

## ISOLATION, CHARACTERIZATION AND INSILICO PHARMACOLOGICAL SCREENING OF MEDICINALLY IMPORTANT BIO-ACTIVE PHYTOCONSTITUENTS FROM THE LEAVES OF *IPOMOEA AQUATICA* FORSK.

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Received: 11 Jan 2014, Revised and Accepted: 26 Feb 2014

### ABSTRACT

**Objective:** Alzheimer's disease (AD) is now considered as a leading cause of dementia in developed and also in developing countries, and is the leading socioeconomic problem in healthcare. The progression of Alzheimer's disease will ultimately lead to dementia, behavioral and cognitive impairments. *Ipomoea aquatica* Forsk (IA) belongs to the family Convolvulaceae is considered to be a potential Indian medicinal herbal source for the supply of biologically and pharmacologically active phytoconstituents for the treatment of AD but only very few scientific studies have been conducted on its clinical aspects. Objective of this current study is to isolate, identify, characterize and virtually screen the medicinally important bio-active constituents from the leaves of IA and explore its activity against AD.

**Methods:** The dried leaf of IA was extracted with hydro alcoholic solvent system using Soxhlet extractor and then re-extracted (liquid: liquid partition) with solvent such as petroleum ether, ethyl acetate and n-Butanol. The fraction was concentrated and subjected to column chromatography for further separation. The isolated compounds were subjected to IR, <sup>13</sup>C NMR, <sup>1</sup>H NMR and UV spectroscopy analysis for structural elucidation. The docking was carried out between the target enzyme acetylcholinesterase (AChE) and isolated bio active compounds by using Donepezil hydrochloride as standard drug.

**Results:** In the present study two major phytoconstituents isolated from the leaves of IA have been identified as Flavonoid derivative such as Quercetin and the phenolic acid such as chlorogenic acid and the docking result reveals that both these compounds have good affinity and binding with target enzyme AChE.

**Conclusion:** Hence it was concluded that IA with interesting biological agents and structural diversity, have often served as valuable and potential lead drug candidate for the treatment of AD by replacing the chemically synthesized drugs with known side effects.

**Keywords:** Alzheimer's disease, *Ipomoea aquatica*, Quercetin, Chlorogenic acid, Docking, Donepezil, Acetylcholinesterase.

### INTRODUCTION

Pathophysiology of the Alzheimer's disease is characterized by selective neuronal cell death and it was further observed that presence of extra cellular amyloid deposits in the core of neuritic plaques and the formation of intra neuronal neurofibrillary tangles in the brain of affected individuals. Neurobiologically, these deficits are often associated with progressive loss of cortically projecting central cholinergic neurons and there by a reduction in the level of presynaptic acetylcholine neurotransmitter, particularly in the areas of the brain related to memory and learning [1].

Acetyl choline (ACh) is considered to be one of the most important neurotransmitter in the body and the primary neurotransmitter in the brain which is responsible for cholinergic transmission. The enzyme AChE plays a key role in the hydrolysis and degradation of the neurotransmitter ACh [2]. Acetylcholinesterase, and in particular the G4 form, which is majorly responsible for terminating the pharmacological action of acetylcholine at cholinergic synapses. The reduction of cholinergic activity in the brain of AD patients correlates with their deterioration scores on dementia rating scales [3].

Search of potential leads from medicinal plant with biologically active constituents at CNS level have been emerged from *Rawolfia serpentina*, *Mucuna pruriens* for Parkinson's disease, *Ocimum sanctum* as an anti-stress agent, *Withania somnifera* as an anti-anxiety agent, *Centella asiatica* and *Bacopa monneria* for memory and learning disorders. *Bacopa monneria* and *Ginkgo biloba* for Alzheimer's disease. The study related to Alzheimer's disease (A.D) is focused towards the traditional use of neurotropic and rejuvenating agents [4]. The recent trends in the neuropharmacological as well as reverse pharmacology research focused towards the molecular and biochemical mechanism which leads to the development of CNS active principles from the herbal source.

Voluminous species belongs to the genus *Ipomoea* (Convolvulaceae) are used in traditional system of medicine all over the world. *Ipomoea aquatica* Forsk (IA) belongs to the family Convolvulaceae grows wild and is cultivated throughout Southeast Asia and it is a widely consumed vegetable in these regions and also considered to be as an important folk medicine for the treatment of various ailments. Only a very few scientific studies have been conducted on its medicinal and pharmacological aspects of this plant. IA widely used as a medicinal plant in the treatment of liver diseases [5], constipation [6]. IA contains several phyto constituents such as vitamins, including A, B, C, E, and "U" (S-methyl methionine) and is used to treat gastric and intestinal disorders [7].

