In Vitro Evaluation of Acetylcholinesterase Inhibitory and Neuroprotective Activity in Commiphora species: A Comparative Study

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ABSTRACT

Introduction: Herbal medicines are widely used in the therapeutic intervention that could delay the onset of Neurodegenerative diseases like, Alzheimer’s disease. Despite intensive advancements in the field of research currently available therapeutic strategies are often limited due to their adverse effects. Hence there is a need for the search for novel compounds for effective medications to treat neurocognitive deficits. Objective: The present study focused on acetylcholinesterase inhibitory and neuroprotective activity of Commiphora species for their possible use in Alzheimer’s disease. Methods: Phytochemical screening, TLC bioautographic and colourimetric assay was performed to quantify their acetylcholinesterase inhibitory activity of ethanolic extracts of the Commiphora species. The MTT assay was carried to evaluate the neuroprotective effect against the Aβ-induced cytotoxicity in SH-SY5Y cell lines. Results: The bark of Commiphora berryi, leaves of Commiphora caudata and Commiphora pubescens were found abundant in flavonoids, glycosides, steroids and terpenoids. Further, the ethanol extract of Commiphora berryi (65.48% ± 0.10) showed the highest acetylcholinesterase inhibitory activity. The Aβ25-35-induced cell damage was as evidenced at a concentration of 20μM. The neuroprotective effect of the ethanol extracts was examined in neurodegenerative cells induced by Aβ25-35. On pretreatment with plant extracts, significant improvement in cell viability was observed as 63.42 ± 2.02% (25µg/ml) for Commiphora berryi. The maximum percentage of in-vitro AChE inhibition and neuroprotective effect was effective in the ethanol bark extract of Commiphora berryi. Conclusion: The results are very rousing to continue the screening of more unexplored plant species could be used for the development of novel bioactive compounds to treat the many diseases, especially for Alzheimer’s disease.

Key words: Acetylcholinesterase, Neuroprotective, Commiphora berryi, Commiphora caudata, Commiphora pubescens, Aβ25-35, SH-SY5Y Cell line.

INTRODUCTION

Alzheimer’s disease is the most common form of dementia in older people and is characterized by progressive and irreversible neurodegeneration. Neuropathologically, Alzheimer’s disease is characterized by the deposition of amyloid-beta (Aβ) and the hyperphosphorylation of tau proteins found within brain cells. The current therapies approved by the Food and Drug Administration (FDA) can only relieve the psychological and behavioural symptoms of dementia associated with Alzheimer’s disease but do not halt the progression of the disease. The compounds approved by the FDA for the treatment of cognitive symptoms associated with Alzheimer’s disease include Memantine and Cholinesterase inhibitors such as Donepezil, Rivastigmine, and Galantamine. In the last decade, several studies have determined the effect of these agents on activities of daily living with improvement in behavioural disturbances.1 Some limitations associated with acetylcholinesterase inhibitors are related to several peripheral side effects, for example, nausea, diarrhoea, and vomiting. Several mechanisms have been suggested to explain the pathogenesis of Alzheimer’s disease, such as “Cholinergic hypothesis” and “amyloidal formation hypothesis”.2 Acetylcholinesterase (AChE) is the enzyme involved in the metabolic hydrolysis of acetylcholine at cholinergic synapses in the central and peripheral nervous systems. Amyloid plaques and neurofibrillary tangles are abnormal protein aggregates defining the pathologic feature of Alzheimer’s disease.3 AChE accelerates the assembly of amyloid β peptide (Aβ) into fibrils, a possible role of the peripheral site of the enzyme. A monoclonal antibody directed against the peripheral acidic binding site of AChE inhibits the effect of the enzyme upon the amyloid formation.4 In-vivo studies have indicated that AChE could enhance amyloid-beta aggregation and amyloid fibril formation. When AChE was infused stereotaxically into the CA1 region of the rat hippocampus, novel plaque-like structures were formed.5 Phystostigmine and donepezil have been reported to inhibit AChE induced Aβ polymerisation.6

