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Research Article

SCREENING AND *IN SILICO* ANALYSIS OF *HYPTIS SUAVEOLENS* METABOLITES FOR ACETYLCHOLINESTERASE INHIBITION

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ABSTRACT

Objective: Current drugs to Alzheimer's disease (AD) are targeted to delay the breakdown of acetylcholine, thereby increasing the concentration of acetylcholine released into synaptic cleft and enhancing cholinergic neurotransmission. This paper deals with screening and identification of acetylcholinesterase (AChE) inhibitors in solvent extracts of *Hyptis suaveolens* (HS).

Methods: In search of natural inhibitors of AChE, this study is focused on extract of HS, a member of Lamiaceae. 1:4 ratio of methanolic extract is prepared with shade dried areal parts of HS plant. The extract was assayed by Ellman's method for inhibition activity and then purified using ammonium sulfate precipitation and chromatography techniques. Gas chromatography-mass spectrometry (GC-MS) identified compounds were analyzed by docking studies.

Results: Methanolic extract showed maximum percentage inhibition of 75.00±4.30 (2.1 mg/ml) with an IC_{50} value of 1.020±0.026 mg/ml. However, saturated ammonium sulfate precipitation of methanolic extract and further fractionation by gel permeation chromatography showed 86.00±1.30% AChE inhibition (AChEI) activity. Reverse phase high-performance liquid chromatography (RP-HPLC) fraction (retention time [RT] 5.170) showed significant inhibition when compared to the other peak (RT 6.643). RP-HPLC fraction (RT 5.170) with significant inhibition was identified as Eugenol by GC-MS analysis. *In silico* analysis of all the GC-MS identified molecules revealed Eugenol as possessing preeminent absorption, distribution, metabolism, elimination properties and a glide (docking) score of −9.14 kcal/mole with AChE enzyme of pacific electric ray (*Torpedo californica* - TcAChE) (PDB ID: 1EVE).

Conclusion: Screening, purification and identification, and identification of diverse phytochemicals of the HS plant, as potent source of AChEI.

Keywords: Acetylcholinesterase, Acetylcholnesterase inhibitor, *Hyptis suaveolens*, Alzheimer's disease, Dementia.

INTRODUCTION

Dementia is a syndrome caused by a number of progressive disorders. Alzheimer's disease (AD) is the most common type of dementia and is a chronic neurodegenerative disease. AD is characterized symptomatically by progressive deterioration of the activities of daily living, behavioral disturbances and cognitive loss in aging populations [1]. It is estimated that worldwide 44.56 million people were suffering with AD in 2015 and were expected to affect 85.7 million by 2030 and 115.4 million by 2050 [2,3].

Enhancing cholinergic activity of brain by inhibiting the acetylcholinesterase (AChE) enzyme is one of the most important symptomatic strategies to treat AD [4,5]. AChE inhibition (AChEI) has resulted in increased levels of acetylcholine within the synaptic region and restoration of the deficient cholinergic neurotransmission [6]. AChE inhibitor drugs with free dose and limiting side effects are scarcely available. Currently, only four Food and Drug Administration (FDA) approved AChE inhibitor synthetic drugs are commercially available and are tacrine [7,8], donepezil [9], rivastigmine [10], and galantamine. Among these, rivastigmine is the most commonly used medicine to treat AD. However, synthetic drugs of AChEI induce side effects such as gastrointestinal reactions, nausea, loss of appetite, anxiety, difficulty in sleeping, fatigue, and weight loss. Moreover, in the treatment of mild to moderate AD, the cost incurred for medication is also expensive [11]. Altogether, currently there is a need to find potent natural inhibitor drugs of AChE to treat AD, which ought to be cost-effective and with trivial side effects.

Most of the phytoprinciples found in medicinal plants (alkaloids, steroids, tannins, and flavonoids) are potent bioactive compounds and act as templates for the synthesis of the therapeutic drugs [12]. These phytoactive substances differ from plant to plant due to vast biodiversity. Around 300 species of such medicinal and aromatic plants are identified and are used in different ethno medicinal preparations [13]. Screening and identification of AChEI in solvent extracts of diverse plant species have been done, but purification of such metabolites is inadequate. *Hyptis suaveolens* (HS) commonly called as *Wilayati tulsi* is a medium sized aromatic, annual shrub belonging to *Lamiaceae* family and is distributed in tropical and sub-tropical regions [14]. It is a traditional medicinal plant used to treat diverse diseases and possess highest percentage of alkaloids (14.5%), phenolics and tannins (0.5%) and approximately 12% of flavonoids [15]. The leaves of HS have been utilized as a stimulant, carminative, galactogogue and as a cure for parasitic cutaneous diseases. Crude leaf extract is used to relieve colic and stomach ache [16]. Leaves and twigs are considered to be antispasmodic and used as anti-rheumatic, anti-inflammatory, anti-fertility agents and also applied as an antiseptic on burns, wounds and various skin complaints [17]. Essential oil of HS possesses potent antioxidant and antifungal activity [18].