It has been evident that acetylcholinesterase (AChE) inhibitors delay the progression of Alzheimer's disease (AD). Disruption in cognitive and mental functions associated with AD is related to the loss of cortical cholinergic neurotransmission. The earliest known AChE inhibitors namely, donepezil, physostigmine and tacrine, showed modest improvement in the cognitive function of Alzheimer's patients [8]. Donepezil hydrochloride inaugurates a new class of AChE inhibitors with longer and more selective action with manageable adverse effects. Currently, there are about 19 new Alzheimer's drugs in various phases of clinical development.

Virtual screening continues to hold greater interest in the field of in-silico modeling and computer based drug design, which screens potential leads by orienting and scoring them in the active binding site of a target protein. As a result of binding affinity of some novel ligands for its protein receptors of known structure were designed and their binding energies were calculated using the scoring functions. Docking score was used to calculate the ligand-binding energies with the relevant amino acid present on active site of the protein target. It is estimated that docking programs like Auto dock, discovery studio currently dock 70 – 80% of ligands correctly [9].

## MATERIAL AND METHODS

### Plant material.

The fresh leaves of *Ipomoea Aquatica* (IA) were collected from (Perambur region of Chennai, Tamilnadu, India). The plant was identified and authenticated by Dr. Sasikala Ethirajulu. Captain srinivasa moorthy research Foundation, Chennai, Tamil Nadu, India. The specimen voucher was deposited in the Department of Pharmacology and toxicology, C.L. Baid Metha College of Pharmacy, Chennai, Tamil nadu, India.

### Preparation of the Hydro alcoholic Extract of IAF.

The fresh leaf of IAF was collected and washed with running water. It was shade dried at room temperature and 1 kg of the dried leaf was made in to coarse powder. The powder was passed through a 60 No mesh sieve. Air dried Powdered drug was extracted with mixture of Ethanol: water (6:4) (hydro-alcoholic extract) by using soxhlet extraction. Then the extract obtained was filtered, concentrated by rotary vacuum pump to get the solid mass. The weight of extract obtained was 20.6 %.

### Extraction and isolation

The total amount of hydro alcoholic extract was filtered and concentrated in a rotary evaporator and fractioned by liquid-liquid partition with solvents of different polarities such as Petroleum ether, Ethyl acetate and n-Butanol. From this petroleum ether fraction was discarded due to high fatty content. The ethyl acetate and n-Butanol fractions were subjected to column chromatography. The ethyl acetate fraction submitted to silica

column chromatography with a ethyl acetate/methanol eluent system. The samples obtained from sub fractions which show crystallization were submitted to thin-layer chromatography in mobile for TLC confirmation (TLC mobile phase ethyl acetate-formic acid-acetic acid, water (100:11:11:27). n-Butanol fraction submitted to silica column chromatography with a methanol/toluene as eluent system The samples obtained from sub fractions were submitted to thin-layer chromatography in mobile for TLC confirmation (TLC mobile phase Chloroform-methanol-water (65:45:12)

### Software's required for docking studies

Various tools and software's are used to analyze the target protein AChE structure and to study the binding energy properties with Donepezil Hydro Chloride, Chlorogenic acid and quercetin. AChE enzyme sequence was obtained from protein data bank ([www.pdb.org/pdb/](http://www.pdb.org/pdb/)).

To get insight the intermolecular interactions, the molecular docking studies were performed for the above mentioned phytoconstituents at the active site 3D space of enzyme of interest AChE using online DOCKING SERVER tool module [10].

### Ligand preparation

The ligands such as Chlorogenic acid, Quercetin, and Donepezil were built using Chemscketch and optimized using Docking server online web tool as shown in Figure 1 and 2 for docking studies by using Geometry optimization method MMFF94 and charge calculation was carried out based on Gasteiger method at PH 7 as shown in Table 1.

Table 1: Ligand Properties

Compounds	Molar weight	H Bond Donor	H Bond Acceptor	Log P	pKa
Chlorogenic Acid	353.301	6	8	0.07	3.33
Quercetin	304.252	5	7	1.62	7.15
Donepezil	380.500	0	4	3.69	17.02