In the present study, pharmacological screening of the plant extracts with antioxidant properties was...
undertaken to discover new sources that could possibly be used in the treatment of Alzheimer’s disease. Free radical reactions have been implicated in the pathology of a large number of disease conditions such as Cancer, Alzheimer’s disease, Diabetes, Inflammation and several cardiovascular diseases. Commiphora berryyi (Arn) Engl., Commiphora caudata (Wight & Arn) Engl. and Commiphora pubescens (Wight & Arn) Engl. are used in traditional Indian medicine to treat diverse ailments conditions. These plants belong to the family of Burseraceae. The name Commiphora is derived from the Greek words kommi (means ‘gum’) and phero (means ‘to bear’). The oleo-gum-resin obtained from these Commiphora species is known as guggul. More than 200 species of Commiphora are widely distributed in dry tropical regions of India, Africa, and Arabia. In India, guggul is found wildly in the arid and semi-arid regions of Rajasthan, Gujarat, Karnataka, and Assam and used as a source of commercial gum guggul. Numerous studies on medicinal herbs Commiphora berryyi, Commiphora caudata, and Commiphora pubescens have shown diverse pharmacological activities. However, no studies have so far been conducted to investigate the selected medicinal plants for their AChE inhibition and Neuroprotective effects of the ethanolic extracts of these plants against β-amyloid-induced cytotoxic effects in SHSY-5Y cells. The present work aimed to investigate the in-vitro effects of acetylcholinesterase inhibitory activity of these plants on the AChE enzyme and to study their neuroprotective effects on the SHSY-5Y cell lines.

MATERIALS AND METHODS

Chemicals and reagents

Acetylthiocholine iodide (A5751), acetylcholinesterase from Electrophorus electricus (Electric eel) (C3389) and eserine (E8375) were obtained from Sigma-Aldrich. Sodium chloride (5891) and magnesium chloride (4467) were purchased from Loba Chemie. 5,5'-Dithiobis (2-nitrobenzoic acid) (RM1677) and bovine serum albumin (RM3155) were procured from Himedia. 3-([4, 5-dimethlthiazol-2-yl]-2 and 5-diphenyltetrazolium bromide (T0793) were purchased from Biobasic. Ham’s F-12 medium, fetal calf serum (FCS) and other cell culture reagents were obtained from Gibco life sciences. Ethanol and all other organic solvents of analytical grade were purchased from Merck. SH-SYSY (Passage) cells were obtained from National Centre for Cell Science (NCCS), Pune, India.

Collection of plant materials

Plants were collected at the end of the flowering stage from various districts of Tamil Nadu, India. The collected plants were authenticated by the Botanical Survey of India, Coimbatore, Tamil Nadu, India. Voucher Specimens of the plants were deposited in the Department of Pharmaceutical Technology, Anna University, BIT Campus, Tiruchirappalli, Tamil Nadu, India.

Preparation of plant extracts

The plant materials were subjected to shade drying and then coarsely powdered. The coarsely powdered plant materials were extracted with 60ml of petroleum ether to remove the fatty substances and were further extracted thrice with 60ml of ethanol (99.9%v/v). The extracts were filtered, cooled, and concentrated at a reduced temperature on a rotary evaporator and then freeze-dried. The residues were re-dissolved in ethanol to the desired test concentrations.

Phytochemical analysis

The phytochemical analysis of alkaloids, flavonoids, phenolic content, saponin, quinine, sterols, cardiac glycoside, tannin, terpenoid, and reducing compounds was carried out by using the standard method.

In-vitro anticholinesterase assay

TLC assay

The acetylcholinesterase inhibitory activity of extracts was performed using Ellman’s assay, which was adopted by Rhee et al. A 2.5mm Silica gel 60 F254 plates was used as a stationary phase and chloroform: methanol (9:1 v/v) was used as the mobile phase. Plant extracts were dissolved in ethanol to a concentration of 10 mg/ml of which 3μl was spotted on the TLC plate and developed in the solvent system. After the plates were developed, enzyme inhibiting activity was detected by spraying a mixture of 1mM of acetylthiocholine iodine (ACTI) and 1mM of 5,5'-Dithiobis [2-benzoic acid] in (DTNB) in 50mM Tris-HCl buffer of pH 8. It was left to dry at room temperature for 5 min. After drying, the plates were sprayed with 3 U/ml acetylcholinesterase enzyme solution. The acetylcholinesterase inhibiting white spots were observed in the yellow background within 15 min after spraying. Such detected spots were recognized due to the hydrolysis of the substrate ACTI by acetylcholinesterase enzyme which leads to the production of thiocholine. The product thiocholine reacts with DTNB to produce 5-thio-2- nitrobenzoate and 2-nitrobenzoate-5-mercaptothiocholine.