The present study supports, HS having inhibitory property against AChE activity. The specific molecule has been identified and the active binding site has been identified in AChE by docking. The neuroprotective role of HS has been studied in this article.

METHODS

Acetylthiocholine iodide (ATCI), AChE, 5,5′-dithio-bis 2-nitrobenzoic acid (DTNB) were obtained from Sigma (India). All organic solvents used in this study (AR grade) were purchased from Merck.

Extraction of plant materials

Fresh leaves of HS were collected from Kunchanpalli village, Vijayawada, Andhra Pradesh, India. A voucher specimen of the HS plant was confirmed by Dr. A. Prasada Rao, Department of Biotechnology, K L University (Voucher no. 20112010). The leaves were shade dried for 7 d and 25 g of grinded fine powder was subjected to soxhlet apparatus using 100 ml of methanol for 5 hrs.

Purification

Crude HS methanolic extract was treated with saturated ammonium sulfate, and the sample was centrifuged at 3000 rpm for 3 minutes. The supernatant was subjected to gel permeation chromatography (0.20 m × 0.01 m) using Biogel P-60 (Bio-Rad). Fractions of the elution (1 ml) were collected and screened by bioassay.

Bioassay

AChE activity was measured using a 96-well microplate reader [19] based on Ellman's method [20]. The enzyme hydrolysis of the substrate acetylthiocholine results in the product thiocholine; which reacts with Ellman's reagent (DTNB) and produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate, which are detected at 405 nm. In 96-well microtiter plate, 80 µl DTNB (3.96 mg of DTNB and 1.5 mg sodium bicarbonate dissolved in 10 ml sodium phosphate buffer pH 7.4), 135 µl of sodium phosphate buffer (200 mM with pH 7.4), 10 µl of 0.22 U/ml of AChE, and 10 µl of HS methanolic extract were added and incubated for 10 minutes. About 15 µl ATCI (100 mM ATCI) in sodium phosphate buffer) was added and the absorbance was recorded using a Multiskan EX microplate (Thermo Scientific, India) reader at 405 nm for every 2 minutes interval time up to 20 minutes.

Phytochemical screening

Phytochemical analysis was performed to detect the presence of various phytoconstituents by adapting protocols from literature [21-23].

High-performance liquid chromatography (HPLC)

The gel permeation chromatography fraction showing inhibition was further analyzed by reverse phase HPLC (RP-HPLC) using C-18 (250 mm × 4.6 mm, 5 µm) column (Hibar) with acetonitrile:water (30:70) as mobile phase. Based on the retention time (RT), fractions were collected separately. The RP-HPLC fractions were vacuum dried and redissolved in 200 mM phosphate buffer (pH 7.4) and bioassay was performed.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS (SHIMADZU QP 2010) employing the electron impact (EI) mode (Ionizing potential 70 eV) and capillary column (Restec-624 MS) (30 m × 0.32 mm and 1.8 µm film thickness) packed with 5% phenyl dimethyl silicone. The ion source temperature was maintained at 45°C for 4 minutes, after that increased to 50°C and then increased to 175°C at the rate of 10°C/minutes for 2 minutes, and then finally programmed to 240°C at a rate of 25°C/minutes, and kept isothermal for 2 minutes. A carrier gas of helium with 99.9% purity was used with flow rate of 1.4 ml/ minute at split ratio of 1:10.

Identification of compounds

GC peak areas were computed for the respective fraction of the sample. Library search was done using the WILEY8, NISTO8S and FAME Libraries.

Protein and ligand structure retrieval and preparation

The three-dimensional (3D) protein structures were obtained from Protein Data Bank (PDB). Crystal structure of AChE enzyme of Pacific electric ray (*Torpedo californica* - TcAChE) (PDB ID: 1EVE) with a resolution of 2.5Å along with respective ligand aricept was retrieved. 3D structures of respective ligands and standard drug ligands, such as donepezil, tacrine, galanthamine, and rivastigmine, were obtained from PubChem (http://pubchem.ncbi.nlm.nih.gov/search/). Molecular modeling software, Schrodinger maestro 9.3 tools were used for predicting absorption, distribution, metabolism, elimination (ADME) properties and docking values.