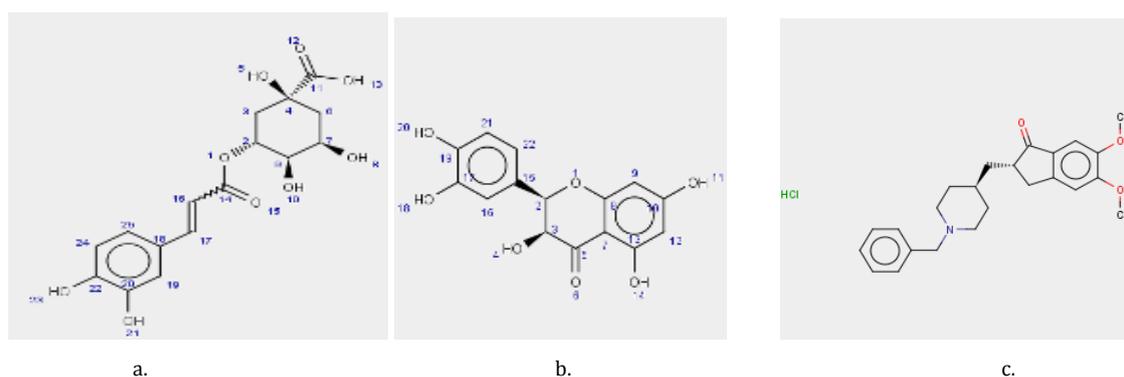


Fig.1: 2D Structure of lead A. Chlorogenic acid B. Quercetin C. Donepezil.

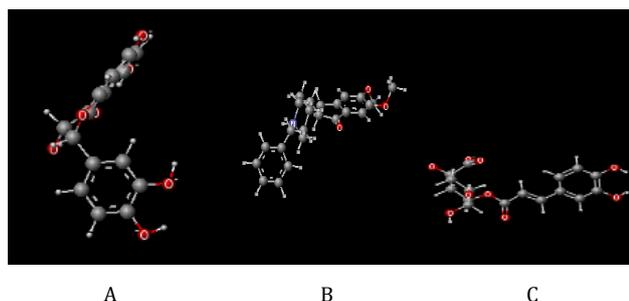


Fig. 2: Showing 3D Structure of lead A. Chlorogenic acid B. Quercetin C. Donepezil.

### Protein preparation

The target protein Human Acetylcholinesterase (PDB Code: 1B41) was retrieved from protein Data Bank ([www.rcsb.org](http://www.rcsb.org)) and crystallographic water molecules were removed from the protein. The chemistry of the protein was corrected for missing hydrogen followed by correcting the disorders of crystallographic structure by filling the valence atoms using alternate conformations and valence monitor options. Total charge on the protein was estimated as -3.847. As shown in Figure 3.

### Active Site Prediction

Active site of enzyme was obtained by LIGSITE web server by using the automatic identification of pockets on protein surface

given 3D coordinates of protein. The potential ligand binding sites in our protein using a probe of radius 5.0. Ligand site prediction was performed by using online tool called Q-Site Finder as shown in Figure 4 and Pockets calculated by GHECOM.As shown in Figure 5.

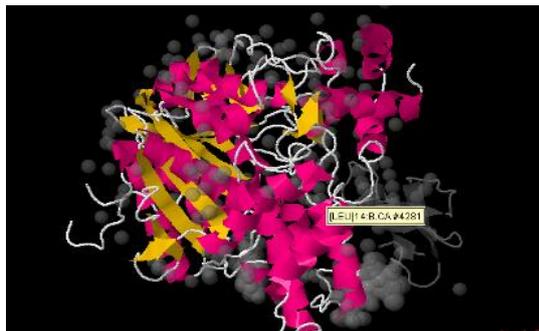


Fig. 3: Target protein Human acetyl cholinesterase 1B41

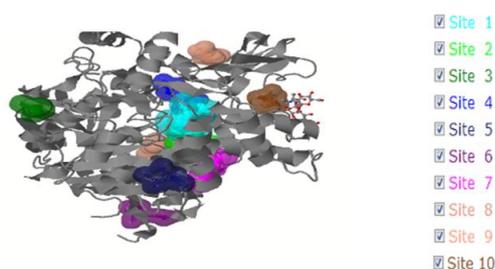


Fig. 4: Function prediction and the computed binding profile of acetylcholinesterase Showing 3D structure of the target protein with ligand pockets coloured based on the ranking performed by using online tool called Q-Site Finder.

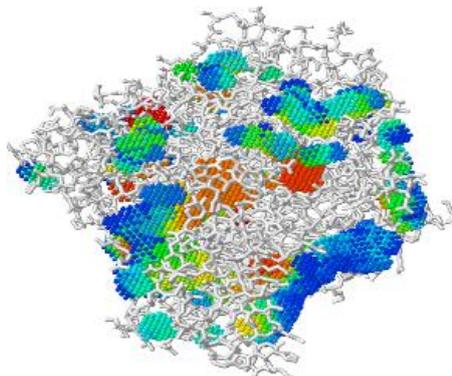


Fig. 5: Showing the possible ligand binding pockets on the surface of target enzyme Acetylcholinesterase. Pockets calculated by GHECOM

#### Docking Methodology

Docking calculations were carried out using Docking Server [11],[12]. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out based on the binding free energy on the following compounds like Chlorogenic acid, Quercetin and Donepezil and their binding affinity towards the enzyme AChE. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools [13]. Affinity (grid) maps of  $\times \times$  Å grid points and 0.375 Å spacing were generated using the Autogrid program. Auto Dock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms,

respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method [14]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

