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# *In-vitro* Screening for acetylcholinesterase enzyme inhibition potential and antioxidant activity of extracts of *Ipomoea aquatica* Forsk: therapeutic lead for Alzheimer's disease

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**ABSTRACT**

Alzheimer's disease (AD) is a primary degenerative disease of the central nervous system. The progression of Alzheimer's disease will ultimately lead to dementia, behavioral and cognitive impairments. Increased level of the enzyme acetylcholinesterase AChE plays a key role in hydrolysis of the neurotransmitter Acetylcholine (ACh) which worsens the condition of cognitive dysfunction. Several drug of natural origin are known to possess AChE inhibition and antioxidant activity. The main objective of the present study is to evaluate AChE inhibition and antioxidant activity of the plant *Ipomoea aquatica* Forsk. Leaves of *Ipomoea aquatica* Forsk was extracted with Chloroform, n-Hexane, Ethanol and mixture of Ethanol: water (6:4) (hydro-alcoholic extract) using soxhlet extraction. All the four extracts were examined for *In-vitro* anti-cholinesterase by Ellman's method and antioxidant activity by DPPH and Hydrogen peroxide radical scavenging assay. Results obtained from the study clearly demonstrates that all four extract has shown promising acetylcholinesterase inhibition activity in hydro alcoholic extract reveals the best inhibition potential with IC50 49.03 µg /ml. Similarly all the extracts projects significant antioxidant activity in DPPH assay with IC50 value ranging from 19.64 to 88.63 µg /ml and in Hydrogen peroxide assay with IC50 value ranging from 56.79 to 137.3 µg /ml.

## INTRODUCTION

Alzheimer's disease is characterized by selective neuronal cell death, the presence of extra cellular amyloid deposits in the core of neuritic plaques and the formation of intra neuronal neurofibrillary tangles in the brain of afflicted individuals. Neuro chemically, these deficits are associated with dramatic losses of cortically projecting cholinergic neurons and by a reduction in presynaptic markers of the cholinergic system, particularly in the areas of the brain related to memory and learning (Iqbal and Grundke, 2008). Acetyl choline (ACh) is the most abundant neurotransmitter in the body and the primary neurotransmitter in the brain which is responsible for cholinergic transmission. The enzyme acetylcholinesterase (AChE) plays a key role in the hydrolysis of the neurotransmitter ACh. AChE tends to become deposited within the neurofibrillary tangles and amyloid plaques associated with Alzheimer's disease **(**Inestrosa *et al.*, 1997).

A wide range of evidence shows that AChE inhibitors can interfere with the progression of Alzheimer's disease (AD). The successful development of these compounds was based on a well-accepted theory that the decline 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 (Hachiro Sugimoto *et al*., 2000). 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.

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The leads of Central Nervous System (CNS) active medicinal plants, which have emerged besides *Rawolfia serpentina*, *Mucuna pruriens* for Parkinson's disease, *Ocimum santum* as an antistress agent, *Withania somnifera* as anxiolytic, *Centella asiatica* and *Bacopa monneria* for learning and memory disorders. *Bacopa monneria* and Ginkgo biloba for Alzheimer's disease. The study related to Alzheimer's disease (AD) is focused towards the traditionally used rejuvenating and neurotonic agents (Thimmappa *et al*., 2005). The recent trends in the pharmacological studies are based on the biochemical and molecular mechanism which leads to the development of CNS active principles from the herbal drugs.

*Ipomoea aquatica* Forsk (IAF) is a member of Convolvulaceae an Indian medicinal herb with versatile phytoconstituents used for the treatment of various disorder as a folklore medicine. Only a very few scientific studies have been conducted on its medicinal aspects. IAF used for treatment of high blood pressure (Perry, 1980), as an emetic in the treatment of opium and arsenic poisoning (Chopra *et al*., 1956). The juice of this plant has reported to be a purgative and ani helminthic activity (Datta and Banerjee, 1978). Oral hypoglycemic (Mital Manvar and Desai, 2013) and anti-oxidant activity (Haung *et al.*, 2005). Treatment on liver diseases (Badruzzaman and Husain, 1992) constipation **(**Samuelsson *et al.*, 1992). IAF is considered a tonic and it is used to treat gastric and intestinal disorders.

The aim of this study is to investigate possible AChE inhibition and Antioxidant activity of the four extracts of *Ipomoea aquatica* Forsk in order to point out the role of these plant as potential sources for the development of therapeutic agents for AD.

