WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 6, 874-882 Research Article ISSN 2278 – 4357

IN-VITRO AND IN-SILICO SCREENING OF ACETYLCHOLINESTERASE INHIBITOR EXTRACTED FROM *EPIPREMNUM AUREUM*; A SOLUTION FOR ALZHEIMER'S

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Article Received on
24 March 2017,

Revised on 13 April 2017,
Accepted on 03 May 2017

DOI: 10.20959/wjpps20176-9233

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ABSTRACT

Acetyl cholinesterase enzyme metabolise the transmission of action potential across the neuromuscular and catabolised the hydrolytic destruction of the cationic neurotransmitter acetylcholine. As acetyl cholinesterase (AChE) inhibitors play a fundamental role in therapeutic alleviate in Alzheimer's disease .The naturally occurring organic compounds from plants are considered to be plenty source of new inhibitors has lead to the discovery of new drugs. In this paper we have isolated the inhibitors from *Epipremnum aureum*. This compound were documented by using GCMS, and simultaneously docking studies were done. We have analysis the efficacy of plant extract in both in-vitro and in-vivo condition by using zebrafish as a model organism. The plant extract has revealed many inhibitory activities in both in-vitro and in-vivo condition. With further research this compound may be used for the treatment of Alzheimer's

KEYWORDS: Acetylcholine, acetyl cholinesterase, GCMS.

INTRODUCTON

Acetyl cholinesterase is a primary cholinesterase which catalyzes the breakdown of acetylcholine. It is involved in the annihilation of impulse transmission by rapid hydrolysis of the neurotransmitter acetylcholine in numerous cholinergic pathways in the both central and peripheral nervous systems.[1] This enzyme is found mainly at neuromuscular junctions and in the chemical synapses. Inhibition of acetyl cholinesterase (AChE) leads to the

accumulation of acetylcholine in the synaptic cleft which results in impeded neurotransmission.

Alzheimer's is an unceasing neurodegenerative disorder which is progressive in nature as well.[2] This provides the major cholinergic innervations to the neocortex, hippocampus and amygdale.[3] It is characterized by memory loss and cerebral abilities grave enough to disturb daily life. Cognitive impairments in humans have been linked to dysfunction of neurons in the basal forebrain cholinergic system (BFCS). Degeneration of these cells may be, in part, responsible for Alzheimer's disease (AD).[4] During the course of disease, proteins construct in the brain to form structures called ''plaques'' and ''tangles''. These structures leads to the loss of nerve cells connections, eventually leading to the loss of brain tissue. Donepezil, rivastigmine or galantamineare some of the drugs that can temporarily assuage some symptoms or slow down their development.[5]

Fig 1: Mode of Enzyme Action.

Loss in acetyl cholinesterase (AChE) from cholinergic and no cholinergic neurons leads to Alzheimer's. Cholinergic projection from the nucleus *basalis* of meynert (in the basal forebrain) to the forebrain neocortex and limbic structures. Deterioration of this pathway is associated with alzheimer's disease.[6] Increase in the acetyl cholinesterase (AChE) activity maybe implication for therapeutic strategies using AChE inhibitors. The expression of AChE is inhibited by beta protein(A beta) which is a major component of amyloid plaques. Deposition of these beta proteins in the brain is dependable for Alzheimer's pathology.[8] An acetylcholinesterase inhibitor is a chemical that inhibits the acetylcholinesterase enzyme from breaking down into acetylcholine. This inhibitor is used to treat Alzheimer's. A potential source of AChE inhibitors is certainly provided by the plenty of plants in nature.[7] It is organic in nature. Methods of extraction are simple and the plant source has no side effects. Zebra fish is used in the experiment. It is an imperative model organism for human neurobehavioral studies and compound screening for neurodegenerative disorders like Alzheimer's disease and dementia.[8] As they are vertebrates they are genetically similar to

humans. Therefore, making it easier in giving a close view into human diseases.[9] The inhibitory activity of ACh was analyzed or determined in Zebrafish.[10] Impact of drugs used on them is easy to observe. We have observe that a mutation in the ache gene of the zebrafish, which eliminate ACh hydrolysis in homozygous animals entirely.[7]

MATERIALS AND METHODS

Procurement of Plant Materials

The plant material ***Epipremnum aureum*** was obtained from Vidyaranyapura of Bangalore district.