## RESULTS

### Physicochemical properties of IPAQ 1

Compound 1 IPAQ 1 weighed about 52 mg obtained as a yellow amorphous powder which was identified between sub-fractions 125-130 with the melting point  $310^{\circ}\text{C} \sim 312^{\circ}\text{C}$ . IPAQ1 dissolves freely in methanol and chloroform and deserves blue color with  $\text{FeCl}_3 \cdot \text{K}_3[\text{Fe}(\text{CN})_6]$  reagent. Isolated compound IPAQ 1 spotted on pre coated TLC plates and developed with the solvent system as mentioned earlier and then air dried and visualized under UV254 nm light which showed blue color fluorescent. Plates then placed in a chamber saturated with I2 vapors to observe the color of spots (yellow brown). Mass spectral value reveals the Mol.wt of IPAQ1 [M-H]<sup>-</sup> 302.2 as shown in Figure 6.

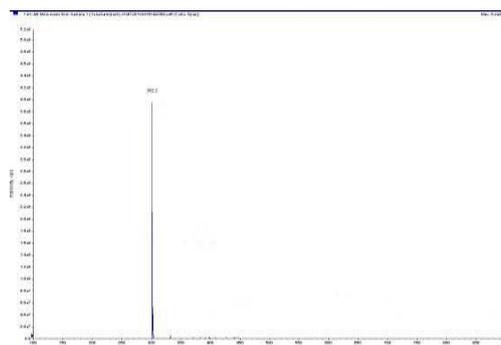


Fig.6: Mass Spectrum of IPAQ 1

### IR spectrum of IPAQ1

The IR spectrum indicated that the IPAQ 1 possessed aromatic C-H stretching  $3042\text{ cm}^{-1}$ , aromatic Hydrocarbons C-H deformations  $895\text{ cm}^{-1}$ , aromatic Hydrocarbons C=C stretching (Aromatic benzene ring)  $1484\text{--}1583\text{ cm}^{-1}$ , aromatic Hydrocarbons C-H bend (meta)  $696\text{--}788\text{ cm}^{-1}$ , aromatic Hydrocarbons C-H bend (para)  $825\text{ cm}^{-1}$ , aromatic Hydrocarbons C-H bend (ortho)  $759\text{--}788\text{ cm}^{-1}$  phenolic C-O stretching  $1043\text{--}1228\text{ cm}^{-1}$ , aromatic O-H Stretching, hydrogen bonded  $3296\text{ cm}^{-1}$ , C=O Aromatic unsaturated ketone  $1641\text{ cm}^{-1}$ , C-OH deformation vibrations  $1380\text{--}1310\text{ cm}^{-1}$ , chromen ring fused with aromatic ring  $1603\text{ cm}^{-1}$  as shown in Figure 7.

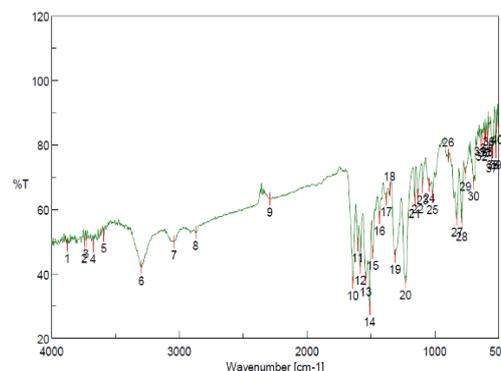


Fig.7: IR Spectrum of Compound IPAQ 1

### <sup>1</sup>H-NMR spectrum of IPAQ1

<sup>1</sup>H-NMR (400 MHz, DMSO)  $\delta$  : 6.189 d ( $J=1.2\text{ Hz}$ , H-6),  $\delta$  6.380 d ( $J=2.73\text{ Hz}$ , H-8),  $\delta$  7.704 d ( $J=4.17\text{ Hz}$ , H-2'),  $\delta$  6.864 d ( $J=1.02\text{ Hz}$ , H-5'),  $\delta$  7.693 dd ( $J=8.82\text{ Hz}$ , H-6') as shown in Figure 8.

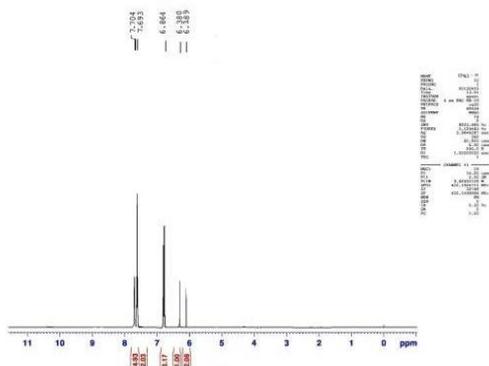


Fig. 8:  $^1\text{H}$ NMR spectra of compound IPAQ 1

#### $^{13}\text{C}$ NMR spectra (100 MHz, DMSO, $\delta$ ppm) of IPAQ1

The  $^{13}\text{C}$ NMR spectra composed of  $\delta$ : 124.33(C-1'), 113.42(C-2'), 146.70(C-3'), 147.43(C-4'), 117.19(C-5'), 120.63(C-6'), 146.70(C-2), 136.86(C-3), 178.45(C-4), 160.11(C-5), 98.48(C-6), 164.04(C-7), 98.05(C-8), 159.91(C-9), 105.63(C-10) as shown in Figure 9.

