led to the isolation of Compound 1.

Compound (**1**) was a white solid, mp 88–90°C and found to be spectroscopically pure as determined from its ^{1}H and ^{13}C NMR spectra. ^{1}H NMR: δ 1.49 (3H, s, 3′-Me), 1.57 (3H, s, 7′-Me), 1.65 (3H, s, 7′-Me), 1.71–1.78 (2H, m, 4′-CH2), 2.13–2.19 (2H, m, 5′-CH2), 2.33 (3H, s, 3-Me), 5.11 (1H, t, J = 6 Hz, 6′-H), 5.65 (1H, d, J = 12 Hz, 2′-H), 6.64 (1H, d, J = 12 Hz, 1′-H), 7.16 (1H, t, J = 6.9 Hz, 6H), 7.27 (1H, t, J = 5.1 Hz, 7H), 7.36 (1H, d, J = 7.8 Hz, 7H), 7.36 (1H, d, J = 7.8 Hz, 8H), 7.65 (1H, s, 4H), 7.90 (1H, d, J = 7.8Hz, 5H), 7.87 (1H, s, -NH). ^{13}C NMR: δ 15.99 (3′-Me), 17.50 (7′-Me), 21.90 (C-4′), 25.41 (7′-Me), 25.85 (3-Me), 39.79 (C-5′), 78.16 (C-3′), 101.06 (C-8), 109.02 (C-7′), 112.14 (C-6′), 116.72 (C-4a), 117.30 (C-5a), 117.59 (C-4), 118.19 (C-1), 119.26 (C-5), 119.29 (C-6), 121.27 (C-7), 125.07 (C-2′), 128.40 (C-1′), 131.60 (C-3), 135.07 (C-9a), 139.67 (C-8a), 150.01 (C-2). MS (m/z, % intensity): m/z 331 (M+, 17), 316 (4), 248 (100), 218 (5), 204 (8), 69 (4), 55 (4), $C_{23}H_{25}NO$. It was identified as mahanimbine, $C_{23}H_{25}NO$, by comparison of its ^{1}H and ^{13}C NMR and Mass spectral data (Roy and Chakraborty 1974; Ramsewak *et al.*, 1999).The structure of carbazole alkaloid mahanimbine (**1**) isolated from *Murraya koenigii* has been shown in Fig. 1.

AChE Inhibitory Activity of *Murraya koenigii* extracts and Compound (1) TLC assay

The extract showed several inhibiting spots in the TLC assay but no corresponding false positive spot. The extract was therefore fractionated over a solvent system of petroleum ether: chloroform (100:0 to 0:100) for the isolation of active Compound (1) identified as mahanimbine. The petroleum ether extract (10 mg/mL) and isolated Compound 1(1 mg/mL) were spotted in another TLC plate and was developed in the mobile phase

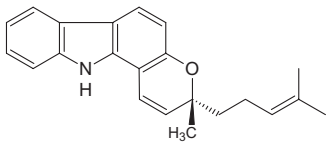

Figure 1. Mahanimbine (**1**).

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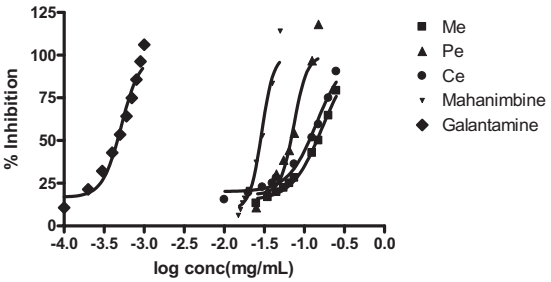
hexane: chloroform (6.5:3.5 v/v). Peak number 2 showed a white inhibitory spot on the TLC assay but no false positive spot and confirmed the real active Compound (**1**).

Enzyme assay

The results of *in vitro* AChE inhibitory activity data of *Murraya koenigii* and the isolated Compound (**1**) were expressed as IC_{50} values. The IC_{50} values for the methanol, petroleum ether, chloroform extract and mahanimbine were 0.15 ± 0.01 mg/mL, 0.07 ± 0.04 mg/mL, 0.12 ± 0.02 mg/mL and 0.03 ± 0.09 mg/mL, respectively. The petroleum ether extract showed a better AChE inhibitory activity probably due to the presence of several alkaloids in this extract which was further supported by the results of TLC assay. The dose-response relationship of the extracts, isolated constituent mahanimbine and galantamine has been shown in Fig. 2. Galantamine was used as standard showed an IC_{50} value of 0.006 ± 0.001 mg/mL. *Murraya koenigii* has been used as a very popular spice in Indian delicacies over the years, and provides nutritional as well as several medicinal values as discussed earlier. This study further explores the AChE inhibitory activity of the extract and mahanimbine isolated from it. Thus this study substantiates further the effect of this plant on central nervous system



[Me = methanolic extract, Pe = petroleum ether extract, Ce - Chloroform extract. Each point represents the mean of the values obtained from three independent experiments].

Figure 2. The dose-response relationships of the extracts, bioactive constituent mahanimbine and standard galantamine.

(Adeleke *et al*., 2004) and as an anti-amnesic agent (Vasudevan and Parle, 2009).

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Acetylcholinesterase enzyme inhibitory potential of standardized extract of *Trigonella foenum graecum* L and its constituents

N. SatheeshKumar, Pulok K. Mukherjee *, S. Bhadra, B.P. Saha

School of Natural Product Studies, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India

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ABSTRACT

Ethno pharmacological approach has provided several leads to identify potential new drugs from plant sources, including those for memory disorders. Acetylcholinesterase inhibitors (AChEI) give a symptomatic relief to some of the clinical manifestations of the disease. The main objective of this study is to standardize the extract of *Trigonella foenum graecum* L with trigonelline by HPTLC method and determine the *in vitro* AChE inhibitory activity of *Trigonella foenum graecum* L and its constituents using galanthamine as a reference. Different concentrations of hydro alcoholic extract of *Trigonella foenum graecum* and trigonelline were subjected to HPTLC analysis using the mobile phase n propanol, methanol and water (4:1:2, v/v). The R_f of trigonelline was found to be 0.43, and the correlation coefficient of 0.99 was indicative of good linear dependence of peak area on concentration. The concentration of trigonelline was found to be 13 mg g^{-1} w/w in the hydro alcoholic extract of *Trigonella foenum graecum*. The AChE inhibitory activity of crude fenugreek seed extracts, fractions and trigonelline was evaluated using Ellman's method in 96-well micro plate's assay and TLC bioassay detection. The ethyl acetate fraction of the alcohol extract (IC_{50} 53.00 $\pm$ 17.33 μg/ml), and total alkaloid fraction (IC_{50} 9.23 $\pm$ 6.08 μg/ml) showed potential AChE inhibition. Trigonelline showed IC_{50} 233 $\pm$ 0.12 μM. Galanthamine was used as standard and it showed inhibition of acetyl cholinesterase with an IC_{50} value of 1.27 $\pm$ 0.21 μM.

Introduction

Alzheimer's disease (AD) is a progressive, neurodegenerative disease of the brain, and is the most common form of dementia among the elderly population (Cummings et al. 1988). AD is a chronic and progressive disease that is characterized symptomatically by progressive deteriorations of activities of daily living, behavioral disturbances and cognitive loss. In the past decade, treatment for AD has largely involved replacement of neurotransmitters that are known to be lacking in AD, mostly based on the cholinergic hypothesis of AD, it is an important approach for the inhibition of acetylcholinesterase (AChE) (Perry 1986). Acetylcholinesterase inhibitors (AchEI) that can increase the cholinergic transmission by blocking the degradation of acetylcholine are therefore used for alleviating the symptoms of patients with Alzheimer's disease. Inhibition of AChE is considered to be a promising approach for the treatment of Alzheimer's disease (AD). Several reports on these aspects for developing natural AChEI have been reviewed by Mukherjee et al. (2007a).

There are a few synthetic medicines, e.g. tacrine, donepezil, and the natural product-based rivastigmine for treatment of cognitive dysfunction and memory loss associated with AD (Li et al. 1977). Several reports on the search for natural AChEI has been made from our laboratory including *Acorus calamus, Clitoria ternetaea, Andrographis paniculata, Centella asiatica, Evolvulus alsinoides, Nelumbo nucifera and Myristica fragrans* (Mukherjee et al. 2007b), *Araucaria bidwillii* (Mukherjee et al. 2007c). *Trigonella foenum graecum* L (Fenugreek; family: Leguminoseae) is one such plant, whose seeds and leaves are not only used as food but also as an ingredient in traditional medicine (Sharma et al. 1996; Warrior and Nambiar 1995). Previously from coffee bean trigonelline was isolated to screen the neurite regenerative activity also demonstrated memory improvement activity in Alzheimer's disease-model mice (Tohda et al. 2005). Seeds of fenugreek are used as a condiment with wheat and maize flour for bread making and as a constituent of the daily diet of general population in Indian subcontinent. Seeds of *Trigonella foenum graecum* L contain tannic acid, fixed and vegetable oils, diosgenin, trigonelline, trigocoumarin, trigomethyl coumarin, steroidal saponin such as gitogenin and traces of trigogenin and vitamin A (Jayaweera 1981). Some of the therapeutic uses of *Trigonella foenum graecum* L include its use as anti ulcer (Almeshal et al. 1985), wound healing (Taranalli and Kuppast 1996), CNS stimulant (Natrajan et al. 2007), immunomodulatory

* Corresponding author. Tel.: +91 33 2429 8313; fax: +91 33 2414 6046.
E-mail address: pulokm@gmail.com (P.K. Mukherjee).

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(Bilal et al. 2003), antioxidant (Kaviarasan et al. 2007), antidiabetic (Al Habori and Raman 1988; Ravikumar and Anuradha 1999; Zia et al. 2001), anti-neoplastic (Sur et al. 2001), anti-inflammatory and anti-pyretic (Ahmadiani et al. 2001). The current study was undertaken to standardize the extract of *Trigonella foenum graecum* L and also to evaluate the AChEI activity to provide a base for the development of natural drugs.

Materials and method

Plant materials

Trigonella foenum graecum L seed were purchased locally. A voucher specimen of the plant material (SNPS–1034) has been retained in the School of Natural Products Studies, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032.

Chemicals

Acetylthiocholine iodide (ATCI), Acetylcholinesterase enzyme (AChE) from bovine erythrocytes, 5,5-dithiobis [2-nitrobenzoic acid] (DTNB), Galanthamine and Trigonelline were obtained from Sigma (Poole, UK). Methanol and all other organic solvents (analytical reagent grade) were purchased from Merck, India.

Extraction of plant materials

Fenugreek seeds were coarsely powdered (1500 g), extracted with ethanol (95%) after standing for 48 h at room temperature, the hydroalcoholic extract was drained off. This process of extraction at ambient temperature was repeated four times. The combined hydroalcoholic extracts were filtered through filter paper and evaporated to dryness under reduced pressure in a rotavapor at 45 °C. The extracts were then freeze-dried which were further used for screening purposes.