Procurement of Zebrafish (Daniorerio): Zebrafish were purchased from A-z Aquarium in kumaraswamy Layout , Bangalore 560078 . The fish were quarantined and stored in a 20L aquarium and fish were fed with shrimp flakes and commercially available feed once every 36 hours.

Purchase of chemicals: Acetylcholine chloride was purchased from Loba chemical.

Isolation of Acetylcholinesterase (AchE): The brain of healthy zebrafish were dissected and homogenized with 3ml phosphate buffer (PB) (0.0M,pH 7.2) using a pre-chilled mortar and pestle. The homogenate was diluted with 20 ml cold PB and then centrifuged at 5000rpm for 15 minutes at 4c.

The supernatant was collected, which serves as the source of the enzyme. The enzyme source was diluted at 2x concentration.

Assay of Acetylcholinesterase: In this assay, to ml of the enzyme, ml of acetylcholine (100nm) was added and incubated for 5 minutes at room temperature and then the enzyme was deactivated by placing in a boiling water bath for 3 – 5 minutes. The terminated mixture was then titrated against NaOH (0.05) with phenolphthalein as the indicator till a pale pink colour obtained. The titre value was noted and the enzyme activity was calculated.

Plant extracts preparation: The leaves ***Epipremnum aureum*** were dried and washed. Methanolic extract of the plant was prepared using soxhlet apparatus.

Screening of Acetylcholinesterase inhibitors

In-vitro screening: Different volumes of the plant extracted were added to 1ml of the enzyme. To this, 0.5ml of acetylcholine was added and incubated for 10 minutes at room temperature. The reaction mixture was then deactivated by keeping in a boiling water bath for 3-5 minutes, cooled, and then titrated with 0.05M NaOH using phenolphthalein as the indicator, and from the tire values obtained, the enzyme activity was calculated. The rate of inhibition was calculated using control.

In-vivo screening: 0.1 ml of the ethanolic extract was added into 1 liter of de-chlorinated water having healthy Zebrafish. The fishes were monitored for 2 days and then the assay was carried out as mentioned above and the enzyme activity was calculated. The extent of inhibition was found in relation to control group.

GC-MS analysis

The GC-MS analysis of ethanol extract *Epipremnum aureum* carried out to determine the compounds present in it.

The GC-MS was run with a column oven temperature of 60^0C and injection temperature of 250^0C with split mode of injection and liner velocity flow control. The pressure applied for GC is 57.4kpa which gives the column flow of 1.00ml/min and linear velocity of 36.5 cm/Sec, with a purge flow of 3.0ml/min and split ratio is 10.0. The ion source temperature was set at 200^0C and the interface temperature is 300^0C, with 2.00min solvent cut time. The mass spectra taken with intervals of 0.50Sec, with scan range of 40- 600 m/z with a scan speed of 1250. The total time has taken is 34.00 min and FTD detector is used for detection.

DOCKING STUDIES

The structures of the compounds obtained from the ethanol *Epipremnum aureum* extract was drawn by using Marvin Sketch and are converted to pdb file. These were subjected to docking studies with the crystal ACE enzyme (PDB108A). The complete docking studies were done by using Auto Dock Vina software.