Protein preparation of 1EVE, 1XLU was done by using protein preparation wizard and Lig Prep tool was used for preparing ligands of Schrodinger maestro 9.3 by applying optimized potential for ligand simulation-2005 Force Field. Desolvation was done by removing crystallized free water molecules beyond 5 Å. Bond orders were added to hydrogen's and zero order bonds were created to metal atoms. Finally, optimization and free energy minimization were done.

Receptor generation and docking

Receptor region was generated by using free energy minimized 1EVE, 1XLU protein structures through receptor grid generation tool of Glide module. The shape and properties of receptor were represented on a grid for more accurate scoring of ligand pose. Receptor Vander Waals radius scaling factor 1-0.25 kcal/mol was assigned. The binding region was defined by 12 Å three dimensional box centered on the mass center of crystallographic ligand to confine the mass center of docked ligand. Amino acids containing hydroxyl groups in receptor region were allowed to rotate so as to minimize the rotational penalty. Prepared ligands were rigidly docked to receptors of 1EVE, 1XLU using Glide extra precision (Glide XP) function. Finally, minimized poses were rescored by Glide scoring function and visualized through XP-Visualizer.

ADME prediction

Qikprop module was used to predict ADME and other molecular properties. About 47 descriptors, which include various pharmacological, pharmacokinetic and physiochemical properties were screened for all the ligands. The most preferable parameters for a central nervous system (CNS) active drugs were CNS (predicted CNS activity), LogBB (predicted blood/brain barrier partition coefficient), MDCK (predicted apparent MDCK cell permeability), Caco-2 (predicted apparent Caco-2 cell permeability), HERG (predicted IC_{50} value for blockage of HERG K^+ channel), Khsa (prediction of drug binding to human serum albumin), metb (number of likely metabolic reactions), Mol.wt (molecular weight of the compound), %oral absop (predicted percentage of human oral absorption), LogS (predicted aqueous solubility), LogPo/w (predicted octanol water partition coefficient), HB accp (hydrogen bond acceptors), HB donr (hydrogen bond donors), PSA (van der Waals surface area of polar nitrogen and oxygen atoms), and Rule of 5 (Lipinski's rule of five).

RESULTS AND DISCUSSION

The phytochemical analysis of a methanolic extract of HS aerial parts confirmed the presence of alkaloids, steroids, terpenoids, flavonoids, glycosides, saponins, tannins, and phenolics (Table 1).

HS methanolic extract was precipitated with ammonium sulfate. Saturated ammonium sulfate precipitation of crude methanolic extract resulted in the removal of all the suspended and charged biomolecular

Table 1: Phytochemical analysis of *H. suaveolens* with different level of purification

Serial number	Phytochemical	Methanolic extract	Step 1	Step 2
1	Alkaloids	+	+	+
2	Steroids	+	+	–
3	Terpenoids	+	+	–
4	Flavanoids	+	+	+
5	Tannins and Phenolics	+	+	–
6	Glycosides	+	+	+
7	Saponins	+	+	+

Step 1: Extract after ammonium sulfate precipitation and Step 2: Fraction after gel-permeation chromatography. "+": Phytochemicals present in extract and "–": Phytochemical not detected. *H. suaveolens: Hyptis suaveolens*

contaminations. The supernatant retained the active principles of AChE inhibition and hence the supernatant was fractionated by gel permeation chromatography (Biogel-P60) and inhibition activity was screened by Ellman's method. The 7th fraction showed maximum inhibition activity. To this end, of all the biogel p60 gel-permeation chromatography fractions bioassayed, the seventh fraction showed the highest percentage of inhibition against AChE (Fig. 1).

Further, the phytochemical analysis of the identified fraction established the presence of the alkaloids, flavonoids, glycosides, and saponins only (Table 1). Gel permeation chromatography had resulted in the exclusion of the steroids, terpenoids, tannins, and phenolics from the methanolic extract, but still holding the AChE inhibition activity in the fraction. The gel permeation fraction exhibiting high AChE inhibition was analyzed by RP-HPLC using C-18 (Hibar) column with acetonitrile:water (30:70) as mobile phase and detector at 220 nm.

RP-HPLC analysis (Fig. 2) of the gel permeation chromatography active fraction evidently resolved into four peaks with RT 4.163, 4.760, 5.170, and 6.630. Bioassay based screening of fractionated peaks reestablished inhibitory activity of peaks with RT 5.170 and 6.630.