Microplate assay

Quantitative evaluation of acetylcholinesterase inhibition by microplate assay

Percentage inhibition was quantified by measuring the amount of thiocholine produced during the hydrolyzation of acetylthiocholine. It was determined by the continuous reaction of thiol with DTNB. Inhibition of acetylcholinesterase enzyme activity was determined using Ellman’s colourimetric method as modified by Rhee et al. Buffer A (Tris/HCl 50mM, pH 8), buffer B (50 mM, pH 8, containing 0.1% bovine serum albumin) and buffer C (50 mM Tris–HCl, pH 8, containing 0.1 M NaCl and 0.02 M MgCl2,6H2O) solution was prepared. In the 96-well plate 25 μl of 15 mM ATC in water, 125 μl of 3 mM DTNB in Buffer C, 50 μl of buffer B and 25 μl of plant extract (0.5, 1.0, 1.5, 2, 2.5 and 3mg/ml) dissolved in ethanol and diluted in buffer A were added. Phystostigmine was used as the standard at concentrations ranging from 0.005 to 0.100 mg/ml. Absorbance was measured spectrophotometrically at 405 nm every 45s, three times consecutively. After that, AChE (0.22 U/ml) was added to the wells, and the absorbance was measured five times consecutively every 45s. Any increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the absorbance before adding the enzyme from the absorbance after adding the enzyme. The percentage of acetylcholinesterase inhibition was calculated by comparing the reaction rates of samples to the negative control (10% ethanol in solution Tris/HCl 50 mM, pH 8, considering 100% as the total activity of acetylcholinesterase. The percentage inhibition was determined using the following equation:

Percentage Inhibition = (1 – A Sample) / A Control) × 100

(where, A Sample - Absorbance of the sample extracts. A Control - Absorbance of the blank [methanol in Buffer A (50 mM Tris–HCl, pH 8)], A Sample = (Absorbance after AChE addition – Absorbance before AChE addition). A BioTek ELISA reader was used to determine the absorbance at the time of reaction. This analysis was carried out in triplicate (n = 6) to calculate the mean and standard deviation. Extract concentration providing 50% inhibition (IC50) was obtained by plotting the percentage inhibition against extract concentration. The IC50 values of the AChE inhibition were calculated using Graph Pad Prism software.

Cell culture

The human neuroblastoma cell line SH-SYSY was obtained from National Centre for Cell Science (NCCS, Pune, India). They have the properties to express human-specific proteins and protein isoforms, which are inherently absent in primary neuronal cell culture of rodents.
The neuroblastoma cell line SH-SY5Y was chosen for this study, as it is extensively used in experimental neurological studies involving the analysis of neuronal differentiation, metabolism, and function related to neurodegenerative and neuron adaptive processes, neurotoxicity and neuroprotection. These cells exhibit biochemical and functional properties of neurons. It also can proliferate effectively in culture for an extended period without contamination. Cells were maintained in culture in Ham’s F-12 supplemented with 2% heat-inactivated fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). Cultures were seeded into flasks containing supplemented medium and maintained at 37°C in 5% CO₂, humidified air. Stock cultures were passaged 1:4 twice weekly.

**Determination of cell viability by MTT assay**

The SH-SY5Y cells was sub-cultured, and after trypsinisation, they are seeded into 96-well plates at a seeding density of 1 × 10⁴ cells/well. These seeded plates were cultured and incubated at 37°C under 5% CO₂ atmosphere for 24 hours until reaching 70% confluence. The initial concentration of extracts of selected Commiphora species was 100 μg/ml in DMSO: PBS: Methanol (9:2:1). Extracts of Commiphora berryi, Commiphora caudata and Commiphora pubescens were serially diluted in complete culture medium to give different concentrations of (3.15, 6.25, 12.5, 25, 50, 100 μg/ml) were added to the cells to a final volume of 100 μl. The blank wells was filled with conditioned medium without cells, and the control well was filled with the cells along with the conditioned medium. After 72 hours incubation, the medium in each well was replaced with 10 μl of MTT (10μg/ml) and again incubated for 4 hours. After incubation, the MTT medium in each well was removed, and 100μl of DMSO was added to dissolve the formed violet formazan crystals within metabolically viable cells. This formazan production is directly proportional to the viable cell number. The plates were shaken for 20 min, and then the optical density was measured at 570 nm with a microplate reader. Wells without cells were used as blanks and were subtracted as background from each sample. Cell viability was expressed as a percentage of the control value.

β-amyloid modulation- Optimization of the dose of Aβ25-35 to induce toxicity

In order to evaluate the neuroprotective activity of plant extracts, Aβ25-35 dose was optimized to induce toxicity in SH-SY5Y cell lines. Dose optimization was also performed by MTT assay in six replicates. Dose level such as 2.5, 5, 10 and 20 µM was selected.