Fractionation

The crude hydro alcoholic extract (300 g) was suspended in distilled water (500 ml) and sequentially partitioned with chloroform (2 × 500 ml), ethyl acetate (2 × 500 ml) to yield chloroform (10.2 g) and ethyl acetate (3.5 g) fractions, respectively.

Total alkaloid fraction

25 g of the hydroalcoholic extract concentrate was extracted with 0.1 N HCl allowing it to stand for 5 h. The aqueous acid extract was partitioned with 100 ml of chloroform in a separating funnel. This procedure was repeated for two more times and the combined chloroform layer was rejected. The aqueous layer was basified with ammonium hydroxide to pH 9.0 and was again partitioned with chloroform. The aqueous layer was rejected and the chloroform layer was collected and evaporated to obtain the concentrate to yield (400 mg) (Ferguson 1956).

Chromatography

Chromatography was performed on 10 × 20 cm aluminum backed silica gel 60F254 HPTLC plates. Before use, the plates were washed with methanol then dried in an oven. Sample were applied to the plates, as 6 mm bands, by spraying with Camag Linomat V sample applicator equipped with 100 μl syringe (Hamilton). Ascending development of the plate, migration distance 84 mm, was performed at 25 ± 2 °C with n propanol, methanol and water in the ratio of 4:1:2 (v/v), as mobile phase in a Camag twin-trough chamber previously saturated with mobile phase for 10 min. The average development time was 25 min. After development the plate was dried and scanned at 254 nm with a Camag TLC scanner III equipped with win cats software, using deuterium light source; the slit dimension was 6.00 × 0.45 mm.

Microplate assay for AChE activity

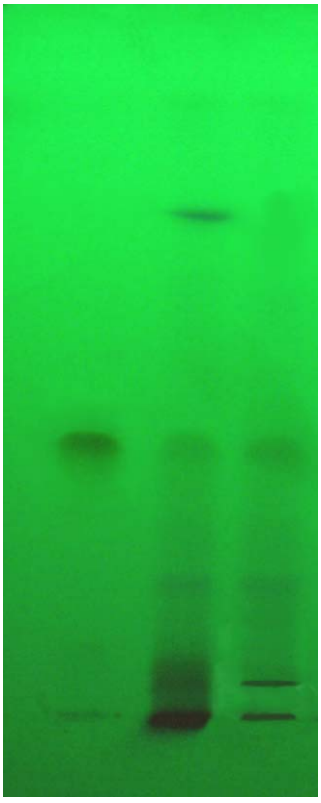
AChE activity was measured using a 96-well microplate reader (Ellman et al. 1961; Mukherjee et al. 2007d). The enzyme hydrolyzes the substrate acetylthiocholine resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 405 nm. In the 96-well plates, 125 μl of 3 mM DTNB, 25 μl of 15 mM ATCI, 50 μl of buffer and 25 μl of sample dissolved in phosphate buffer were added. The absorbance was measured at 405 nm every 13 s for 65 s. 25 μl of 0.22 U/ml of AChE enzyme was then added and the absorbance was again read every 13 s for 104 s. The absorbance was read using a Bio Rad microplate reader at 405 nm. Absorbance was plotted against time and enzyme activity was calculated from the slope of the line so obtained and expressed as a percentage compared to an assay using a buffer without any inhibitor.

Thin layer chromatography (TLC) with bioassay detection for AChE inhibition

The TLC with bioassay detection for AChE inhibition was modified from the study (Rhee et al. 2001). A 2.5 mm silica gel plate F_{254} (Merck, Darmstadt, Germany) was used as a stationary phase. The plant extracts, fractions and trigonelline (1 mg/ml) were spotted in the TLC plate and it is developed in the mobile phases n -proponal: methanol: water 4:1:2 (v/v/v). After the plate was developed, it was dried at room temperature and then sprayed with 30 mM ATCI followed by 20 mM DTNB. The plate was dried at room temperature for 45 min, and then sprayed with AChE. After 20 min, the plate was observed under visible light. A positive spot indicating AChE inhibitor was a colorless spot on the yellow background. A false-positive test was carried out in order to confirm any AChE inhibiting activity (Rhee et al. 2003). A TLC plate was developed and sprayed with 5 mM DTNB in 50 mM Tris–HCl, pH 8. After drying, the plate was sprayed with 5 mM ATCI and 3 U/ml AChE in 50 mM Tris–HCl, pH 8 at 37 °C. After a few minutes a yellow background appeared; occurrence of white spots indicated false positive reactions.

Results and discussion

Standardization of trigonelline was modified based on the Chopra et al. (2006). TLC profile and HPTLC fingerprints were developed for hydro alcoholic extract. Different concentrations of extract and trigonelline were subjected to HPTLC analysis using the mobile phase n propanol, methanol and water (4:1:2, v/v). The R_f of trigonelline was found to be 0.43, and the correlation coefficient of 0.99 was indicative of good linear dependence of peak area on concentration. The percentage of the active marker was calculated by using peak area, and the trigonelline content was found to be not less than 13 mg g^{-1} w/w in the hydro alcoholic extract of *Trigonella foenum graecum* and the HPTLC chromatograms obtained from trigonelline, hydroalcoholic extract and total alkaloid fraction of *Trigonella foenum graecum* are shown in Fig. 2(A, B, C). The hydroalcoholic extract, fractions of ethyl acetate, chloroform, total alkaloid and the trigonelline were tested



A = Trigonelline

B = Methanolic Extract

C = Total alkaloid fraction

Fig. 1. TLC Plate of *Trigonella foenum graecum* L.

for AChE inhibitory activity in the microtitre assay and TLC bioassay detection. The results obtained through this study for the AChE inhibitory activity is shown in Table 1. The results were expressed as IC_{50} values, calculated from the regression equations prepared from the concentrations of the samples. All the fractions and trigonelline showed significant AChE inhibitory activity. The hydro alcoholic extract and its chloroform fraction showed weak inhibition of acetyl cholinesterase with IC_{50} values of 140.26 ± 17.52 μg/ml and IC_{50} 146.94 ± 17.33 μg/ml. The ethyl acetate fractions gave a strong AChE inhibition (IC_{50} 53.00 ± 17.33 μg/ml), as did total alkaloid fraction (IC_{50} 9.23 ± 6.08 μg/ml) and trigonelline (IC_{50} 233 ± 0.12 μM), respectively. Galanthamine was used as standard and it shows inhibition of acetyl cholinesterase with an IC50 value of 1.27 ± 0.21 μM. The plant extracts, total alkaloid fractions and trigonelline (1 mg/ml) were spotted in the TLC plate and developed in the mobile phases n -proponal: methanol: water 4:1:2 (v/v/v) as shown in Fig. 1. The presence of cholinesterase inhibitory activity was determined by the formation of well-defined white spots made visible by spraying with DTNB, which gave a yellow background. In the TLC assay we determined the most suitable enzyme concentration as 3 U/ml. Even though the TLC assay is a qualitative method, the extracts which contained possible active compounds other than galanthamine gave the yellow background became more intense and white spots were more easily found when more concentrated enzyme were used, 3 U/ml gave acceptable results. The TLC assay demonstrated AChE inhibitory activity and no false positive reaction was detected.

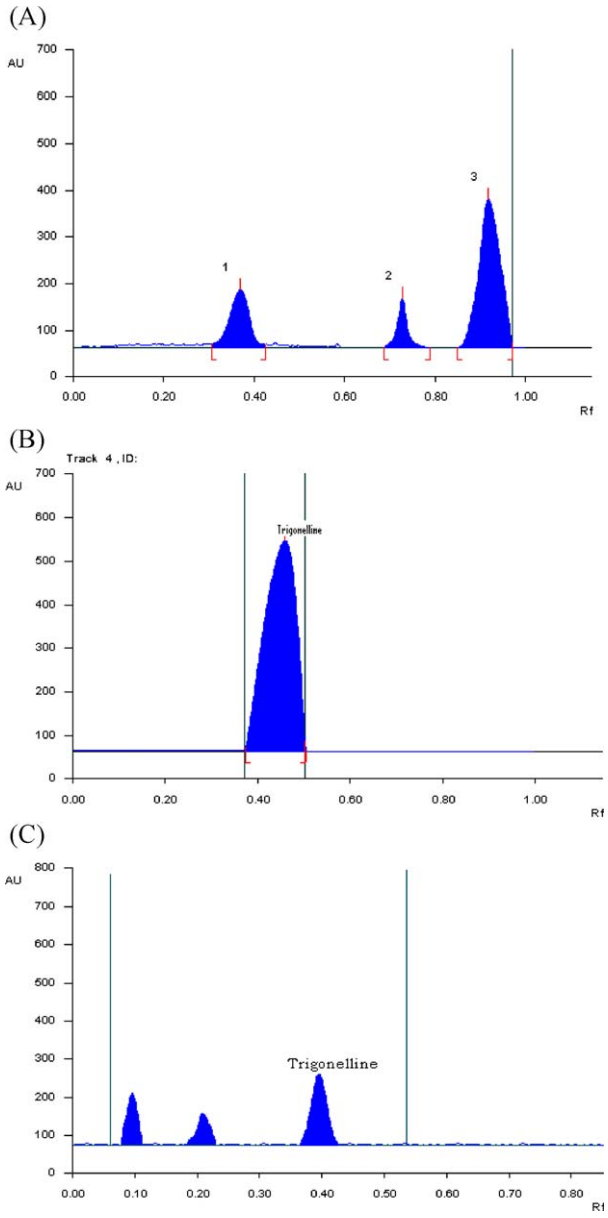


Fig. 2. HPTLC chromatograms of hydroalcoholic extract of *Trigonella foenum graecum* Fig. 2(A); HPTLC chromatograms of trigonelline in *Trigonella foenum graecum* Fig. 2(B); HPTLC chromatograms of total alkaloid fraction of *Trigonella foenum graecum* Fig. 2(C).

Table 1
Acetylcholinesterase Inhibition by the crude extract, fractions and trigonelline of *Trigonella foenum graecum* L.

Sample *Trigonella foenum graecum*	IC_{50} value
Hydro alcoholic extract	140.26 ± 17.52 μg/ml
Ethyl acetate fraction	53.00 ± 17.33 μg/ml
Chloroform fraction	146.94 ± 17.33 μg/ml
Total alkaloid fraction	9.23 ± 6.08 μg/ml
Trigonelline	233 ± 0.12 μM
Galanthamine	1.27 ± 0.21 μM.

Dilutions were made to find out the minimum quantity required to produce white inhibition spot. Trigonelline and Galantamine showed white inhibition spot at the concentration of 0.9864 nmole and 0.0041 nmole respectively. Another advantage

of the TLC assay is that there is no disturbance of solvent which dissolves the sample before the assay. Since most of the acetyl cholinesterase inhibitors are known to contain nitrogen, the higher activity of these extracts may be due to their rich alkaloid content. From the results, it can be postulated that the fractions and trigonelline of T*rigonella foenum graecum* (fenugreek) seed has a potential AChE inhibitory activity and could be used for the treatment of Alzheimer's disease (AD).