Commercially available AChEI from synthetic source includes donepezil, tacrine; whereas, galanthamine, rivastigmine and physostigmine are from natural sources. These compounds exhibit different chemical structures such as piperidine, acridine, benzazipine, methylcarbamate, and indole alkaloid. Among these physostigmine and tacrine showed low bioavailability and narrow therapeutic index, whereas donepezil and rivastigmine were useful only in mild AD conditions. Therefore, to increase the potency and efficacy with low adverse effects, many bioactive compounds are screened from various sources. Fumariaceae, Lycopodiaceae, Ericaceae and Papaveraceae (whole plant), Amaryllidaceae (bulbs), and Apocynaceae (roots) showed high inhibitory rates in methanolic and chloroform and methanol (1:1) extracts [24].

Isoquinoline alkaloids such as hydrastine, bulbocapnine, fumarophycine, corydaldine, protopine, ophiocarpine N-oxide, β-allocryptopine, ophiocarpine, and berberine from *Fumaria vaillantii* showed synergistic enzyme inhibition up to 94.23%. Among these alkaloids, ophiocarpine showed the highest inhibition. Other than huperzine A, α-oncocerin from *L. clavatum* also showed high dose-dependent inhibition against AChE. Moreover, alkaloids of Amaryllidaceae are expected to possess more compatibility due to structural similarity with phenylalanine and tyrosine metabolites. Among different alkaloids named, lycorine, tazettine, crinine, galanthamine, 3-epi-hydroxybulbispermine and 2-demethoxymountanine from *Galanthus ikariae*, galanthamine and lycorine exhibited potent inhibitory activity [25,26].

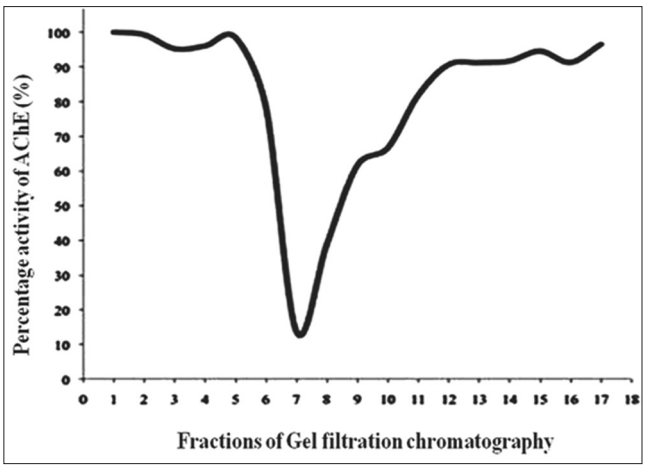

Fig. 1: Acetylcholinesterase inhibition activity of gel permeation chromatography fractions. The fraction with less percentage of activity has been considered for further analysis

GC-MS

GC-MS analysis resulted in the identification of 17 compounds with different RTs. Out of all the compounds, oleic acid has higher percentage area of 27.83; whereas, Eugenol has 0.93% of peak area. On the other hand, tetradecanoic acid, 2-hydroxy-3-[(9E)-9-octadecenoyloxy] propyl (9E)-9-octadeconoate and n-hexa decanoic acid had peak areas of 1.89%, 5.46%, and 15.77%, respectively. Glycidol stearate and 15-hydroxypentadecanoic acids were retained at 21.785 and 20.117 minutes with an area of 1.56% and 4.02%, respectively.

***In silico* analysis**

The GC-MS identified compound structures were retrieved from FLAME library for analysis. Protein preparation and docking analysis were performed for selected compounds through Schrodinger maestro 9.3 tools. Galantamine has the highest glide score of −13.79 among all the other compounds. Huperzine A has minimum glide score among FDA drugs. Whereas plant extract Eugenol had a glide score −9.14, which is approximately equal to huperzine A. 2-hydroxy-3-[9E] has minimum glide score of −1.53 out of all other compounds. Based on glide scores Eugenol has been selected as one of the inhibitor for AChE. Apart from this glycidol stearate has second highest glide score plant compounds. Even though, glycidol has CNS score −2, Eugenol (CNS = 0) had more glide score. Blood brain-barrier partition coefficient and MDCK cell permeability of Eugenol was similar to that of FDA drug tacrine (Table 2). Potassium channel blocking IC_{50} and serum albumin binding efficiency of Eugenol was −4.066 and −0.107, respectively. Methoxy pyrrolidin-2-one, glycidol stearate, n-hevadecanoic acid and tetradeconoic acids were showing high metabolic interference of 5 whereas; Eugenol has shown 3 metabolic reaction interference only. The FDA approved drugs also showed metabolic reactions in the range of 3-6 metabolisms. The $CaCO_2$ score represents the gut permeability of the compound. Out of all the compounds, glycidol stearate had greater permeability and second best permeable compound was Eugenol with 1616.207.