RESULT AND DISCUSSION

In-vitro assessment of AChE acitivity

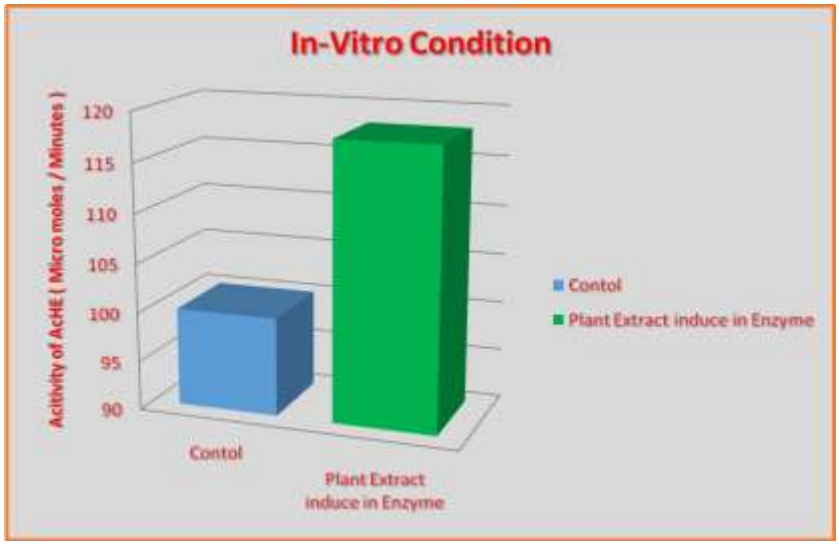

FIG 2: In-Vitro Condition.

In-vivo assessment of AcHE activity

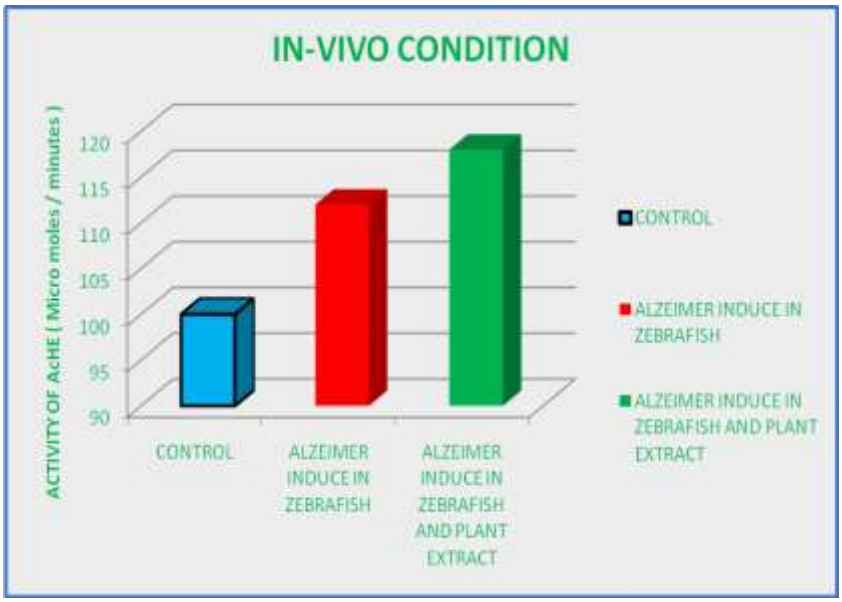

Fig 3: In-Vivo Condition.

GC-MC analysis

The ethanol extract of ***Epipremnum aureum*** was subjected to GC-MC analysis and it show 28 different compounds. The complete details of GC-MS chromatogram was shown in figure 4. The list of 28 compounds along with the retention time and the area was shown in the table 1.