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Lead Finding for Acetyl Cholinesterase Inhibitors from Natural Origin: Structure Activity Relationship and Scope

P.K. Mukherjee*, N. Satheeshkumar, P. Venkatesh and M. Venkatesh

School of Natural Product Studies, Department of Pharmaceutical Technology, Jadavpur University, Kolkata - 700032, India

Abstract: Acetylcholinesterase (AChE) inhibitors are considered as promising therapeutic agents for the treatment of several neurological disorders such as Alzheimer's disease (AD), senile dementia, ataxia and myasthenia gravis. There are only few synthetic medicines with adverse effects, available for treatment of cognitive dysfunction and memory loss associated with these diseases. A variety of plants has been reported to possess AChE inhibitory activity and so may be relevant to the treatment of neurodegenerative disorders such as AD. Hence, developing potential AChE inhibitors from botanicals is the need of the day. This review will cover some of the promising acetylcholinesterase inhibitors isolated from plants with proven *in vitro* and *in vivo* activities with concern to their structure activity relationship.

Keywords: Acetylcholinesterase inhibitors, Alzheimer's, structure activity relationship, ethnomedical.

INTRODUCTION

Work on new bioactive compounds from medicinal plants has led to the isolation and structure elucidation of a number of exciting new pharmacophores. Alzheimer's disease (AD) is one of the most common forms of dementia affecting so many elderly people. Besides the neuropathologic hallmarks of this disease, namely neurofibrillary tangles and neuritic plaques, it is characterized neurochemically by a consistent deficit in cholinergic neurotransmission, particularly affecting cholinergic neurons in the basal forebrain [1]. Principal role of acetylcholinesterase (AChE) is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of acetylcholine (ACh). Inhibition of AChE serves as a strategy for the treatment of Alzheimer's disease (AD), senile dementia, ataxia, myasthenia gravis and Parkinson's disease [1]. Even though mankind always relies on nature for their basic requirements, rediscovery of the connection between plants and health is responsible for launching a new generation of botanical therapeutics that include plant-derived pharmaceuticals, multicomponent botanical drugs, and plant-produced recombinant proteins. There is great interest in finding better AChE inhibitors from natural product showing low toxicity, good brain penetration and high bioavailability. Natural products have inspired many developments in organic chemistry leading to advances in synthetic methodologies in developing several therapeutically potential analogues of lead compounds. The research lead on ayurvedic drugs yielded numerous drug candidates that are prevailing in the market. There are several plants of ayurvedic origin with potential therapeutic activity, which are widely used as ayurvedic medicine. Nature has provided several leads as potential AChE inhibitors from plant sources, including those for memory disorders. This article highlights several aspects of lead identification for Acetyl cholinesterase Inhibitors, their structure activity relationship and potential uses.

NATURAL REMEDIES FOR AD

Natural sources genetic codes contain the recipes for chemical compounds of potential value in pharmaceutical products. Pharmaceutical research in natural products is more often intended to develop leads and to identify those plants, which can be used in unmodified form. Plant made pharmaceuticals (PMPs) are the result of a breakthrough application of biotechnology on plants to enable them to produce therapeutic proteins that could ultimately be used by the medical community to combat life-threatening illnesses [2]. The leads from plants are promising molecules that must be modified to increase efficacy or reduce side effects. Based on these aspects several works on the search for natural AChEI has been reported from our laboratory [3-6].

Alzheimer's disease (AD) is the fourth leading cause of death among the elderly worldwide, accounting for the most common form of dementia diagnosed after the age of 60. Currently, several kinds of AChE inhibitors, such as donepezil (Fig. (**1a**)), galantamine (Fig. (**3b**)), and rivastigmine (Fig. (**2e**)) are available for the symptomatic treatment of patients with mild-to-moderate AD.

There are a number of approaches to the treatment of the cholinergic deficit in Alzheimer's disease, most of which have initially focused on the replacement of ACh precursors (choline or lecithin) but these agents failed to increase central cholinergic activity. Other studies have investigated the use of AChE inhibitors that reduce the hydrolysis of ACh for example, physostigmine. Further researches are being conducted in order to find a suitable remedy for this ailment. The most recent investigational compounds for treatment of cholinergic deficit include specific M1 muscarinic or nicotinic agonists, M2 muscarinic antagonists, or improved "sec-

*Address correspondence to this author at the School of Natural Product Studies, Dept. of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India; Tel: +91 33 24298313; Fax: + 91 33 24146046;
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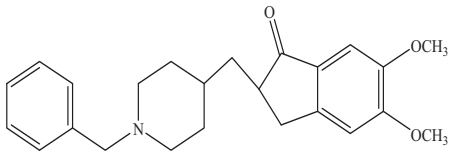
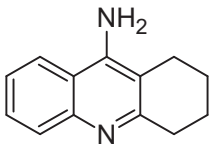


(a) Donepezil



(c) Tacrin, (IC_{50} 1 μM)

Fig. (1).

ond generation" AChE inhibitors [7]. This type of cholinesterase inhibitors are found in abundance in natural products which encourages the researchers towards lead finding for AD from the natural products.

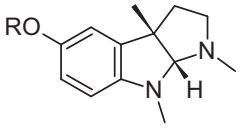
Although a variety of AChE inhibitors have been developed as potential treatments for Alzheimer's disease, their pharmacological activities differ. One of the most fundamental differences between them is in the mechanism of ChE inhibition. For example, enzyme kinetic studies have shown that tacrine (Fig. (**1c**)), an acridine compound, and donepezil, a novel piperidine class agent, are "mixed type" reversible inhibitors of ChE. These compounds inhibit ChE *via* both non-competitive (by blockade of the deacetylation process) and ACh competitive mechanisms [7].

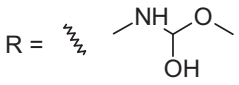
STRUCTURE-ACTIVITY RELATIONSHIP OF NATURAL ACETYLCHOLINESTERASE (AChE) INHIBITORS

The ethno medicinal plant used in the treatment of AD contains numerous molecules, they exhibit inhibitory effect due to the potential structural characteristics of the individual molecule and due to the synergistic action of the molecules collectively. As the phytochemicals can be structurally classified based on their chemical groups, it will be useful to study the effect of these molecules based on the groups under which it can be classified. Several potential molecules has been isolated from various ethnomedical plants and studied for their AChE inhibitory activity. Activities of some therapeutically active phytochemical groups have been discussed in the following sections:

ALKALOIDS

Many alkaloids like physostigmine (Fig. (**2a**)), eseroline (Fig. (**2b**)), neostigmine (Fig. (**2c**)), pyridostigmine (Fig. (**2d**)), rivastigmine (Fig. (**2e**)), eptastigmine (Fig. (**2f**)), as shown in Fig. (**2**) have been proved to possess AChE inhibitory activity. The plant alkaloid galantamine is a phenanthrene similar to codeine, which has been isolated from *Galanthus nivalis* L., the European daffodil or common snowdrop [8, 9]. Galantamine is the last drug approved for the treatment of AD. It is a tertiary alkaloid with a unique, dual mode of action. It is a reversible, competitive AChE inhibitor, and also an allosteric modulator of nicotinic acetylcholine receptors [10, 11]. The efficacy of galantamine has been

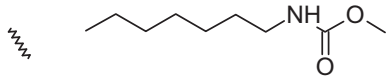




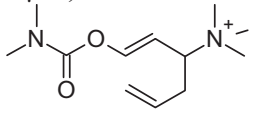
(a) Physostigmine (IC_{50} 0.25 μM)

R = H

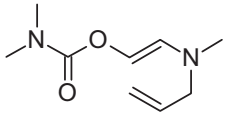
(b) Eseroline



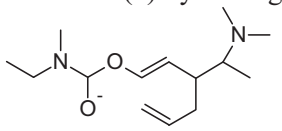
(c) Neostigmine



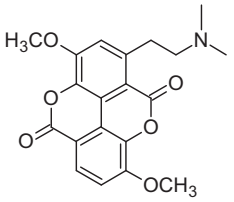
(d) Pyridostigmine



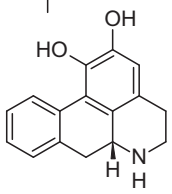
(e) Rivastigmine



(f) Eptastigmine



(g) Taspine
IC_{50} 0.33± 0.07 μM



(h)Asimilobine

Fig. (2).

extensively studied in clinical trials that have demonstrated that a dosing regimen of 16–24 mg/day consistently produced beneficial effects on cognitive and non-cognitive AD symptoms. Galantamine exhibits favorable pharmacokinetic characteristics including predictable linear elimination kinetics at the recommended maintenance doses (16 and 24 mg/day), a relatively short half-life (approximately 7 h), and high bioavailability, and side effects include the predictable gastrointestinal upset which is transient with mild intensity, and easily controllable using the recommended slow dose-escalation scheme [12,13].

Physostigmine, prototype acetylcholinesterase inhibitor (also known as serine) is isolated from the seeds of *Physostigma venenosum* Balf (Papilionaceae). The structure of physostigmine was determined and shown to have a pyrroloindole skeleton. Physostigmine is distributed throughout the body and produce general cholinergic effect. Because of its polarity it is not distributed in large concentrations in the CNS. In bovine erythrocytes, it showed AChEI activity with IC_{50} of 0.25 μM. it was found that the carbamate portion is essential for cholinesterase inhibitory activity. When the ester link is hydrolyzed to the product eseroline inhibitory activity was not observed. However, the carbonyl group interacts with the hydroxyl (-OH) of a serine to form ester in the AChE with urethane part of the molecule. The ester form is slowly hydrolyzed and regenerates the active parent form, which interfere the AChE activity of the enzyme. The carbamate moiety has been a key factor in the use of eserine as a lead molecule for dramatization or synthesis of longer-lasting or more selective drugs, insecticides and other agents. To make a possible binding the presence of an aromatic ring and an N atom is required.

Two alkaloids, taspine (Fig. (**2g**)) and asimilobine (Fig. (**2h**)) were detected in *Magnoliax soulangiana* Hort extract. Of these two, taspine showed significantly higher effect on AChE than galantamine and selectively inhibited the enzyme with time and concentration-dependant manner with an IC_{50} value of 0.33± 0.07 μM. Further studies also have showed that the ligand bind in an alternative orientation when compared to galanthamine. It was also found to be stabilized by sandwich like π stacking interaction between the planar aromatic ligand and the Trp84 and Phe330 of the enzyme, esteratic site anchoring with the amino acid side chain and hydrogen bonding network [14-16].