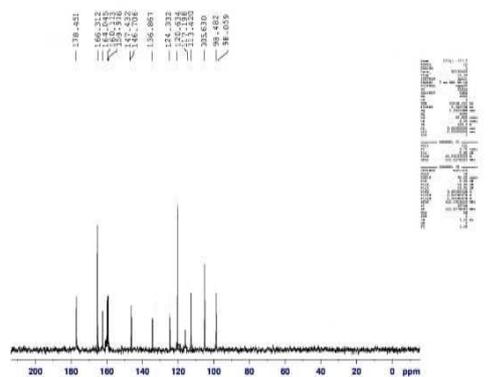


Fig. 9:  $^{13}\text{C}$ NMR spectra of compound IPAQ 1

#### Physicochemical properties IPAQ2

Compound 2 IPAQ 2 weighed about 48 mg obtained as a white amorphous powder which was identified between sub-fractions 180-190 with the melting point melting point  $207^{\circ}\text{C} \sim 209^{\circ}\text{C}$ . IPAQ2 dissolves freely in methanol. Isolated compound IPAQ 2 spotted on pre coated TLC plates and developed with the solvent system as mentioned earlier and then air dried and sprayed NP/PEG reagent and visualized under UV 365nm light which showed blue color fluorescent denotes the presence of phenolic acid. Mass spectral value reveals the mol wt of IPAQ2 [M-H]<sup>-</sup> 355.4 as shown in Figure 10.

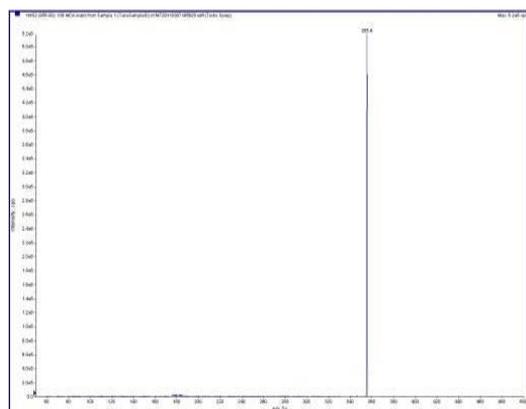


Fig. 10: Mass Spectrum of IPAQ 2

#### IR spectrum of IPAQ2

Aromatic C-H stretching  $3061\text{ cm}^{-1}$ , aromatic Hydrocarbons C-H deformations  $770$  and  $742\text{ cm}^{-1}$  aromatic Hydrocarbons C=C stretching (Aromatic benzene ring)  $1582-1459\text{ cm}^{-1}$ , aromatic Hydrocarbons C-H bend (meta)  $891\text{ cm}^{-1}$ , aromatic Hydrocarbons C-H bend (para)  $830 - 845\text{ cm}^{-1}$ , Phenolic C-O stretching  $987\text{ cm}^{-1}$ , aromatic O-H Stretching /hydrogen bonded  $3526 - 3316\text{ cm}^{-1}$ , Carboxylic acid OH stretching  $2364 - 2329\text{ cm}^{-1}$ , cyclo hexane =C-H stretching  $2956\text{ cm}^{-1}$ , cyclo hexane C-H stretching  $2924\text{ cm}^{-1}$ , cyclo hexane C=C stretching  $1635\text{ cm}^{-1}$ , cyclo hexane C-H deformation in CH<sub>2</sub>  $1442\text{ cm}^{-1}$ , C=O stretching in alkenes  $1612\text{ cm}^{-1}$ , C=C Stretching aliphatic side chain linkage  $1635\text{ cm}^{-1}$  as shown in Figure 11.