Predicted pharmacological parameters support that, of all plant extract compounds, Eugenol inhibition efficiency was high. In the case of predicted pharmacokinetic parameters from (Table 3); 5-methoxy pyrrolidin-2-one had the lowest molecular weight among all others. FDA approved drugs donepezil, rivastigmine and tacrine had a greater percentage of oral absorption. Eugenol, 2-Hydroxy-3-[9E] and glysidol stearates were having a higher percentage of absorption when compared to other plant compounds. Eugenol has aqueous solubility of −2.436 and only on hydrogen bond donating molecule. Apart from this, Eugenol had interactions with three amino acid residues in the ligand-binding site. PHE288, ARG289 and PHE331 are three residues having H-bonding with 'OH' and 'O' groups of Eugenol. All these predicted ADME parameters were supporting Eugenol's efficient inhibition activity in protein-ligand docking (Fig. 3).

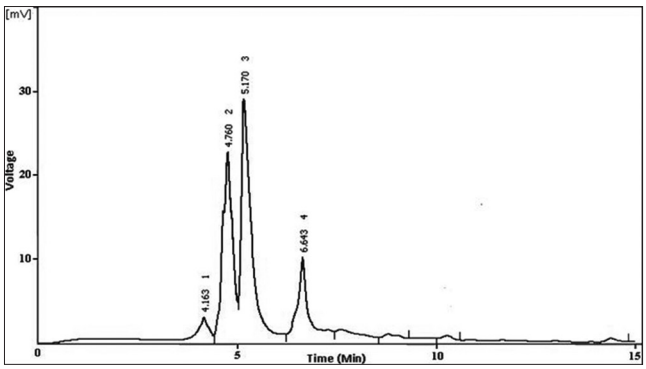

Fig. 2: Reverse phase high-performance liquid chromatography of gel-permeation fraction

Table 2: Pharmacological properties: FDA approved drugs and HS extracted GC-MS compounds

Serial number	Ligand	Protein ligand interaction docking by Glide			Predicted pharmacological parameters by Qikprop						
		Glide scores (kcal/mol)	H	Residues	CNS	QP log BB	QP MDCK	QP Caco	QP log HERG	QP log Khsa	Metb
					−2 (inactive) +2 (active)	−3-1.2	<25 poor >500 great	<25 poor >500 great	>−5	−1.5-1.5	1-8
FDA approved drugs	Galantamine	−13.79	-	-	1	0.368	381.71	716.50	−4.564	0.04	4
	Donepezil	−12.95	-	-	1	0.259	707.03	1267.3	−6.776	0.76	6
	Huperzine A	−9.280	-	-	0	−0.17	82.011	172.71	−4.905	0.03	6
	Rivastigmine	−10.76	-	-	2	0.459	756.04	1348.4	−5.447	−0.13	3
	Tacrine	−10.07	-	-	1	0.047	1608.6	2977.1	−4.092	0.06	4
1	Eugenol	−9.14	−1.81	PHE 288, ARG 289, PHE 331	0	−0.142	1616.207	2990.01	−4.066	−0.107	3
2	4H-pyran-4-one	−8.79	−2.77	PHE 288, ARG 289	−1	−0.628	210.896	454.387	−2.6	0.802	3
3	5-methoxy pyrrolidin-2-one	−7.20	−1.18	PHE 288, ARG 289	0	−0.102	900.23	737.688	−1.987	−1.139	1
4	15-hydroxy pentadecanoic acid	−6.69	−0.93	ARG 289, PHE 288, TYR 130, GLU 199	−2	−2.134	35.484	69.934	−3.272	−0.024	2
5	2-hydroxy-3-[9E]	−1.53	−0.49	TYR 70	−2	−4.254	163.866	359.787	−7.696	3.131	7
6	Glycidol stearate	−8.02	−0.44	SER 122	2	−1.132	1810.334	3320.849	−5.749	0.927	1
7	n-hexadecanoic acid	−4.6	−0.7	PHE 288, ARG 289	−2	−1.474	134.55	240.007	−3.366	0.549	1
8	Tetradecanoic acid	−4.95	−0.74	PHE 288, ARG 289	−2	−1.29	−134.54	240.006	−3.024	0.296	1