1. Amaryllidaceae Alkaloids

Twenty three pure amaryllidaceae alkaloids shown in (Fig. (**3a-3w**)) and 26 extracts from different species of the genus *Narcissus* were tested for their acetylcholinesterase inhibitory activity using galanthamine as a standard. Among them seven alkaloids, belonging to the galantamine and lycorine skeleton types, exhibited cholinesterase inhibitory effect. When compared with galantamine (IC_{50}= 0.33 μM), sanguinine (Fig. (**3a**)) was observed to be most active (IC_{50} = 0.72 μM). An *in vitro* structure-activity relationship study involving sanguinine and other synthetic analogues of galantamine indicated that properly placed hydrophilic groups on galantamine contribute to its effective binding to the AChE molecule [15-17].

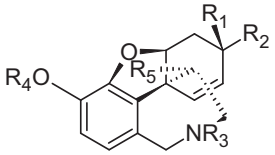
The structural features of the amaryllidaceae alkaloids have been explained by Lo´pez *et al*, [19]. It has been shown that the relatively tight binding in the structure appears to come from a number of moderate to weak interactions with the protein, including classical and non-classical hydrogen bonds. Therefore, the extra hydroxyl group of sanguinine available for potential interaction with AChE can explain the strong inhibitory activity of this alkaloid. Assoanine, oxoassoanine has been reported as the most active amongst the lycorine-type alkaloids, which could be due to the aromatic ring C which gives a certain planarity to those molecules [18, 19].

The acetylcholinesterase inhibitory effect of 23 amaryllidaceae alkaloids has been reported [20]. Among them, the alkaloid, 1-O-acetyllycorine (Fig. (**$3a_1$**)) (IC_{50}: 0.96±0.04) was found to show significant inhibitory activity in micro molar concentration. Beside these, compounds shown in (Fig. (**$3b_2$**), (**$3c_3$**), (**$3d_4$**), (**$3e_5$**), (**$3f_6$**), (**$3g_7$**), (**$3h_8$**), (**$3i_9$**), (**$3j_{10}$**), (**$3k_{11}$**), (**$3l_{12}$**), (**$3m_{13}$**), (**$3n_{14}$**) and (**$3a_1$**) were also found to have weak activity. It seems that lycorine was found to be a potent inhibitor of AChE because of the different ring type when compared with that of other alkaloids. Further the presence of an acetoxy group and a hydroxyl group at positions 1 and respectively are required in 1-O-acetyllycorine for proper binding and inhibition of activity of the enzyme. On the other hand, the aromatic ring C that gives certain planarity to assoanine and oxoassoanine explains the higher activity of the molecules in comparison with lycorine type alkaloids. In Crinine type alkaloids, the stereochemistry of 5,10 b-ethano bridge has no effect whereas 15-carbon ring system of crinine is important for their activity.

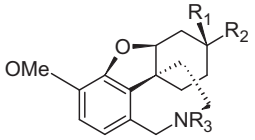
2. Indole Alkaloids

Andrade *et al.* (2005) reported ten indole alkaloids from the chloroform extract of stalk of *Tabernaemontana australis* Miers, as depicted in (Fig. (**4a-4j**)) were studied for their acetylcholinesterase inhibitory activity. Of these the first four showed potent acetylcholinesterase activity. The activity of these alkaloids seems to be related to their antagonist effect in subtype a3b4 nicotinic receptors (nChRs), binding with low affinity to other types of receptors, including a4β2 nChRs. [21].

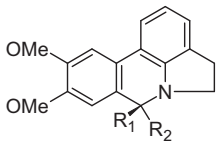
Compounds shown in (Fig. (**4k-4q**)) are the fungal metabolite isolated from *Aspergillus terreus*. Among this territrem B (TRB) (Fig. (**4l**)), shown to be a potent and irreversible inhibitor of acetylcholinesterase (AChE). Omura's group additionally showed that several analogs of territrem isolated from rice culture broth of Penicillium sp. FO-4259, called arisugacins, which are highly specific and potent AChE inhibitors [22, 23]. Both territrems and arisugacins are composed of a basic structure that includes a benzyl group, a pyran, and a terpenoid. The mechanism of TRB AChE inhibition using both kinetic and molecular modeling studies indicates that TRB does not form a covalent bond with the enzyme. It is consistent with its lack of a carbamate and a phosphate moiety, which could otherwise react with the active serine of the enzyme. By searching for a probable binding mode between TRB and AChE through extensive docking simulations, a structural model of their complex was derived. The notable absence of nitrogen in these compounds is



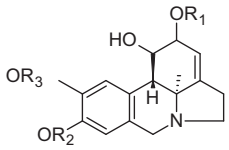
(a) Sanguinine: R^1=OH, R^2=H, R^3=Me, R^4=H, R^5=H, (IC_{50} = 0.72 μM)
(b) Galanthamine: R^1=OH, R^2=H, R^3=Me, R^4=Me, R^5=H, (IC_{50} 0.33 μM)
(c) 11-Hydroxygalanthamine: R^1=OH, R^2=H, R^3=Me, R^4=Me, R^5=OH
(d) Epinorgalanthamine: R^1=H, R^2=OH, R^3=H, R^4=Me, R^5=H



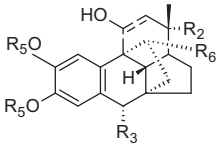
(e) Lycoramine: R^1=OH, R^2=H, R^3=Me
(f) Epinorlycoramine: R^1=H, R^2=OH, R^3= H



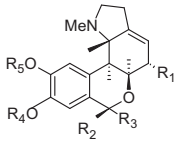
(g) Assoanine:R^1=R^2=H
(h) Oxoassoanine:R^1+R^2=O



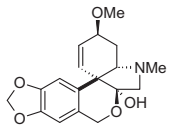
(i) Lycorine: R^1=H,R^2+R^3=CH_2
(j) Pseudolycorine:R^1=H,R^2=Me,R^3=H
(k) 2-O-Acetylpseudolycorine: R^1=Ac,R^2=Me,R^3=H



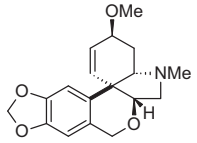
(l) Haemanthamine: R^1=OMe,R^2=H,R^3=H,R^4+R^5=CH_2,R^6=OH
(m) Crinamine: R^1=H,R^2=OMe,R^3=H,R^4+R^5=CH_2,R^6=OH, (IC_{50}: 697±12)
(n) Papyramine: R^1=OMe,R^2=H,R^3=OH,R^4=Me,R^5=Me,R^6=H
(o) Haemanthidine: R^1=OMe,R^2=H,R^3=OH,R^4+R^5=CH_2,R^6=OH



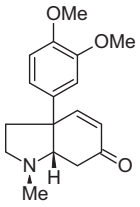
(p) 8-O-Demethylhomolycorine: R^1=H, R^2+R^3=O,R^4=H,R^5=Me
(q) 9-O-Demethyl-2α-hydroxyhomolycorine:R^1=OH,R^2+R^3=O, R^4=H,R^5=Me
(r) Dublusine: R^1=OAc,R^2+R^3=O, R^4=Me,R^5=OOC-CH2-CHOH-Me
(s) Hippeastrine: R^1=OH,R^2+R^3=O,R^4+R^5=CH_2
(t) O-Methyllycorenine: R^1=H,R^2=H,R_3=OMe,R^4=Me,R^5=Me



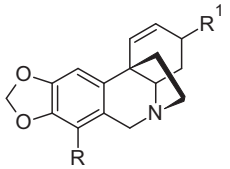
(u) Tazettine, (IC_{50}: 705±63)



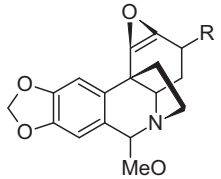
(v) Pretazettine



(w) Mesembrenone

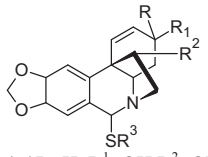


(b_2)R=H,R^1=OH Crinine, (IC_{50}: 461±140)
(c_3)R=H, R^1=OMe Epibuphanisine, (IC_{50}: 547± 5),

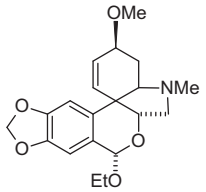


(d_4)R=OH Crinamidine, (IC_{50}:300±27)

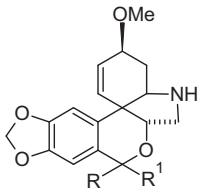
(Fig. 3). Contd…..



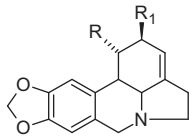
(e_5)R=H, R^1=OH,R^2=OH,R^3=H Hamayne, (IC_{50}: 533±3)
(f_6)R= R^3=H, R=H, R^1=OAc,R^2=OH 3-O-Acetylhamayne, (IC_{50}: 594±8)
(g_7)R=H, R^1=OH,R^2=R^3=H Epivittatine, (IC_{50}:239±9)
(h_8)R=H, R^1=OMe,R^2=OH,R^3=OH (α,β) 6-Hydroxycrinamine, (IC_{50}:490±27)



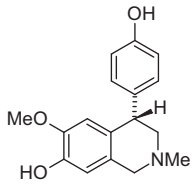
(i_9) 8α-ethoxypretazettine, (IC_{50}: 234±13)



(j_{10}) R= H, R^1= OEt N-desmethyl-N-desmethyl-8α-ethoxypretazettine, (IC_{50}: 234±13)
(k_{11}) R= OEt, R^1= H N-desmethyl-N-desmethyl-8β-ethoxypretazettine, (IC_{50}: 419±8)



(l_{12})R=R^1=OH Lycorine, (IC_{50}:213±1)
(a_1) R=OAc,R^1=OH 1-O-acetyllycorine, (IC_{50}: 0.96±0.04)
(m_{13})R=R^1=OAc 1,2-di-O-acetyllycorine, (IC_{50}:211±10)



(n_{14}) Cherylline, (IC_{50}:407±32)

Fig. (3). Amaryllidaceae alkaloids.

unlike other known AChE inhibitors. It was found that the inhibition of TRB on AChE is mediated by a tight non covalent binding that is kinetically irreversible, at least within the time duration of our experiments.

3. Isoquinoline Derivatives

Cocculus pendulus (J. R. & G. FORST.) results two new alkaloids namely kurramine-2'-β -N-oxide (Fig. (**5a**)), kurramine-2'-α-N-oxide (Fig. (**5b**)), and three known bisbenzylisoquinoline alkaloids (1,2-dehydroapateline (Fig. (**5 c)**), cocsoline (Fig. (**5d**)) and cocsuline (Fig. (**5e**)) during phytochemical investigation. Compounds (Fig. (**5d**)) (IC_{50} 6.1μM) and (Fig. (**5e**)) (IC_{50} 12.0 μM) were found to be active against butyrylcholinesterase, while compounds (Fig. (**5a**)) (IC_{50} 10.0μM) and (Fig. (**5d**)) (IC_{50} 47.6μM) have inhibited acetyl cholinesterase significantly [24]. The activity of the bisbenzylisoquinoline alkaloids is found to be associated with the quaternary nitrogen group present in the compounds. Quantitative structure-activity relationship studies have shown that the inhibitory potency of isoquinoline derivatives was determined by steric, rather than electrostatic, properties of the compounds. Tubocurarine isolated from *Chondodendron tomentosum* Ruiz & Pav (Menispermaceae) is a classical example for the potential activity of the benzylisoquinoline ring. A number of other bisbenzylisoquinoline (BBIQ) alkaloids; such as fangchinoline (Fig. (**5f**)), atherospermoline (Fig. (**5g**)), and fenfangjine E (Fig. (**5h**)) isolated from root of *Stephania tetrandra* S. Moore, Menispermaceae family, were also found to inhibit acetylcholinesterase enzyme in micro molar range [25].