## 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 two botanist one Dr. Sasikala Ethirajulu. Captain srinivasa murthy research Foundation, Chennai, Tamil nadu, India and Dr. A. Aravind, Assistant Professor ,Department of Medicinal Botany ,National institute of Siddha, Chennai 600047,Tamilnadu, India. Reg no: NIS/MB/73/2012. 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 Plant Extracts

The fresh leaf of IA 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 the following solvents like Chloroform, n-Hexane, Ethanol and mixture of Ethanol: water (6:4) (hydro-alcoholic extract) by using soxhlet extraction. Then the extracts obtained such as Chloroform extract of *Ipomoea aquatica* (CEIA), n-Hexane extract of *Ipomoea aquatica* (HEIA),Ethanol extract of *Ipomoea aquatica* (EEIA) and Hydro-alcoholic extract of *Ipomoea aquatica* (HAEIA) was filtered, concentrated by rotary vacuum pump to get the solid mass.

### Assay of AChE enzyme activity by spectrophotometric method.

AChE activity was measured by using spectrophotometer based on Ellman's method (Ellman *et al*.,1961). The enzyme hydrolyses 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 412 nm. In test tube 1710 µL of 50 mM Tris–HCl buffer pH 8.0 and 250 µL of plant extracts at the concentrations of 25 – 400 µg/ mL,10 µL 6.67 $UmL^{-1}$ AChE and 20 µL of 10 mM of DTNB (5,5'-dithio-bis[2-nitrobenzoic acid]) in buffer were added. Positive control namely galanthamine were prepared in serial concentration as same as test extract by dissolving in 50 mM Tris–HCl buffer pH 8.0. The mixture was incubated for 15 min at 37$^{o}$C.Then,10 µL of acetylthiocholine iodide (200 mM) in buffer were added to the mixture and the absorbance was measured at 412 nm every 10 sec for 3 mins, for a blank with buffer instead of enzyme solution was used.

The enzyme inhibition (%) was calculated from the rate of absorbance change with time (V= Abs/Δt) the calculation as follows.

$$\text{Inhibition (\%)} = 100 - \frac{\text{Change of sample absorbance}}{\text{Change of blank absorbance}} \times 100$$

The experiment was done in triplicate and concentrations of the test extract that inhibit the hydrolysis of the substrate (acetylcholine) by 50% (IC50) were determined by linear regression analysis between the inhibition percentage versus the extract concentration by using the Excel program.

### DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay

The antioxidant activity of four different extracts of *Ipomoea aquatica* plant was determined using the 1, 1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging assay (Badami *et al*.,2005, Sharan Suresh *et al*.,2011, Renukadev *et al*.,2011) . DPPH• is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH•, reacts with an antioxidant compound that can donate hydrogen and gets reduced. The change in colour (from deep violet to light yellow) was measured. The intensity of the yellow colour depends on the amount and nature of radical scavenger present in the sample or standard compounds. The four different extracts namely Chloroform extract, n-Hexane extract, Ethanol extract and Hydro alcoholic extracts of *Ipomoea aquatica* was mixed with 95% methanol to prepare the stock solution in required concentration (10mg/100ml or 100µg/ml). From the stock solution 1ml, 2ml, 4ml, 6ml 8ml and 10ml of this solution were taken in five test tubes and by serial solution with same solvent were made the final

volume of each test tube up to 10 ml whose concentration was then10 µg/ml, 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml respectively. Ascorbic acid were used as standard was prepared in same concentration as that of the sample extract by suing methanol as solvent. Final reaction mixture containing 1 ml of 0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. Decrease in the absorbance in the presence of sample extract at different concentration of (10 µg, 20 µg, 40 µg, 60 µg, 80 µg and 100 µg/ml) was noted after 15 min incubation period at $37^0$C. Methanol (1.0 ml) plus plant extract solution (2.5 ml) was used as a blank. Decrease in absorbance is the presence of sample extract, and standard at different concentrations was noted after Absorbance was read out at 517 nm using double-beam U.V Spectrophotometer. (SHIMADZU UV-1700, UV-visible spectrophotometer).

$$\% \text{ Inhibition} = (A \text{ Blank} - A \text{ Test}) / A \text{ Blank} \times 100$$

**Hydrogen peroxide scavenging (H2O2) assay**

Human beings are exposed to H2O2 indirectly via the environment nearly about 0.28 mg/kg/day with intake mostly from leaf crops. Hydrogen peroxide may enter into the human body through inhalation of vapour or mist and through eye or skin contact. H2O2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\bullet}$) that can initiate lipid peroxidation and cause DNA damage in the body (Ruch *et al*., 1989). The ability of plant extracts to scavenge hydrogen peroxide can be estimated. A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. All the four extracts of the plant in different concentrations is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\% \text{ Scavenged } (H_2O_2) = [(A_i - A_t) / A_i] \times 100$$

where $A_i$ is the absorbance of control and $A_t$ is the absorbance of test.

## RESULT AND DISCUSSION

One of the characteristic changes that occurs in AD is increase in acetyl cholinesterase (AChE) activity, the enzyme responsible for acetylcholine hydrolysis, from both Cholinergic and non-cholinergic neurons of the brain (Curcio *et al*., 1984).