FDA: Food and Drug Administration, HS: *Hyptis suaveolens*, GC-MS: Gas chromatography-mass spectrometry, HB: Hydrogen bond, CNS: Central nervous system, QP logBB: Qikprop predicted blood/brain barrier partition coefficient, QP MDCK: Qikprop predicted apparent MDCK cell permeability, QP logHERG: Qikprop predicted IC_{50} value for blockage of HERG K^+ channel, QP log Khsa: Prediction of drug binding to human serum albumin, Metb: Number of likely metabolic reactions

Table 3: Pharmacokinetic properties: FDA approved drugs and HS extracted GC-MS compounds

Serial number	Ligand	Protein ligand interaction docking by Glide			Predicted pharmacokinetic parameters by Qikprop							
		Glide scores (Kcal/mol)	HB	Residues	Mol. Wt.	% oral absorp	QP log S	QP log Po/w	HB accp	HB donor	PSA	Rule of 5
					130-725	<25 poor >80 great	−6.5-0.5	−2-6.5	2-20	0-6	7-200	0-4
FDA approved drugs	Galantamine	−13.79	-	-	287.3	90.0	−2.20	2.042	5.2	1.0	44.74	0
	Donepezil	−12.95	-	-	379.4	100.0	−4.75	4.876	5.5	0.0	46.21	0
	Huperzine A	−9.280	-	-	242.3	75.35	−2.38	1.429	3.5	3.0	64.08	0
	Rivastigmine	−10.76	-	-	250.3	100.0	−2.25	2.479	5.0	0.0	40.78	0
	Tacrine	−10.07	-	-	198.2	100.0	−3.07	2.569	2.0	1.5	33.88	0
1	Eugenol	−9.14	−1.81	PHE 288, ARG 289, PHE 331	164.20	100	−2.436	2.664	1.5	1	29.884	0
2	4H-pyran-4-one	−8.79	−2.77	PHE 288, ARG 289	144.12	71.24	−0.707	−0.558	5.2	2	79.356	0
3	5-Methoxy pyrrolidin-2-one	−7.20	−1.18	PHE 288, ARG 289	115.132	74.452	0.939	−0.653	4.2	1	51.554	0
4	15-Hydroxy pentadecanoic acid	−6.69	−0.93	ARG 289, PHE 288, TYR 130, GLU 199	258.4	81.927	−4.278	3.751	3.7	2	73.706	0
5	2-hydroxy-3-[9E]	−1.53	−0.49	TYR 70	620.99	100	−15.845	12.327	5.7	1	94.784	2
6	Glycidol stearate	−8.02	−0.44	SER 122	340.545	100	−6.904	6.312	4	0	48.92	1
7	n-Hexadecanoic acid	−4.6	−0.7	PHE 288, ARG 289	256.428	87.572	−5.609	5.292	2	1	50.59	1
8	Tetradecanoic acid	−4.95	−0.74	PHE 288, ARG 289	228.374	95.983	−4.716	4.515	2	1	50.59	0

FDA: Food and Drug Administration, HS: *Hyptis suaveolens*, GC-MS: Gas chromatography-mass spectrometry, HB: Hydrogen bond, PSA: Prostate-specific antigen, Mol. Wt.: Molecular weight of the compound, % oral absorp: Predicted percentage of human oral absorption, QP LogS: Qikprop predicted aqueous solubility, QP logPo/w: Qikprop predicted octanol water partition coefficient, HB accp: Hydrogen bond acceptors, HB donor: Hydrogen bond donors

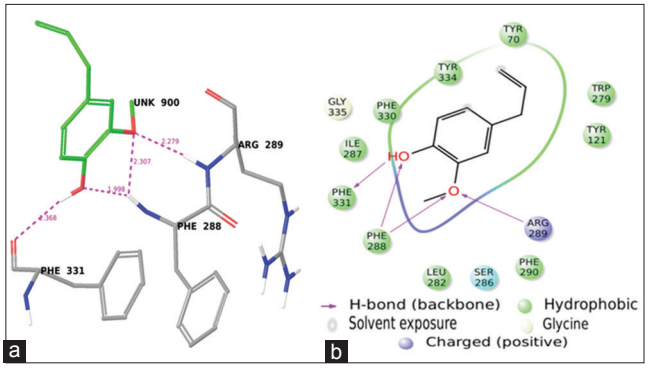

Fig. 3: (a) 1EVE protein prepared for docking, (b) interactions of protein with Euginol (PHE288, ARG289 and PHE331 are residues involving)