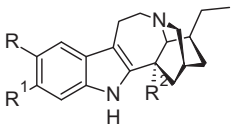
4. Lycopodium Alkaloids

Three lycopodium alkaloids isolated from the club moss *Lycopodium casuarinoides* Spring were tested for their inhibitory activity against acetylcholinesterase. Lycoparins A (Fig. (**6a**)) and lycoparins B (Fig. (**6b**)) possessing a carboxylic acid at C-15 and one or two N-methyl groups did not show such activity (IC_{50} > 200 μM) . Whereas lycoparin C (Fig. (**6c**)) was found to inhibit acetylcholinesterase with the IC_{50} 25 μM. [26]. Carinatumins A (Fig. (**6d**)), B (Fig. (**6e**)) and C (Fig. (**6f**)), another three lycopodium alkaloids isolated from the club moss *Lycopodium casuarinoides* were also studied. Among this, carinatumins A and B inhibited acetylcholinesterase with IC_{50} 4.6 and 7.0 μM, respectively, whereas carinatumin C did not show such activity (IC_{50} > 100 μM). Carinatumin A possessing a hydroxyl at C-10 showed less potent inhibition compared with huperzine A (IC_{50} 0.8 μM), while carinatumin B showed inhibition of acetylcholinesterase comparable to that of huperzine B, IC_{50} 8 μM [27].

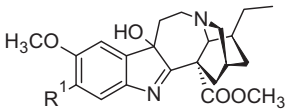
5. Pregnane Alkaloids

Choudhary *et al.* [28] reported that *Sarcococca hookeriana* contains four new pregnane type of alkaloids as shown in (Fig. (**7a -7c**).This has been reported by bioassay guided isolation and the same showed wide variation in AChE inhibitory activity with IC_{50} values ranging from 1.5 to 148.2 μM.

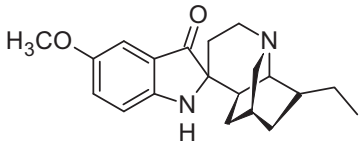
The mechanism of inhibition of acetylcholinesterase enzymes by 23 pregnane-type alkaloids including compounds



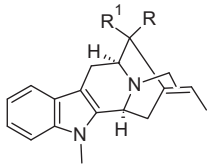
(a) Coronaridine: $R=R^1=H,R^2=COOCH_3$
(b)Voacangine:$R=H,R^1=OCH_3,R^2=COOCH_3$
(e) Ibogamine: $R=R^1=R^2=H$
(f) Ibogaine:$R=OCH_3,R^1=R^2=H$
(g) Ibogaline: $R=R^1=OCH_3,R^2=H$
(h) Desethyl-voacangine:$R=H,R^1=OCH_3,R^2=COOCH_3$



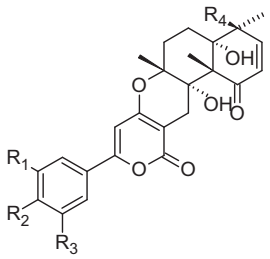
(c) Voacangine hydroxyindolenine



(d) Rupicoline



(i) Voachalotine: $R=COOOH_3,R^1=CH_2OH$
(j) Affinisine: $R=CH_2OH,R^1=H$



	R^1	R^2	R^3	R^4
(k)Territrem A (TRA)	$-OCH_2O-$		$-OCH_3$	$-CH_3$
(l)Territrem B (TRB)	$-OCH_3$	$-OCH_3$	OCH_3	$-CH_3$
(m)Territrem C (TRC)	$-OCH_3$	-OH	$-OCH_3$	$-CH_3$
(n)Arisugacin A	-H	$-OCH_3$	–H	$-CH_3$
(o)Arisugacin B	-H	$-OCH_3$	–H	$-CH_3$
(p)MB2	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-CH_2OH$
(q)MB2-Succinate	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-CH_2OCOCH_2CH_2COOH$

Fig. (4). Indole alkaloids.

as shown in Fig. (**7e-7z**), (**7z$_1$**) were isolated from the *Sarcococca saligna* (D. Don) Muell. From the study, it was established through SAR that, the major interaction of the enzyme-inhibitor complexes are due to the hydrophobic and cation - interactions inside the aromatic narrow passage of these cholinesterases.

Khalid *et al.* [29] has reported that the structures with amino nitrogens at C-3 and/or C-20 positions are the most important features that determine the inhibitory potency of these compounds, which are expected to be protonated at physiological pH. This finding has further established the fact that the replacement of any of these two amino substituents with oxygen function, as in compounds 2-dydroxysalignarine-E and salignamine exhibit decrease in the IC_{50} and Ki values against AChE. C-20 amino group may remain close to the aromatic rings of Tyr-70 and Trp- 279, near the top of the narrow passage, thus allowing the substrate to be accommodated at the active site of the enzyme. The most active members of this series were found to be compounds axillaridine-A (Fig. (**7y**)), sarsalignone (Fig. (**7z**)) and sarsalignenone (Fig. (**7z$_1$**)). They have a carbonyl or acetoxy substituents at C-4 (29).

When compared to the compounds containing aliphatic side chain such as tigloyl or sencioyl groups at C-3, benzamide moieties at C-3 of compounds 2β-Hydroxyepipachysamine-D (Fig. (**7g**)), axillarine-C (Fig. (**7j**)) and epipachysamine-D (Fig. (**7u**)) appears to cause a steric hindrance, which may result in some decrease in the activity. The steric effect of C-3 benzamide substituent may be due to the limited possible flexibility of the molecule within the aromatic narrow passage of the enzyme. This study proves that the affinity of the compound which is bulkier than the existing inhibitors could be rationalized by their flexibility [29, 30].

6. Protopine

A total methanolic extract of tuber of *Corydalis ternate* Nakai (papaveraceae) was found to have anti cholinesterase activity. An alkaloid, protopine (Fig. (**8a**)) was isolated and it was found to be responsible for the acetylcholinesterase

inhibitory activity in a dose dependant manner. The IC_{50} value was 50 μM protopine was found to be specific, reversible and competitive inhibitor of acetylcholinesterase. Protopine was also proved to have an efficacy almost identical to a marketed tacrine derivative, velnacrine [31-34]. Similarly, protopine derived from Korean natural resource *Corydalis* speciosa through bioactivity guided isolation, showed acetylcholinesterase activity in a dose-dependent manner, with IC_{50} values of 16.1 μM [33].

7. Steroidal Alkaloids

Methanolic extract of the aerial parts of the *Sarcococca coriacea* (Hook f.) gave two new steroidal alkaloids, shown in (Fig. (**9a -9b**)) and two known compounds funtumafrine C [(20S)-20-(N,N-dimethylamino)-5a-pregna-3-one] (Fig. (**9c**)) and N-methylfuntumine (Fig. (**9d**)). The compounds epoxynepapakistamine-A (IC_{50}>200), funtumafrine C (IC_{50} 45.75±1.122) and N-methylfuntumine (97.61±1.731) were found to have cholinesterase inhibitory activity [35]. Two new cevanine steroidal alkaloids, impericine (Fig. (**9e**)) and forticine (Fig. (**9f**)) along with known bases delavine (Fig. (**9g**)), persicanidine A (Fig. (**9h**)), and imperialine (Fig. (**9i**)) were isolated from the bulbs of *Fritillaria imperialis* Rubra. These steroidal bases also showed anti-acetylcholinesterase and anti-butyrylcholinesterase inhibitory activity [36, 37].

FLAVANONES

Hispidone (Fig. (**10a**)), a new flavanone, has been isolated from *Onosma hispida* WALL. In addition, (2S)-5,2'-dihydroxy-7,5'- dimethoxyflavanone, benzoic acid, and 4-hydroxy benzoic acid are also reported for the first time from this species. Both compounds hispidone and (2S)-5,2'-dihydroxy-7,5'- dimethoxyflavanone were found to be potent cholinesterase inhibitors and inhibited enzymes in a concentration-dependent manner with the IC_{50} values 11.6 and 28.0 μM against AChE and 15.7 and 7.9 μM against BChE, respectively. The activity of these compounds depends upon the structure of the flavanone. The SAR of flavanones shows that the inhibitory efficiency of the compounds depends on the hydroxyl group and their potential [38].

PREGNANE GLYCOSIDE

It is reported that, cynatroside B was isolated from the methanol extract of the roots of *Cynanchum atratum* Bunge (Fig. (**11a**)), and it significantly inhibited the AChE activity. Cynatroside B was found to be the most potent of these isolated pregnane glycoside inhibitors and its mode of AChE inhibition was also characterized. Cynatroside B inhibited AChE activity in a dose-dependent manner and its IC_{50} value was 3.6 μM. The mode of AChE inhibition by cynatroside B was reversible and non-competitive. Therefore, cynatroside B has anti-AChE activity that may ultimately hold significant therapeutic value in curing certain memory impairments observed in Alzheimer's disease [39].

TERPENOIDS

1. Monoterpenoids

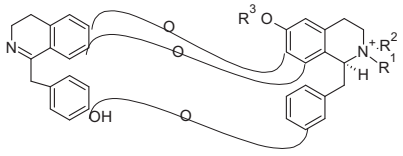
Monoterpenes were first reported to exhibit AChE inhibitory activity by Perry *et al* [40]. The essential oil of *Salvia lavandulaefolia* Vahl. was reported to exhibit uncompetitive, reversible inhibition of AChE in erythrocytes. This study confirmed, the low molecular weight compounds inhibit AChE. Similar reports of AChE inhibitory activity has been exhibited by oil of Salvia (Sage) species. The phytochemical constituents present in the oil (21.5%) such as 1,8 cineole exhibited marked AchE activity at IC_{50} value of 0.06±0.007 mg/ml. From the dose response curve plotted against 1,8 cineole, it was concluded that 1,8 cineole produced only 20% inhibition of AChE, hence it could be reasoned out that the synergistic activity exerted by other phytoconstituents could have provided a high IC_{50} values.