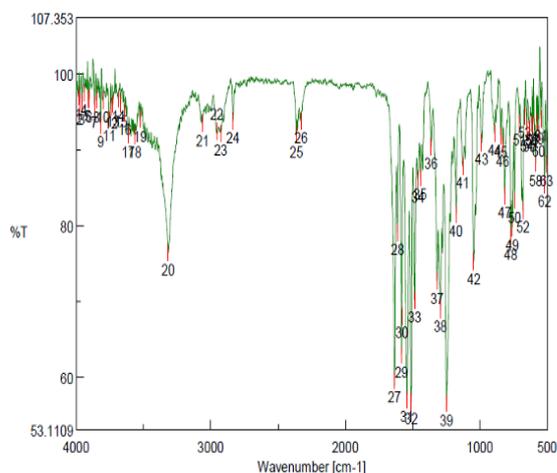


Fig. 11: IR Spectrum of Compound IPAQ 2

#### $^1\text{H}$ -NMR spectrum of IPAQ2

$^1\text{H}$ -NMR (400 MHz, DMSO)  $\delta$  : 1.998 (1H, m,  $J=12.10\text{ Hz}$ ),  $\delta$  2.052 (1H, q,  $J=11.38\text{ Hz}$ , H-6),  $\delta$  2.11 (1H, d,  $J=11.37\text{ Hz}$ ),  $\delta$  2.207 (1H, m, d,  $J=12.50\text{ Hz}$  H-2),  $\delta$  3.059 (1H, d,  $J=0.71\text{ Hz}$ ),  $\delta$  3.214 (1H, d,  $J=0.71\text{ Hz}$ , m, H-4),  $\delta$  3.617 (1H, d,  $J=0.70\text{ Hz}$ , m, H-5),  $\delta$  3.763 (br, 1H),  $\delta$  4.242 (br, 1H),  $\delta$  5.455 (1H, d,  $J=2.97\text{ Hz}$ , H-3),  $\delta$  6.201 (1H, d,  $J=15.00\text{ Hz}$ , H-2'),  $\delta$  6.752 (1H, d,  $J=2.44\text{ Hz}$ ),  $\delta$  6.935 (1H, dd,  $J=8.20, 0.99\text{ Hz}$ , H-9'),  $\delta$  7.149 (1H, d,  $J=1.04\text{ Hz}$ , H-3'),  $\delta$  7.589 (1H, d,  $J=15.87\text{ Hz}$ , H-5'),  $\delta$  8.213 (1H, d,  $J=1.60\text{ Hz}$ ),  $\delta$  9.129 (1H, s, broad,  $J=0.68\text{ Hz}$ ),  $\delta$  11.09 (1H, s, broad, COOH) as shown in Figure 12.

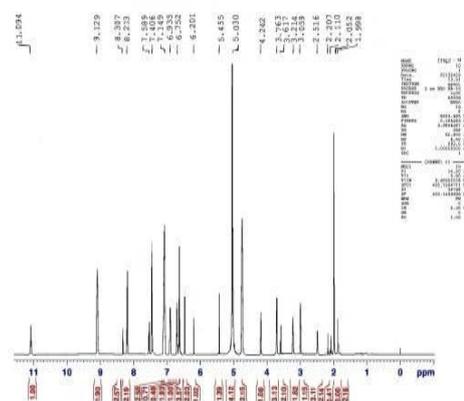


Fig. 12:  $^1\text{H}$ NMR spectra of compound IPAQ 2

#### $^{13}\text{C}$ NMR spectra (100 MHz, DMSO, $\delta$ ppm) of IPAQ2

The  $^{13}\text{C}$ NMR spectra composed of  $\delta$ : 177.32(C-1'), 115.06 C-2'), 145.48(C-3'), 126.99 (C-4'), 117.86(C-5'), 158.24(C-6'), 145.48(C-7'), 115.06 (C-8'), 125.05(C-9'), 76.51(C-1), 38.91(C-2), 76.51(C-3), 81.55 (C-4), 67.99(C-5), 41.92(C-6), 177.32(C-7) as shown in Figure 13.

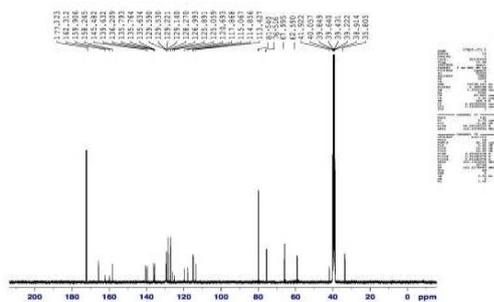


Fig. 13: <sup>13</sup>C NMR spectra of compound IPAQ 2

Table 2: Summary of the molecular docking studies of compounds against AChE

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki mM*/ $\mu$ M	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol
Chlorogenic acid	-3.49	2.79*	-0.09	-4.64
Quercetin	-3.69	1.99*	-0.18	-4.18
Donepezil	-5.15	169.18	-0.74	-6.98

The docking calculations of both the ligands at the active sites of AChE revealed that the compounds bound to the active site of enzyme with lower docking (D energy) when compared with standard donepezil. Inhibition constant is directly proportional to binding energy. Inhibition constant ranges from (169.18  $\mu$ M to 1.99 mM). Thus from the report it was clear that both the phytoconstituents having promising AChE inhibition activity when compared to standard donepezil with inhibition constant 169.18  $\mu$ M. Intermolecular energy of all three compounds ranging between -6.98 to -4.18 kcal/mol which was lesser when compared to the standard -6.98 Kcal/mol. Intermolecular energy is also directly proportional to binding energy. We found a decrease in intermolecular energy of both the selected compounds coincide with the binding energy.