Eugenol ($C_{10}H_{12}O_2$) is a phenylpropanoid, having an allyl chain-substituted guaiacol (weakly acidic), soluble in organic solvent and water solubility is low [27]. Eugenol has been identified in several aromatic plants such as *Myristica fragrans* Houtt. (Nutmeg), *Cinnamomum verum* J.Presl. (True Cinnamon), *Cinnamomum loureirii* Nees. (Saigon Cinnamon), *Ocimum gratissimum* Forssk. (Basil) and *Ocimum basilicum* L. (Sweet Basil) [28]. However, *Eugenia caryophyllata* is considered as the principal natural source of this compound as it represents 45-90% of the total oil [29,30].

Several *in vitro* and *in vivo* studies have been conducted to determine the pharmacological properties such as antimicrobial, anti-inflammatory, analgesic, antioxidant, and anticancer activity. Present work supports, the role of euginol as AChE inhibitor and further its effect on the CNS encompassing seizure control and anti-depressant effects can be further explored *in vivo*.

The HS methanolic extract showed IC_{50} value 1.02±0.026 mg/ml. The maximum percentage inhibition of the enzyme was found to be 75±4.30 (2.1 mg/ml). Among the Lamiaceae members, *Salvia officinalis* (ethanolic extract), *Salvia aucheri, Salvia ceratophylla, Salvia migrostegia, Salvia syriaca,* and *Organum vulgare L.* methanolic extracts showed 68.20±15.60 (2.5 mg/ml), 39.90±1.17 (1 mg/ml), 27.80±2.82 (1 mg/ml), 23.60±0.61 (1 mg/ml), 12.10±1.22 and 3.00±0.06 (0.1 mg/ml) AChE inhibition, respectively [31,32]. HS showed potent IC_{50} value when compared with other members of the family. The plant extracts having 60% or more AChE inhibition are considered to be significantly strong inhibitors [33] and hence further purification of phytochemical constituents of methanolic extract was considered.

CONCLUSION

Neurotransmitter carry-over in dementia people can be possible by AChE inhibition. The current study aimed at screening and purification of natural AChE inhibitors. Among the selected plants sources, HS had greater inhibitory property. Through GC-MS and *in silico* analysis, Eugenol is identified as a positive inhibitor against AChE. HS phytochemicals acting as AChEI is reported and purification of such diverse plant phytochemicals with salt (ammonium sulfate) precipitation followed by gel filtration is novel and could be used in large scale process development for the purification of phytochemicals and augments the search for the better drug to treat AD.