2. Diterpenoids

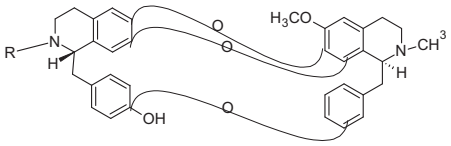
Four inhibitory compounds dihydrotanshinone (Fig. (**12a**)), cryptotanshinone (Fig. (**12b**)), tanshinone I (Fig. (**12c**)) and tanshinone IIA (Fig. (**12d**)) were isolated from the dried roots of *Salvia miltiorhiza* Bunge called as Danshen in China [41]. Among these, the activity of dihydrotanshinone and cryptotanshinone were found to be dose dependant with IC_{50} values of 1.0 and 7.0 μM respectively, while tanshinone I and tanshinone II A showed an weaker inhibition at >50 and >140 μM concentration. The structures of dihydrotanshinone differs only by double bond and dihydro furan ring with tanshinone, which reflects the activity of the compound. Cryptotanshinone and tanshionone IIA show similar difference of activity, so it proves that dihydro furan ring is accountable for the acetylcholinesterase activity. Further, the hydrophobicity clogP values of dihydrotanshinone, cryptotanshinone, tanshinone I and tanshinone IIA were calculated as 2.4, 3.4, 4.8 and 5.8 respectively. This indicates that the compounds have the potential to penetrate blood-brain barrier [41].

Seven abietane and seco-abietane diterpenes were isolated from the methanolic extract of aerial part of *Salvia candelabrum* Boiss [38]. Besides these several terpenes like those shown Fig. (**12e-12k**) were also isolated from *Salvia candelabrum.* The enzyme dependent assay were performed for diterpenes candesalvoquinone (Fig. (**12e**)), candelabroquinone (Fig. (**12f**)), candesalvone B methyl ester (Fig. (**12h**)), candelabrone (Fig. (**12i**)), candesalvone B (Fig. (**12j**)) and candesalvolactone (Fig. (**12k**)). The effects were measured for enzyme dependent assay and was found to indicate similar inhibitory activity, while 12-O-methylcandesalvone B (Fig. (**12g)**) showed weak inhibitory activity. The IC_{50} values were found to be 3.49-10.42 μM [42].

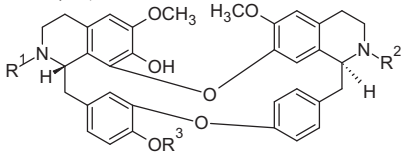
AChE inhibiting properties of natural products buxamine-B (Fig. (**12l**)) and buxamine-C (Fig. (**12m**)) isolated from *Buxus papillosa* C.K. Schneider and *Buxus hyrcana* Pojark species were studied. The buxamine-B and buxamine-C have been found to inhibit AChE noncompetitively in a concentration dependent fashion. The IC_{50} values of buxamine- B and buxamine-C were 74 and 7.5 μM respectively. Buxamine- B and buxamine-C have two amino groups situated at both ends of the cyclopentanophenanthrene ring system. The structures of these compounds differ only at the C-3 and C-20 amino substituents. Structurally similar triterpenoidal compounds lacking the C-3 and C-20 amino groups could not inhibit the AChE (*Torpedo californica*) in concen-



(a): R^1=α-CH_3, R^2= β-O, R^3=H kurramine-2'-β -N-oxide, (IC_{50} 10.0μM)
(b): R^1= β-CH_3, R^2=α-O, R^3=H kurramine-2'-α-N-oxide
(c): R^1=CH_3, R^2= R^3=CH_3 1,2-dehydroapateline

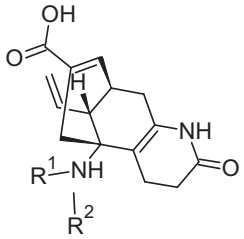


(d) R=H Cocsoline, (IC_{50} 47.6μM)
(e) R=CH_3 Cocsuline, (IC_{50} 12.0 μM)

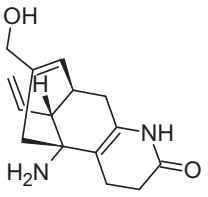


Compound	R^1	R^2	R^3	
Fangchinoline	CH_3	CH_3	CH_3	(f)
Atherospermoline	CH_3	CH_3	H	(g)
Fenfangline E	CH_3	H	CH_3	(h)

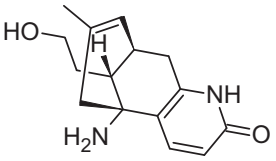
Fig. (5). Isoquinoline derivatives.

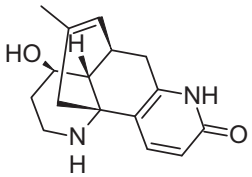


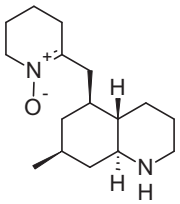
(a) R^1=R^2=CH_3 Lycoparin A
(b) R^1=CH_3,R^2=H Lycoparin B



(c) Lycoparin C , (IC_{50} 25 μM)







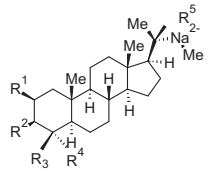
(d) Carinatumins A, IC_{50} 4.6 μM) (e) Carinatumins B, IC_{50} 7 μM) (f) Carinatumins C, (IC50 > 100 μM)

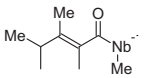
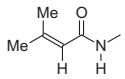
Fig. (6). Lycopodium alkaloids.

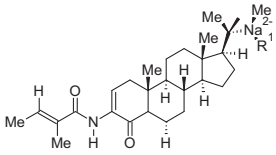
trations up to 1 μM, which demonstrates the importance of these amino groups on the inhibitory activities of these compounds. The distances of C-3 amino nitrogen in both alkaloids and quaternary ammonium of decamethonium are 2.8 and 1.2 A°, respectively. Therefore, it can be predicted that the C-3 tertiary ammonium of buxamine-C is better positioned as compared to secondary amino group of buxamine-B [43,15].

3. Triterpenoids

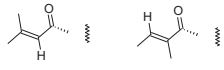
The ethanol extract of *Origanum majorana* L. was screened for its inhibitory activity on acetylcholinesterase. It showed the highest inhibitory effect on AChE *in vitro* among the herbs, edible plants and spices screened. By sequential fractionation of *Origanum majorana* L., the active component was identified as ursolic acid (3 -Hydroxyurs-12-en-28-oic acid) (Fig. (**13a**)). The ursolic acid of *Origanum*



	R^1	R^2	R^3	R^4	R^5	unsaturation
(a)	H		H	H	CHO	
(b)	OAc		H	H	Me	$\Delta^{14,15}$
(d)	H		OAc	H	Me	



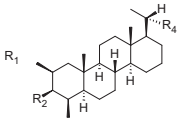
	R^1	unsaturation
(c)	H	$\Delta^{14,15}$



Senecioyl

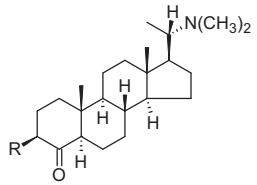
Tigloyl

Cinnamoyl



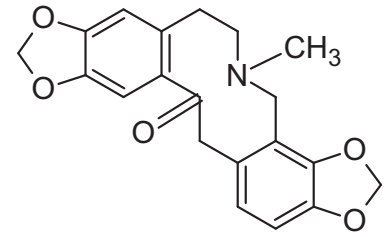
R_3

Compound	R^1	R^2	R^3	R^4	Unsaturation
(e) Salignenamide-C	OH	HN-Tigloyl	OAc	$N(CH_3)_2$	$\Delta^{14,15}$
(f) Salignenamide-D	α-OH	HN-Tigloyl	H	$N(CH_3)_2$	$\Delta^{4,5}$& $\Delta^{16,17}$
(g) 2β-Hydroxyepipachysamine-D	OH	HN-Benzoyl	H	$N(CH_3)_2$	
(h) Salignenamide-E	H	$NCH_3COCH=CCH_3CH(CH_3)_2$	H	$N(CH_3)2$	$\Delta^{16,17}$
(i) Salignenamide-F	H	$NCH_3COCH=CCH_3CH(CH_3)_2$	H	$N(CH_3)2$	
(j) Axillarine-C	OH	HN-Benzoyl	OAc	$N(CH_3)_2$	
(k) Axillarine-F	OH	HN-Tigloyl	OAc	$N(CH_3)_2$	
(l) Sarcorine	H	NHAc	H	$N(CH_3)_2$	
(m) Na-Demethylsaracodine	H	$NHCH_3$	H	NCH_3Ac	
(n) Saligcinnamide	H	CH_3N -Cinnamoyl	H	$N(CH_3)_2$	
(o) Salignenamide-A	H	$NHCOCH=C(CH_3)CH(CH_3)_2$	H	$N(CH_3)_2$	
(p) Vaganine-A	H	HN-Senecioyl	OAc	$N(CH_3)_2$	
(q) 5,6-Dehydrosarconidine	H	$NH(CH_3)$	H	N(CH3)2	$\Delta^{16,17}$
(r) 2-Dydroxysalignarine-E	OH	OCH_3	H	$N(CH_3)_2$	$\Delta^{5,6}$& $\Delta^{16,17}$
(s) Salignamine	H	OCH_3	H	$N(CH_3)_2$	$\Delta^{5,6}$& $\Delta^{16,17}$
(t) 2-Hydroxysalignamine-E	OH	HN-Tigloyl	H	$N(CH_3)_2$	$\Delta^{4,5}$
(u) Epipachysamine-D	H	HN- Benzoyl	H	$N(CH_3)_2$	
(v) Dictyophlebine	H	$NHCH_3$	H	$N(CH_3)_2$	
(w) Iso-N-formylchoneformine	OH	$N(CH_3)_2$	H	NHCHO	
(x) Sarcodinine	H	$N(CH_3)_2$	H	$N(CH_3)_2$	$\Delta^{5,6}$



Compound	R	Unsaturation
(y) Axillaridine-A	HN-Benzoyl	$\Delta^{2,3}$
(z) Sarsalignone	HN-Tigloyl	$\Delta^{5,6}$
(z_1) Sarsalignenone	HN-Tigloyl	$\Delta^{5,6}$ & $\Delta^{14,15}$

Fig. (7). Pregnane alkaloids.



(a) Protopine, IC_{50} 50 μM

Fig. (8). Protopine.

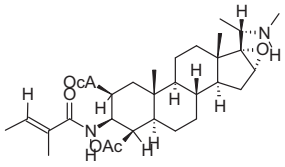
majorana L. inhibited AChE activity in a dose-dependent and competitive/non-competitive type. The Ki value (representing the affinity of the enzyme and inhibitor) of *Origanum majorana* L. ursolic acid was 6 pM, and that of tacrine was 0.4 nM. The IC_{50} value of the active compound is 7.5 nM and tacrine was 1 μM.

From the Km and Vmax value it was found that the compound inhibited the acetyl cholinesterase in a mixed (competitive / non-competitive) manner [44].