#### Hydrogen bond interaction

The hydrogen bond interaction is contributed as major parameter. The Hydrogen bonding interaction of the compounds (Figure 14, 15 and 16) was analyzed for possible involvement of hydrogen bond formation with amino acid residues on receptor protein surface.

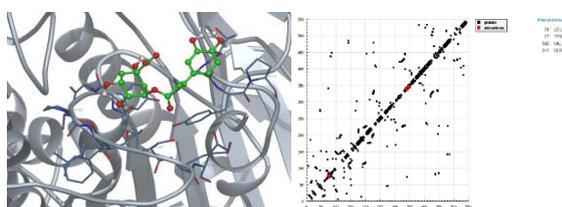


Fig. 14: Hydrogen bond interaction between AChE 1B41 with Chlorogenic acid

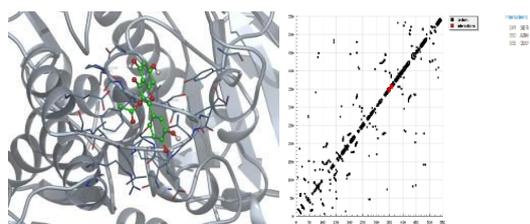


Fig. 15: Hydrogen bond interaction between AChE 1B41 with Quercetin

Chlorogenic acid involved in hydrogen bond formation with aminoacids residues on the protein like Leu 76, Tyr 77, Val 340 and Ser 347. Total interaction surface of about 463.254. Quercetin involved in hydrogen bond formation with aminoacids residues on

#### Docking scores

The Protein-Ligand interaction plays a significant role in structure based drug designing. In the present work, Human AChE was selected as a target protein and it was docked with retrieved compounds of *Ipomoea Aquatica* plant. The different score such as binding free energy, inhibition constant, intermolecular energy and electrostatic energy values represented in Table 2. The results showed that all the selected compounds showed binding energy ranging between -3.69 kcal/mol to -3.49 kcal/mol when compared with that of the standard (-5.15 kcal/mol). Electrostatic energy (-0.74 kcal/mol to -0.09 kcal/mol) of the ligands also coincide with the binding energy. Both the selected compounds contributed acetylcholinesterase inhibitory activity because of its structural parameters.

the protein like Ser 347, Asn 350 and Glu 358. Total interaction surface of about 482.748.

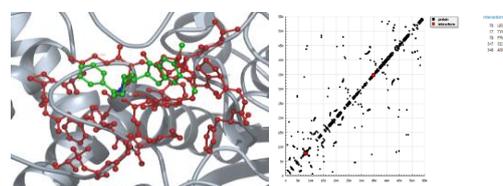


Fig. 16: Hydrogen bond interaction between AChE 1B41 with Donepezil

Donepezil involved in hydrogen bond formation with aminoacids residues on the protein like Leu 76, Tyr 77, Pro 78, Ser 347 and Asp 349. Total interaction surface of about 564.883.

The result obtained from the hydrogen bond interaction study shows that the phytoconstituents in particular chlorogenic acid possesses great AChE inhibition activity by binding with the active site pocket on target protein. Further this compound may have a direct action on target enzyme by binding to the potentially active amino acid residue in the same way as that of the standard donepezil as listed in the Table 3.

Table 3: Interaction of lead compounds with active site amino acid residue of AChE

Compounds	Target binding Amino acid residue
Chlorogenic acid	Leu 76, Tyr 77, Val 340 and Ser 347
Quercetin	Ser 347, Asn 350 and Glu 358
Donepezil (Standard)	Leu 76, Tyr 77, Pro 78, Ser 347 and Asp 349

#### DISCUSSION

The first compound isolated (IPAQ1) as a yellow amorphous powder with melting point 310<sup>o</sup> C has a molecular formula of C<sub>15</sub>H<sub>10</sub>O<sub>7</sub> based on the Mass spectrum exhibiting a molecular ion peak at m/z 302.2 and the IR spectrum exhibited a strong absorption band corresponding to OH group (3296 cm<sup>-1</sup>), Aromatic C-H stretching (3042 cm<sup>-1</sup>), Phenolic C-O stretching (1043-1228 cm<sup>-1</sup>), Aromatic Hydrocarbons C-H bend (ortho) 759-788 cm<sup>-1</sup>, C=O Aromatic unsaturated ketone 1641 cm<sup>-1</sup>.