However AChE activity has been shown to be increased within and around amyloid plaques to promote the assembly of amyloid beta-peptides into fibrils and to increase the cytotoxicity of these peptides. The results obtained from the four extracts of IA against AChE enzyme inhibition activity and the percentage inhibition was evaluated and tabulated. HAEIA showed very potent inhibition (76.22 ± 1.66 %) at the concentration of 400 µg/ml when compared with galanthamine (95.53 ± 0.8094%) followed by this EEIA has showed a moderate inhibition of AChE (62.54 ± 1.44%). CEIA shown very minimal AChE inhibition activity (42.34 ± 1.712%) and HEIA extract shows mild inhibition activity (22.81 ± 3.053).As shown in Table 1.

Inhibitory concentration (IC 50) data of all the four extract reveals that strongest AChE inhibition activity were exhibited by HAEIA with (IC 50 = 49.03 µg/ml) followed by this EEIA with (IC50= 165 µg/ml) when compared to Galanthamine (IC 50= 2.727 µg/ml) as shown in Table 2. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry stable anti-oxidant drug can be able to reduce the free radical generated by this assay (Huang *et al.*,2005), so it can be useful to assess various products at a time. All the four extracts were screened for DPPH radical scavenging activity with percentage inhibition ranges from 31.28 to 83.96 % in which the highest activity was detected in Hydroalcoholic extract (IC 50 value 19.64 µg/ml) followed by ethanol and chloroform extract (IC 50 value 42.6 and 71.95 µg/ml respectively). The t-test analysis showed that there is significant difference in the DPPH radical scavenging activity among the different extract of test sample and standard ascorbic acid with (IC 50 value 7.089 µg/ml).As shown in Table 3 and 4.

Brain aerobic metabolism normally render hydrogen peroxide as a bi product. H2O2 is often considered a toxic molecule for a wide range of living systems. It has also been reported to be implicated in severe pathological conditions such as cancer, ischaemia and neurodegenerative diseases (Halliwell and Gutteridge, 1999, Halliwell *et al.*, 2000). Hence a drug which quenches the H2O2 radical may serves as better therapeutic lead for the stress related disorders. All the four extracts were screened for H2O2 radical scavenging activity in which the highest activity was detected in Hydroalcoholic extract (IC 50 value 56.79 µg/ml) followed by ethanol and chloroform extract (IC 50 value 78.85 and 102.8 µg/ml respectively). The t-test analysis showed that there is significant difference in the H2O2 radical scavenging activity among the different extract of test sample and standard Butylated hydroxyanisole (BHA) with (IC 50 value 17.67 µg/ml).As shown in Table 5 and 6.

**Table 1:** Percentage inhibition of extracts on AChE enzyme activity.

| Concentration (µg/ml) | % Inhibition of CEIA | % Inhibition of HEIA | % Inhibition of EEIA | % Inhibition of HAEIA | % Inhibition of Galanthamine |
|---|---|---|---|---|---|
| 25 µg/ml | 21.75 ± 0.88 | 3.216 ± 0.52 | 29.59 ± 4.26 | 33.71 ± 0.95 | 58.55 ± 0.57 |
| 50 µg/ml | 31.23 ± 2.08 | 5.595 ± 2.48 | 41.93 ± 5.46 | 52.61 ± 1.23 | 63.96 ± 1.91 |
| 100 µg/ml | 35.76 ± 2.27 | 13.18 ± 1.77 | 53.2 ± 3.68 | 64.71 ± 1.53 | 75.23 ± 1.95 |
| 200 µg/ml | 40.63 ± 1.14 | 17.73 ± 2.31 | 57.51 ± 1.73 | 70.56 ± 0.64 | 84.18 ± 1.57 |
| 400 µg/ml | 42.34 ± 1.71 | 22.81 ± 3.05 | 62.54 ± 1.44 | 76.22 ± 1.66 | 95.53 ± 0.80 |

Data are given as Mean ± SEM (n=3)

**Table 2:** IC50 Values for AChE inhibition by plant extracts and standard.

| Extract/Positive control | $IC_{50}$ Value AChE inhibition activity ± SEM (µg $mL^{-1}$) |
|---|---|
| HEIA | 926 ± 98.4 |
| CEIA | 507.8 ± 36.22 |
| EEIA | 165 ± 17.68 |
| HAEIA | 49.03 ± 7.06 |
| GALANTHAMINE (Standard) | 2.727 ± 0.08 |

Data are given as Mean ± SEM (n=3)