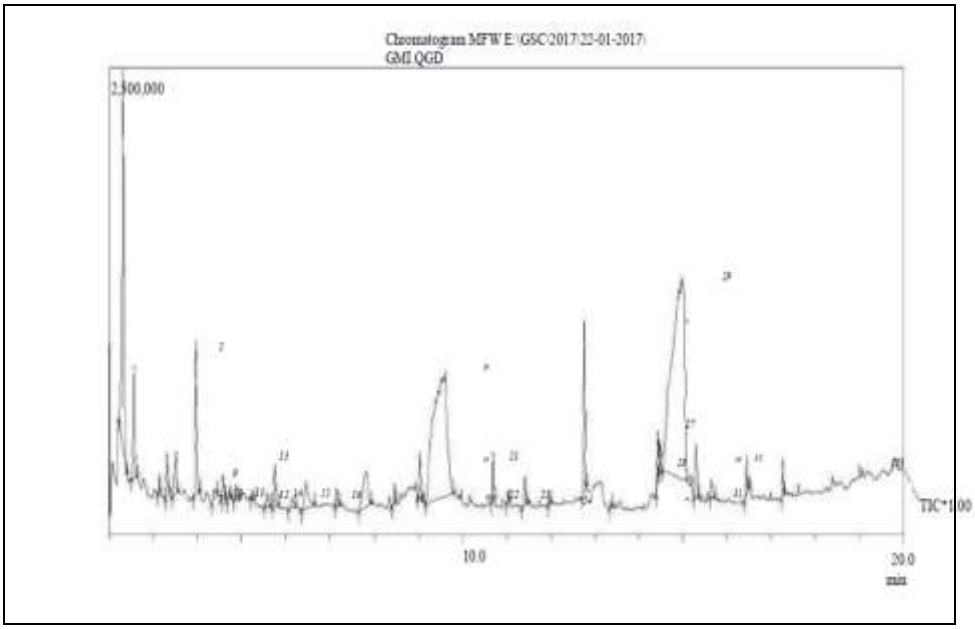

Fig 4: The GC-MS chromatogram showing peaks at different time intervals.

TABLE 1: List of compounds identified in ethanol of *Epipremnum aureum* during GC-MS analysis.

PEAK	R.TIME	I.TIME	F.TIME	AREA	AREA %	NAME
1	2.302	2.208	2.408	5814446	9.89	6-Ethoxy-9H-PURINE
2	2.555	2.492	2.633	1904590	3.24	N-Phenylaniline
3	3.127	3.092	3.167	210048	0.36	Viridiflorol
4	3.299	3.258	3.367	660083	1.12	Viridiflorol
5	3.499	3.425	3.558	759895	1.29	3,4Dichloro(1,6)Naphthyridine
6	3.96	3.892	4.042	1987461	3.38	3,4Dichloro(1,6)Naphthyridine
7	4.378	4.317	4.45	199306	0.34	6-(dimethylnitroryl)-4-4diphenyl-3-heptanone
8	4.569	4.525	4.642	339022	0.58	Nicotine
9	4.709	4.65	4.767	181179	0.31	Anabasine
10	4.873	4.817	4.933	229537	0.39	Catharanthine
11	5.237	5.208	5.283	79512	0.14	Salinorin A
12	5.575	5.517	5.642	206318	0.35	Salinorin A
13	6.137	5.7	5.825	531723	0.9	Octadecanamide
14	6.445	6.05	6.217	194795	0.33	Dihydro-oxodemethoxy haemanthamine
15	7.151	6.35	6.642	954174	1.62	Oxoassoanine
16	7.812	7.117	7.217	207501	0.35	α-Bergamotol
17	0.458	7.642	7.933	1554287	2.64	Delta.-Cadinene
18	9.04	8.4	8.492	185226	0.32	CIS-.alpha.-bisabolene
19	9.614	8.983	9.1	445091	0.76	Nerolidol B (CIS OR TRANS
20	10.695	9.167	9.808	79207	0.13	(-)-Caryophyllene oxide
21	11.036	10.658	10.733	1754365	2.98	Trans-Caryophyllene
22	11.036	10.992	11.067	85250	0.15	Beta.-Bisabolol

23	11.424	11.35	11.475	526340	0.9	Alpha.-bisabolol
24	11.972	11.942	12.008	174861	0.3	2-Methyl-6-(trimethylsilyl)benzophe
25	12.766	12.708	12.817	21364354	36.35	1-NAPHTHALENECARBONITRILE, 8 -AMINO
26	13.36	13.325	13.4	973529	1.66	Octadecadienoic acid, methyl ester, (E,E)-
27	14.428	14.383	14.45	294391	0.5	Acetyl-3,3-epoxymethano-6,6,7-t
28	14.466	14.45	14.483	585980	1	1,2-Benzenedicarboxylic acid, dibut

Docking studies

The 28 compounds obtained from GC-MS shows different binding affinity towards ACE. Among these Viridiflorol (-7.8kcal/mol) shows maximum affinity towards the ACE. The structure of Viridiflorol was shown in the figure 5 . The binding of viridiflorol to ACE table was shown in the table 2.