The <sup>1</sup>H NMR spectral displayed a characteristic signals at H-6 with 6.18  $\delta$ , H-8 with 6.38  $\delta$ , H-2' with 7.70  $\delta$ , H-5' with 1.02  $\delta$ , and H-6' with 7.69  $\delta$  thus shows the presence of aromatic protons. The <sup>13</sup> C NMR indicated the presence of 15 carbons, including 5 carbon attached with hydroxyl group (C7 -164.04  $\delta$ , C5 -160.11  $\delta$ , C3 -136.86

$\delta$ , C3'-146.70  $\delta$  and C4'- 147.43  $\delta$ ), further carbon attached with oxygen (C-4 178.45  $\delta$ ).  $\lambda$  max from UV spectrum indicated the presence of conjugation and two chromophores which is a specific character of flavonoids. The data correlates the structure of the isolated compound is a flavonoid with phenyl group.

On comparison of complete spectral detail of the compound it was concluded that the proposed structure was identified as Quercetin (compound with flavonol skeleton ring). Quercetin is believed to protect against several degenerative diseases by preventing lipid peroxidation. Quercetin is considered to be a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions. These properties of quercetin allow it to inhibit lipid peroxidation [15],[16]. Quercetin, being a major constituent of the flavonoid from *Ipomoea Aquatica* plant intake, could be key in fighting several chronic degenerative diseases [17].

The second compound (IPAQ2) isolated as a white amorphous powder with melting point 207<sup>o</sup> C. has a molecular formula of C<sub>16</sub>H<sub>18</sub>O<sub>9</sub> based on the Mass spectrum exhibiting a molecular ion peak at m/z 355.4 and the IR spectrum exhibited a strong absorption band corresponding to aromatic C-H stretching (3061 cm<sup>-1</sup>), Aromatic O-H Stretching and hydrogen bonded (3526 - 3316 cm<sup>-1</sup>), Carboxylic acid OH stretching (2364 - 2329 cm<sup>-1</sup>), Cyclo hexane C- H stretching (2924cm<sup>-1</sup>) and C = O stretching in alkenes (1612 cm<sup>-1</sup>). The <sup>13</sup>C NMR indicated the presence of 16 carbons, including 5 carbon attached with hydroxyl group (C7,C8 -145.48  $\delta$ , C4' -126.99  $\delta$ , C5' -117.86  $\delta$ , and C2' -115.06  $\delta$  ), further carbon attached with carboxylic acid group (C1'-4 177.32  $\delta$ ). The <sup>1</sup>H NMR spectral displayed a characteristic signals at H-7 with 5.45  $\delta$ , H-8 with 4.24  $\delta$ , H-4' with 1.99  $\delta$ , H-5' with 2.05  $\delta$ , H-2' with 2.11  $\delta$  and H-1' with 11.09 (attached with carboxylic acid group). Protons present in the aromatic ring with signals ranges from H5 with 6.201  $\delta$ , H-9 with 6.935  $\delta$ . Thus shows the presence of aromatic protons, hydroxyl protons and proton attached with carboxylic acid group.  $\lambda$  max from UV spectrum of isolated chlorogenic acid and standard appears very similar. From the correlation of all data's it was concluded that the isolated compound shows the presence of phenolic acid group such as chlorogenic acid. The results of the present docking study clearly demonstrated the in silico molecular docking studies of donepezil and selected phytoconstituents with AChE enzyme exhibited binding interactions and warrants further studies needed for the development of potent AChE inhibitors for the treatment of neurodegenerative disorders like Alzheimers disease. These results clearly indicate that the leads especially Chlorogenic acid have similar binding sites and interactions with AChE compared to the standard drug donepezil. It was reported that chlorogenic acid possess hepato protective activity [18]. It also strongly inhibits lipid peroxidation [19]. From the literature it was also concluded that the compound chlorogenic acid has anti-hyperlipidemic [20] and anti-ulcer activities [21]. Even though the chlorogenic acid and quercetin is consider as a prominent phyto-constituents of numerous medicinal plant species, it was the first time that this potential constituents got isolated from the genus of *Ipomoea aquatica* Forsk belongs to the family Convolvulaceae. Hence from this study it was concluded the IA may serve as promising source for these noble constituent like chlorogenic acid and quercetin because of its current commercial sources from plants are very limited and much expensive. In silico virtual docking studies is actually an added advantage to screen the potential lead against AChE inhibition activity. Now a day's plant phytoconstituent derived from natural herbal source may serves as useful leads in the development of clinically useful AChE inhibitor. Further investigations on the above compounds and in vivo studies are necessary to develop potential chemical entities for the prevention and treatment of Alzheimer's disease.

#### ACKNOWLEDGEMENT

The authors are grateful to Dr. Grace Rathinam (Principal of C.L.Baid Metha College of Pharmacy, Chennai) and Dr. Venkataraman (Director, C.L.Baid Metha college, Research wing ) for their technical and secretarial assistance.

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