**Table 3:** Percentage inhibition of extracts on DPPH radical scavenging assay.

| Concentration (µg/ml) | % Inhibition of CEIA | % Inhibition of HEIA | % Inhibition of EEIA | % Inhibition of HAEIA | % Inhibition of Ascorbic Acid |
|---|---|---|---|---|---|
| 10 µg/ml | 34.25 ± 0.07 | 31.28 ± 0.08 | 38.91 ± 0.47 | 43.98 ±0.02 | 48.78 ± 0.26 |
| 20 µg/ml | 36.63 ± 0.11 | 33.51 ± 0.11 | 44.77 ± 0.04 | 49.79 ± 0.31 | 52.11 ± 0.08 |
| 40 µg/ml | 40.97 ± 0.10 | 40.45 ± 0.06 | 45.83 ± 0.31 | 61.42 ± 0.36 | 72.84 ± 0.71 |
| 60 µg/ml | 43.92 ± 0.08 | 43.01 ±0.02 | 58.04 ± 0.06 | 68.21 ± 0.50 | 79.76 ± 0.13 |
| 80 µg/ml | 46.28 ± 0.04 | 45.76 ±0.27 | 61.09 ± 0.13 | 80.19 ± 0.11 | 86.36 ± 0.08 |
| 100 µg/ml | 63.91 ± 0.20 | 53.97 ± 0.38 | 68.92 ± 0.22 | 83.96 ± 0.69 | 90.97 ± 0.16 |

Data are given as Mean ± SEM (n=3)

**Table 4:** IC50 Values for DPPH radical scavenging assay by plant extracts and standard.

| Extract/Standard | $IC_{50}$ Value DPPH assay ± SEM (µg /ml) |
|---|---|
| HEIA | 88.63 ± 0.45 |
| CEIA | 71.95 ± 0.35 |
| EEIA | 42.6 ± 0.40 |
| HAEIA | 19.64 ± 0.55 |
| ASCORBIC ACID (Standard) | 7.089 ± 0.36 |

Data are given as Mean ± SEM (n=3)

**Table 5:** Percentage inhibition of extracts on H2O2 radical scavenging assay.

| Concentration (µg/ml) | % Inhibition of HEIA | % Inhibition of CEIA | % Inhibition of EEIA | % Inhibition of HAEIA | % Inhibition of BHA |
|---|---|---|---|---|---|
| 10 µg/ml | 5.181 ± 1.24 | 7.075 ± 0.46 | 14.8 ± 0.40 | 23.02 ± 0.87 | 41.64 ± 0.66 |
| 20 µg/ml | 8.631 ± 0.72 | 11.35 ± 0.30 | 24.21 ± 0.13 | 29.77 ±1.66 | 52.47 ± 0.35 |
| 40 µg/ml | 12.7 ± 1.04 | 18.32 ± 0.10 | 31.26 ± 0.48 | 42.28 ± 0.91 | 64.68 ± 2.62 |
| 60 µg/ml | 22.02 ± 1.38 | 30.32 ± 0.16 | 42.7 ± 0.32 | 53.22 ± 0.45 | 76.96 ± 2.90 |
| 80 µg/ml | 27.89 ± 1.28 | 38.72 ± 1.17 | 49.93 ± 1.38 | 61.52 ± 0.27 | 83.82 ± 2.38 |
| 100 µg/ml | 38.2 ± 1.01 | 49.53 ± 0.43 | 59.48 ± 1.24 | 73.42 ± 2.23 | 92.05 ± 2.88 |

Data are given as Mean ± SEM (n=3).

**Table 6:** IC50 Values for H2O2 radical scavenging assay by plant extracts and standard.

| Extract/Standard | $IC_{50}$ Value H2O2 radical scavenging assay ± SEM (µg /ml) |
|---|---|
| HEIA | 137.3 ± 7.46 |
| CEIA | 102.8 ± 1.66 |
| EEIA | 78.85 ± 0.79 |
| HAEIA | 56.79 ± 0.88 |
| BHA (Standard) | 17.67 ± 1.70 |

Data are given as Mean ± SEM (n=3).

## CONCLUSION

From the present investigation it was concluded that all four extracts has shown promising antioxidant activity in DPPH and H2O2 radical scavenging assay in which the HAEIA shown significant percentage of inhibition in DPPH and H2O2 assay when compare to that of standard ascorbic acid and BHA. Results from AChE enzyme inhibition activity clearly indicates that HAEIA showed very potent inhibition followed by this EEIA has showed a moderate inhibition of AChE when compared to galanthamine. When compare to all the four extract the HAEIA has shown significant activity in both the AChE inhibition and anti-oxidant activity this is because of the reason that the hydro alcoholic extract of *Ipomoea aquatica* may have several active chemicals which possess potent antioxidant and also has anticholinesterase property. This indicates that his plant have wide margin of medicinal value and also has capabilities for the production of novel drugs for the treatment of Alzheimer's disease and also for other neurodegenerative disorders.

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