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REFERENCES

1. Johnson N, Davis T, Bosanquet N. The epidemic of Alzheimer's disease. How can we manage the costs? Pharmacoeconomics 2000;18(3):215-23.
2. Wimo A, Winblad B, Jönsson L. The worldwide societal costs of dementia: Estimates for 2009. Alzheimers Dement 2010;6(2):98-103.
3. The WHO Report Dementia. Available from: http://www.who.int/en. [Last accessed on 2015 Mar].
4. Krall WJ, Sramek JJ, Cutler NR. Cholinesterase inhibitors: A therapeutic strategy for Alzheimer disease. Ann Pharmacother 1999;33(4):441-50.
5. Giacobini E. Cholinergic foundations of Alzheimer's disease therapy. J Physiol Paris 1998;92(3-4):283-7.
6. Easwaramoorthy B, Pichika R, Collins D, Potkin SG, Leslie FM, Mukherjee J. Effect of acetylcholinesterase inhibitors on the binding of nicotinic alpha-4 beta-2 receptor PET radiotracer, (18) F-nifene: A measure of acetylcholine competition. Synapse 2007;61(1):29-36.
7. Qizilbash N, Whitehead A, Higgins J, Wilcock G, Schneider L, Farlow M. Cholinesterase inhibition for Alzheimer disease: A meta-analysis of the tacrine trials. Dementia trialists' collaboration. JAMA 1998;280(20):1777-82.
8. Arrieta JL, Artalejo FR. Methodology, results and quality of clinical trials of tacrine in the treatment of Alzheimer's disease: A systematic review of the literature. Age Ageing 1998;27(2):161-79.
9. Jones RW, Soininen H, Hager K, Aarsland D, Passmore P, Murthy A, *et al.* A multinational, randomised, 12-week study comparing the effects of donepezil and galantamine in patients with mild to moderate Alzheimer's disease. Int J Geriatr Psychiatry 2004;19(1):58-67.
10. Polinsky RJ. Clinical pharmacology of rivastigmine: A new-generation acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. Clin Ther 1998;20(4):634-47.
11. Castro DM, Dillon C, Machnicki G, Allegri RF. The economic cost of Alzheimer's disease. Dement Neuropsychol 2010;4 Suppl 4:262-7.
12. Sofowora A. Research on medicinal plants and traditional medicine in Africa. J Altern Complement Med 1996;2(3):365-72.
13. Robbers J, Speedie M, Tyler V. Pharmacognosy and Pharmacobiotechnology. Baltimore, USA: Williams and Wilkins; 1996.
14. Kingston DG, Rao MM, Zucker WV. Plant anticancer agents. IX. Constituents of *Hyptis tomentosa*. J Nat Prod 1979;42(5):496-9.
15. Edeoga H, Omosun G, Uche L. Chemical composition of *Hyptis suaveolens* and *Ocimum gratissimum* hybrids from Nigeria. Afr J Biotechnol 2006;5 Suppl 10:892-5.
16. Das PK, Sahoo S, Sethi R, Nayak PS, Nayak S, Joshi A. Phytochemical and pharmacological investigation of the protective effect of plant *Hyptis suaveolens* against duodenal ulceration. J Glob Pharm Technol 2009;1 Suppl 1:99-103.
17. Shirwaikar A, Shenoy R, Udupa AL, Udupa SL, Shetty S. Wound healing property of ethanolic extract of leaves of *Hyptis suaveolens* with supportive role of antioxidant enzymes. Indian J Exp Biol 2003;41(3):238-41.
18. Nantitanon W, Chowwanapoonpohn S, Okonogi S. Antioxidant and antimicrobial activities of *Hyptis suaveolens* essential oil. Sci Pharm 2007;75 Suppl 1:35.
19. Ren Y, Houghton PJ, Hider RC, Howes MJ. Novel diterpenoid acetylcholinesterase inhibitors from *Salvia miltiorhiza*. Plant Med 2004;70 Suppl 3:201-4.
20. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-95.
21. Parekh J, Chanda SV. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol 2007;31 Suppl 1:53-8.
22. Raghavendra M, Satish S, Raveesha K. Phytochemical analysis and antibacterial activity of *Oxalis corniculata*; a known medicinal plant. Science 2006;1 Suppl 1:72-8.
23. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. New Delhi: Springer; 1998.
24. Mukherjee PK, Kumar V, Mal M, Houghton PJ. Acetylcholinesterase inhibitors from plants. Phytomedicine 2007;14(4):289-300.
25. Orhan I, Terzioglu S, Sener B. Alpha-onocerin: An acetylcholinesterase inhibitor from *Lycopodium clavatum*. Planta Med 2003;69(3):265-7.
26. Sener B, Orhan I. Discovery of drug candidates from some Turkish plants and conservation of biodiversity. Pure Appl Chem 2005;77(1):53-64.
27. Barceloux DG. Medical Toxicology of Natural Substances: Foods, Fungi, Medicinal Herbs, Plants and Venomous Animals. Hoboken, NJ, USA: Wiley; 2008.

28. Kamatou GP, Vermaak I, Viljoen AM. Eugenol – From the remote Maluku Islands to the international market place: A review of a remarkable and versatile molecule. Molecules 2012;17(6):6953-81.
29. Wright DA, Payne JP. A clinical study of intravenous anaesthesia with a eugenol derivative, G.29.505. Br J Anaesth 1962;34:379-85.
30. Zheng GQ, Kenney PM, Lam LK. Sesquiterpenes from clove (*Eugenia caryophyllata*) as potential anticarcinogenic agents. J Nat Prod 1992;55(7):999-1003.
31. Orhan I, Sener B, Choudhary MI, Khalid A. Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants. J Ethnopharmacol 2004;91(1):57-60.
32. Adsersen A, Gauguin B, Gudiksen L, Jäger AK. Screening of plants used in Danish folk medicine to treat memory dysfunction for acetylcholinesterase inhibitory activity. J Ethnopharmacol 2006;104(3):418-22.
33. Khan RA, Bukhari IA, Nawaz SA, Choudhary MI. Acetylcholinesterase and butyrylcholinesterase inhibitory potential of some Pakistani medicinal plants. J Basic Appl Sci 2006;2:7-10.