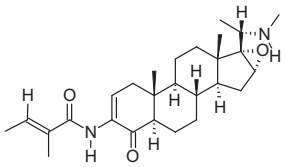
The methanolic extract of the twigs of *Vaccinium oldhami* Miquel, a Korean natural product was found to have significant acetylcholinesterase (AChE) inhibitory activity. Bioassay-guided fractionation of the extract resulted in the isolation of two compounds, taraxerol (Fig. (**13b**)) and scopoletin as active constituents. These compounds were found to inhibit AChE in a dose-dependent manner, and the IC_{50} values of compounds taraxerol and scopoletin were 33.6 (79 μM) and 10.0 (52 μM) mg/mL, respectively. These compounds exhibit good activity due to their low molecular weight, as they can easily reach the site of action by crossing the blood-brain barrier [45].

4. Meroterpenoid

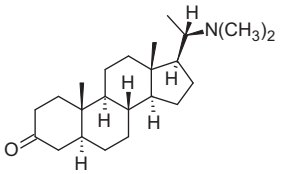
A seven-membered lactone type meroterpenoid isoterreulactone A (Fig. (**14a**)), was isolated from the solid state fermentation of *Aspergillus terreus* [46]. Meroterpenoids such as pyripyropene and oxalicine, and seven- membered lactone type terpenoids such as andilesins, anditomin, fumigatonin, and obacunol have been isolated from fungi [47,48]. Isoter-



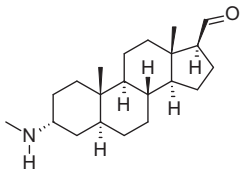
(a) Epoxynepapakistamine-A



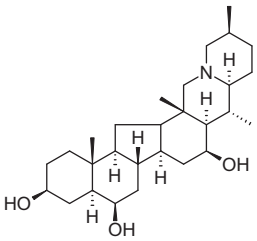
(b) Epoxysarcovagenine-D



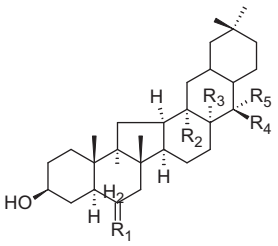
(c) Funtumafrine C, (IC_{50} 45.75±1.122)



(d) N-methylfuntumine, (IC_{50} 97.61±1.731)

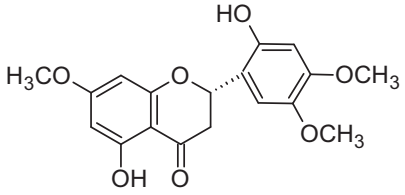


(e) Impericine



	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	
(f)	OH / H	β -H	α-H	CH_3	H	CH_3	H	α-H	Forticine
(g)	OH / H	β -H	β -H	H	CH_3	CH_3	H	α-H	Delavine
(h)	OH / H	α-H	β -H	H	CH_3	H	CH_3	α-H	Persicanidine A
(i)	=O	β -H	β -H	OH	CH_3	CH_3	H	α-H	Imperialine

Fig. (9). Steroidal Alkaloids.



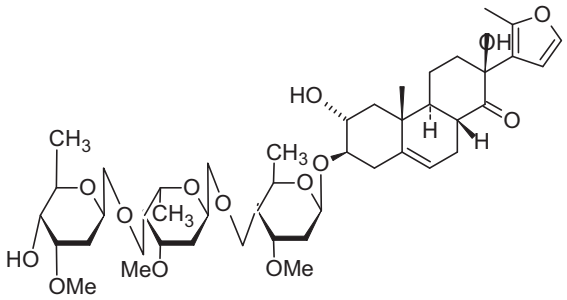
(a) Hispidone, (IC_{50} 11.6 μM)

Fig. (10). Flavanones.

reulactone A inhibited acetylcholinesterase with an IC_{50} value of 2.5 μM while did not inhibit butyrylcholinesterase even at 500 μM. Isoterreulactone A inhibited acetylcholinesterase in a dose-dependent mode with an IC_{50} (μM) value of 2.5. Anti-acetylcholinesterase activity of isoterreulactone A was 10 times weaker than that (0.23 μM) of terreulactone A, which suggested the important role of the ring A in acetylcholinesterase inhibitory activity.

STEROLS

Haloxysterols A (Fig. (**15a**)), B (Fig. (**15b**)), C (Fig. (**15c**)) and D (Fig. (**15d**)) have been isolated from the chloroform soluble fraction of *Haloxylon recurvum* Bunge ex Boiss, along with five known sterols, and all of them were found to inhibit AChE and BChE enzymes in a concentration-dependent manner. They found a non-competitive type



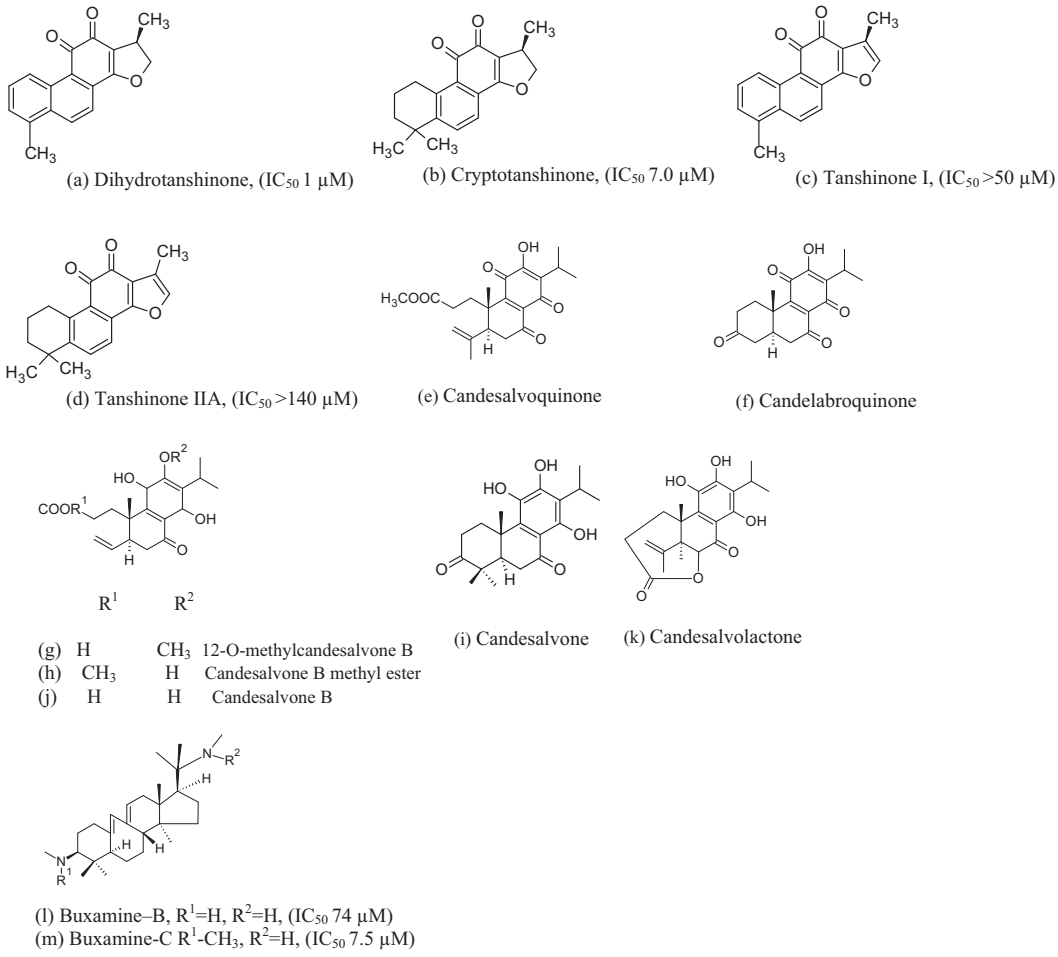
(a) Cynatroside B, (IC_{50} 3.6 μM)

Fig. (11). Pregnane glycoside.

of inhibition. Their similar binding mode is not surprising because all of them have almost similar structures with minor differences only in the basic skeleton of the compounds. The inhibitory potential of these compounds can be the cumulative effect of hydrogen bonding and π-π stacking interactions. The hydroxyl moieties present can be involved in hydrogen bonding with the amino acid residues of the active site of the AChE [49].

STILBENES

Two active stilbene oligomers α –viniferin (Fig. (**16a**)) and kobophenol A (Fig. (**16b**)) were isolated from the un-

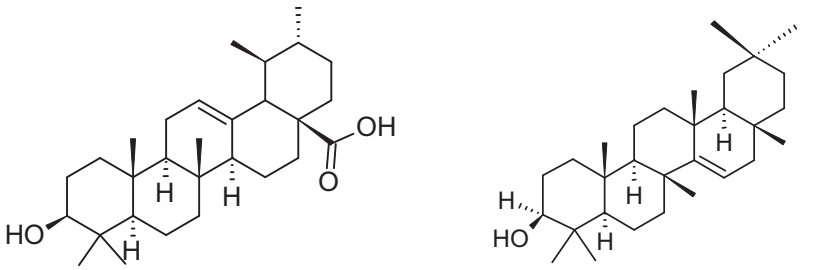


(a) Dihydrotanshinone, (IC_{50} 1 μM)
(b) Cryptotanshinone, (IC_{50} 7.0 μM)
(c) Tanshinone I, (IC_{50} >50 μM)
(d) Tanshinone IIA, (IC_{50} >140 μM)
(e) Candesalvoquinone
(f) Candelabroquinone

	R^1	R^2	
(g)	H	CH_3	12-O-methylcandesalvone B
(h)	CH_3	H	Candesalvone B methyl ester
(j)	H	H	Candesalvone B

(i) Candesalvone
(k) Candesalvolactone
(l) Buxamine–B, R^1=H, R^2=H, (IC_{50} 74 μM)
(m) Buxamine-C R^1-CH_3, R^2=H, (IC_{50} 7.5 μM)

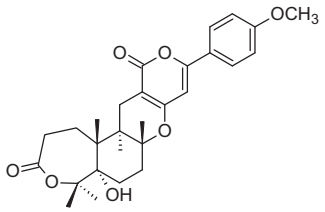
Fig. (12). Diterpenoid.



(a) Ursolic acid, (IC_{50} 7.5 nM) (b) Taraxerol, (IC_{50} 79 μM)

Fig. (13). Triterpenes.

derground parts of *Caragana chamlague* LAMRK (Leguminosae). Both α –viniferin and kobophenol A inhibited AChE activity in a dose-dependent manner, and the IC_{50} values were found to be 2.0 and 115.8 mM respectively. Among the two, the AChE inhibitory activity of α –viniferin was found to be specific, reversible and noncompetitive. α – viniferin has an appropriately bulky structure that masks AChE and was supposed to prevent acetylthiocholine iodide from binding to AChE in a noncompetitive manner. In contrast, in the case of kobophenol A, while it has a bulky structure, its activity may be lowered due to the simple difficulty of accessibility to AChE [50].



(a) Isoterreulactone A,(IC_{50} 2.5 μM)

Fig. (14). Meroterpenoid.