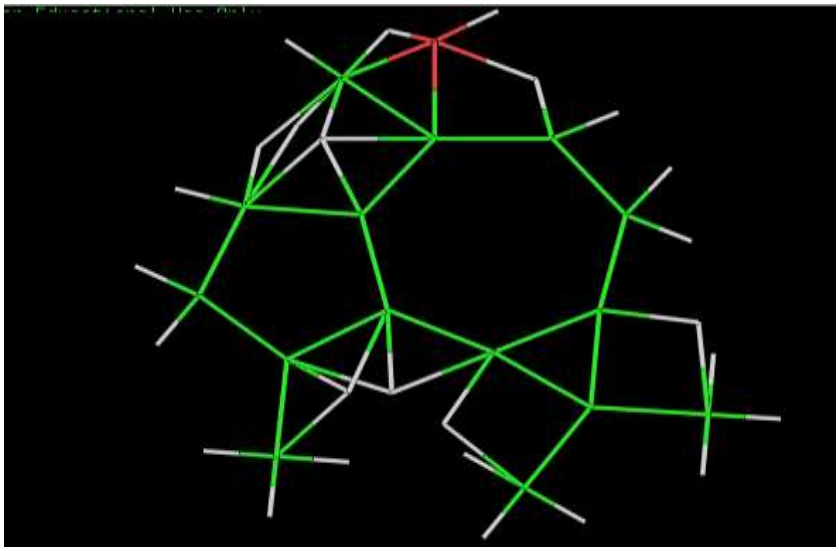

Fig 5 – 2D structure of Viridiflorol.

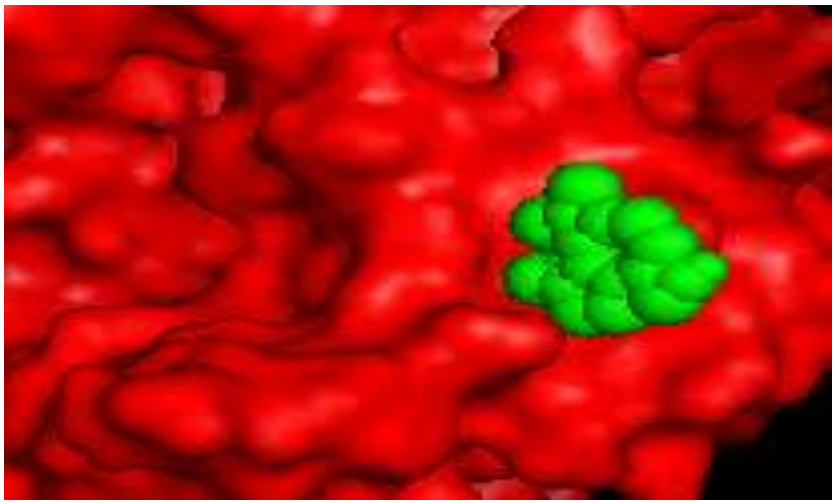

Fig 6: Docking of crystal ACE protein with Viridiflorol ligand.

Table 2: Represents the docking results of Viridiflorol to ACE.

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.9	0.000	0.000
2	-6.0	27.340	28.400
3	-5.7	31.277	33.989
4	-5.7	31.874	34.224
5	-5.6	31.565	34.106
6	-5.6	31.437	33.644
7	-5.4	31.850	34.372
8	-5.3	11.226	13.155
9	-5.2	28.356	30.593

Writing output ... done.

CONCLUSION

The methanolic extracts from leaves of *Epipremnum aureum* were checked for Acetylcholinesterase inhibition on the acetylcholinesterase from brain homogenate of Danio rerio.The plant extract showed the inhibition of AChE whose activity is elevated during disorders like Alzheimer's disease, Parkinson's disease, and Huntington's disease. *Epipremnum aureum* is therefore, a potential source of AChE inhibitors that can be used for the treatment of neurodegenerative disorders. Further studies are required to identify the specific compounds/molecules that act as acetylcholine esterase inhibitors.

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