WITHANOLIDES

The withanolides are a group steroids present in, with a lactone-containing side chain of nine carbons attached at C-17. A total of six withanolides were isolated from the whole plant of *Withania somnifera* (L.) Dunal. Their structures were characterized as shown Fig. (**17a-17$_f$**), respectively. Compounds 5b, 6b -epoxy-4b, 17a, 27-trihydroxy-1-oxowitha- 2, 24-dienolide, withaferin-A, 6a,7a-epoxy-5a, 20b -dihydroxy-1-oxowitha- 2,24-dienolide and 5b, 6b -epoxy-4b-hydroxy-1-oxowitha-2,14,24-trienolide displayed inhibitory potential against butyrylcholinesterase, but only compounds withaferin-A, 2,3-dihydrowithaferin-A and 5b ,6b -epoxy-4b -hydroxy-1-oxowitha-2,14,24-trienolide were found to be active against acetylcholinesterase. Compounds (**17a-17f**) were screened for their anti-cholinesterase activity in a mechanism-based assay. Compounds 5b, 6b -epoxy-4b, 17a, 27-trihydroxy-1-oxowitha- 2,24-dienolide (IC_{50} 161.5 μM), withaferin-A (IC_{50} 84.0 μM), 6a,7a-epoxy-5a,20b -dihydroxy-1-oxowitha- 2,24-dienolide (IC_{50} 50.5 μM), and 5b ,6b -epoxy-4b -hydroxy-1-oxowitha-2,14,24-trienolide (IC_{50} 124.0 μM) were found to be active against AChE. Similarly, compounds withaferin-A (IC_{50} 125.0 μM), 2,3-dihydrowithaferin-A (IC_{50} 500. μM), and 5b,6b -epoxy-4b -hydroxy-1-oxowitha-2,14,24-trienolide (IC_{50} 62.5μM) inhibited the activity of BchE significantly [51].

XANTHONES

The methanol extract of *Gentiana campstris* (L.) DC leaves were found to exhibit significant inhibition of AChE activity. Four xanthones as shown in Fig. (**18a-18$_d$**) were found to be responsible for the activity. It was found that all

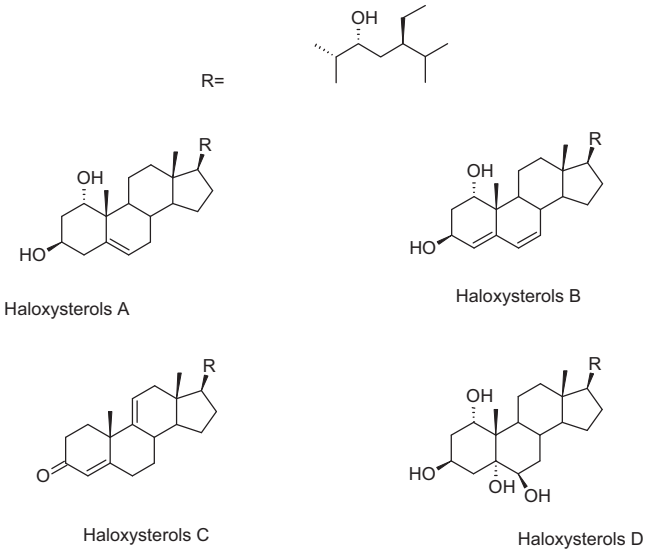
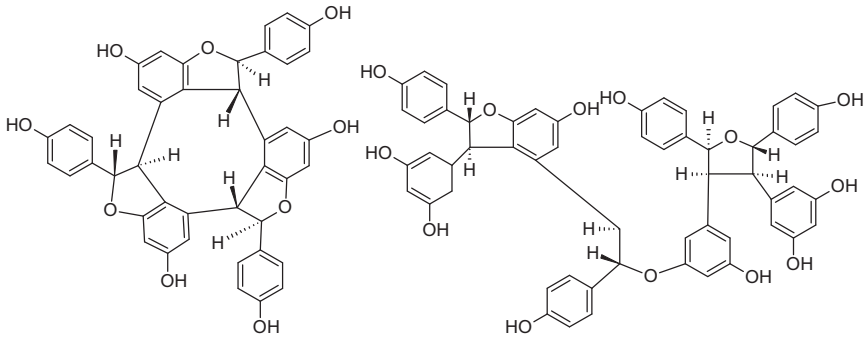
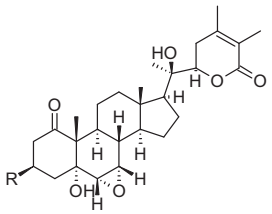


Fig. (15). Sterols.

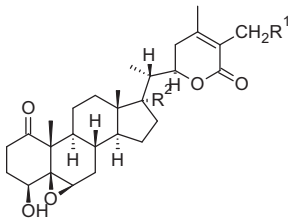


(a) α –Viniferin, (IC_{50} 2.0 mM) (b) Kobophenol A, (IC_{50} 115.8 mM)

Fig. (16) Stilbenes.



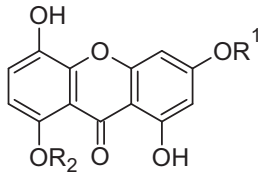
(a) R=OH (6a,7a-epoxy-3b,5a,20b -trihydroxy-1-oxowitha-24-enolide)
(e) R=H, $\Delta^{2,3}$ (6a,7a-epoxy-5a,20b -dihydroxy-1-oxowitha- 2,24-dienolide), (IC_{50} 50.5 μM)



(b) R^1=R^2=OH, $\Delta^{2,3}$ (5b ,6b -epoxy-4b ,17a,27-trihydroxy-1-oxowitha- 2,24-dienolide), (IC_{50}161.5 μM)
(c) R^1=OH, R^2=H, $\Delta^{2,3}$ (withaferin-A), (IC_{50} 84.0 μM)
(d) R^1=OH, R^2=H (2,3-dihydrowithaferin-A), (IC_{50} 50.5 μM)
(f) R^1=R^2=H, $\Delta^{2,3}$, $\Delta^{14,15}$ (5b ,6b -epoxy-4b -hydroxy-1-oxowitha-2,14,24-trienolide), (IC_{50} 124.0 μM)

Fig. (17). Withanolides.

the xanthones inhibited the enzyme at less than 0.5μg and bellidifolin was more active than bellidin and bellidin 8-O-βglucopyrnoside was more active than belllidin 8-O-β-glucopyranoisde suggesting the significance of methoxy group in position C-3 [52, 3].



(a) R^1 =CH_3, R_2 = β-glucopyranosyl
(b) R^1 = R_2 = H
(c) R^1 = CH_3, R_2 = H
(d) R^1= H, R_2 = β-glucopyranosyl

Fig. (18). Xanthone.

ZEATIN

Zeatin (Fig. (**19**)) is a member of the plant growth hormone family known as cytokinins. The methanol extract from *Fiatoua villosa* (Thunb.) Nakai among 100 traditional edible plants tested were showed the most potent inhibitory effect (51%) on acetylcholinesterase *in vitro*. Zeatin was isolated from this extract and tested for acetylcholinesterase inhibitory activity, which could easily reach the site of action after oral or transdermal administration, because the molecule could cross the blood-brain barrier. Thus this active component could slow down the decline of cognitive function and memory in some patients with mild or moderate AD [53].

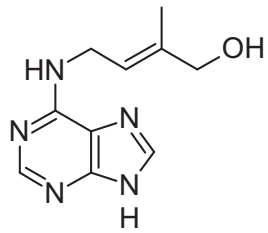


Fig. (19). Zeatin.

CONCLUSION

Alzheimer's disease (AD) is one of the most common forms of dementia affecting a large number of geriatric

population, which is due to the depletion of ACh. The ACh has a very short half-life due to the presence of large amounts of acetylcholinesterase (AChE), an enzyme which hydrolyses the ester bond in the molecule, thus leading to loss of stimulatory activity. Even though amyloid pathway is also a suggested pathway for the disease, AChE pathway is so far the most promising hypothesis. The cholinergic hypothesis of Alzheimer's disease is based on the presynaptic deficits found in the brains of patients with Alzheimer's disease and studies of the role of ACh in animal and human behaviour. Although it is now clear that cholinergic dysfunction may not cause cognitive impairment directly, but rather indirectly, by interfering with attentional processing, the hypothesis predicted that cholinomimetic drugs would improve cognitive function. This prediction was not fully realized with compounds because the emergence of side effects that may have constrained the dosing regimen to subefficacious doses. Poor tolerability seems to be less of an issue for the second generation compounds of the type now being licensed for the treatment of Alzheimer's disease. With improved diagnosis, careful patient selection, and fewer side effects, such compounds will establish its cholinomimetic therapy. Moreover, the emerging relation between neurotransmission and metabolism of two key proteins involved in Alzheimer's disease, APP and tau, raises the possibility that second generation ChE inhibitors may alter disease pathology and progression. However, acetylcholinesterase inhibitors have been accepted to be the most effective for the treatment of AD, up to the present. The effect on the activities of AChE not associated with nervous transmission is more difficult to predict, but inhibitors of these aspects of its function have been predicted as worth exploring in the search for novel drugs.

Ethno medicinal plants have been a huge resource for AChE inhibition activity [3, 54] and thus used widely for the treatment of Alzheimer's disease. The search for the plant derived molecules has accelerated in view of the benefits of these drugs not only in the treatement of AD but in other forms of dementia also [55-58]. More likely is the fact that discovery of AChE inhibitory compounds in traditional remedies, may explain their use in improving memory and other cognitive functions associated with cholinergic stimulation. Several phytomolecules from therapeutically potential plants play major role as AChE inhibitors. These phytomolecules of different chemical classes act by binding to various active sites of the receptor. The structure of these phytomolecules plays a major role in this type of inhibition. The use of the enzyme kinetics for determining the inhibition by isoenzymes with known variations and mutations in the amino acid sequence has helped the comparison of several binding areas. This has been especially facilitated by the use of molecular modeling programs. By using these techniques it has been clarified that the positively-charged nitrogen in these phytomolecules binds to the active site, even though several molecules have potential activities even in the absence of nitrogen. Further this was confirmed by earlier hypotheses about the structural features necessary for a molecule to have such type of inhibitory effects.

The data concerning AChE inhibitors isolated from the plants are complicated by the complexity of their molecular forms. In spite of the deficiencies in the literature concerning the correlation of structure with its activity the overall observation of current status in the research in this regard provide necessary inputs for boosting strategies to develop a valuable and more useful molecule from the ethno medicinal resources. Though a good number of AChE inhibitors have been developed from plants, the present drugs available for the treatment of AD possess some side effects and are effective only against the mild type of AD. Hence it is required to develop a potential phytomolecule from the yet to be explored ethnomedicinal plants. A potential molecule can be developed at ease when the relationship between the structure and their activity is well understood. With this in their mind scientists will have to look towards nature for another diverse molecule with a novel mode of action to tackle this alarming disease.

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