**Determination of neuroprotective effects on treatment with Aβ25-35**

The neuroprotective effect of the selected plant extracts of Commiphora species on Aβ25-35 – induced neurotoxicity was also measured by MTT Assay in SH-SY5Y cells. Aβ25-35 was reconstituted in DMSO to a concentration of 1mM. Aliquots were incubated at 37°C for 72 hours to form aggregated amyloid. During the experiment, Aβ25-35 was directly added to the culture medium to achieve a final concentration of 20 μM. Three concentrations of each of the plant extract that presented low toxicity (as determined from the tests above) were selected to assess their possible protective effects. The cells were plated as described above and pre-treated with the plant extracts for 2 hours before adding Aβ25-35 and then incubated for 72 hours. The viability of the cells was conducted according to the MTT assay method as described above. Cell viability was expressed as a percentage of the control value. Three different concentrations (6.25, 12.5, 25μg/ml) of the plant extracts that presented low toxicity on human neuroblastoma cells as assessed by the MTT assay were selected to determine the neuroprotective effects and to determine whether they could decrease the neuronal cell death induced by Aβ in SH-SY5Y cells.

**Statistical analysis**

The analysis of the data was carried out using Graph Pad Prism 5. All experiments were performed in triplicate, and the results were expressed as mean ± SD. A significant difference in the data was evaluated by performing a one-way ANOVA analysis by Dunnet’s multiple comparison tests.

**RESULTS**

**Phytochemical screening**

The preliminary qualitative phytochemical screening of ethanolic extracts of Commiphora berryi, Commiphora caudata, (EECC) and Commiphora pubescens (EECP), revealed the presence of alkaloids, flavonoids, terpenoids, steroids, tannins and along with other phytonutrients (Table 1).

**TLC bioautographic assay**

The preliminary investigation of acetylcholinesterase inhibitory activity of ethanol extracts of selected plants was determined qualitatively by the thin layer chromatography and the results are presented in Figure 1. The white spots were observed in the yellow background for all the plant extracts tested, thus it indicated that the presence of acetylcholinesterase. The RF values were recorded as 0.97, 0.93, and 0.96 Commiphora berryi, Commiphora caudata and Commiphora pubescens, respectively.

**Table 1: Phytochemical analysis of ethanol extracts of Commiphora species.**

| Phytoconstituents | EECB | EECC | EECP |
|-------------------|------|------|------|
| Alkaloids         | -    | -    | -    |
| Amino Acids       | -    | +    | +    |
| Anthraquinones    | -    | -    | -    |
| Flavonoids        | +    | +    | +    |
| Tannins           | -    | +    | +    |
| Glycosides        | +    | +    | +    |
| Proteins          | -    | +    | +    |
| Reducing Sugars   | +    | +    | +    |
| Saponins          | -    | +    | +    |
| Starch            | +    | +    | +    |
| Steroids          | +    | +    | +    |
| Terpenoids        | +    | +    | +    |

**Figure 1:** The qualitative results of the TLC bioautographic assay of acetylcholinesterase inhibition activity of the crude ethanol extracts of Commiphora Species.
Microplate assay to assess inhibition of acetylcholinesterase enzyme

The percentage of acetylcholinesterase (AChE) inhibition of the plant extracts and also standard drug physostigmine was determined at different concentrations. The percentage inhibition increases with an increase in concentration of doses in a dose dependent manner for all the plant extracts and also the standard drug. The maximum percentage AChE inhibition (65.48%) was observed the plant extract of *Commiphora berryi* at 3 mg/ml concentration when compared with physostigmine (99.72%) at 0.100 mg/ml concentration. The moderate AChE inhibition 52.61% and 62.60% were observed for the plant extracts of *Commiphora caudata* and *Commiphora pubescens*, respectively. AChE inhibition of the plant extracts to 50% i.e. IC50, for physostigmine was 2.1 μg/mL. However, the IC50 values at 2.29, 2.92, and 2.59 mg/mL were obtained for the plant extract of *Commiphora berryi*, *Commiphora caudata* and *Commiphora pubescens*, and respectively (Table 2).

Cell viability assay

**Determination of cell viability - MTT Assay**

The gradual dose-response of the SH-SY5Y cell viability for EECB, EECC, and EECP was determined. All extracts showed > 85% of viability in terms of control in lower doses, specifically at 3.15 μg/mL of concentrations. There was a slight reduction in the percentage viability at 6.25 and 12.5 μg/mL of concentrations. A gradual reduction in cell viability was observed in all plant extracts at 25 and 50 μg/mL. Minimum viability was observed at 100 μg/mL concentrations. No saturation in viability was observed in all plant extracts at 25 and 50 μg/ml. Minimum concentrations. There was a slight reduction in the percentage viability in terms of control in lower doses, specifically at 3.15 μg/ml of concentrations. The percentage of acetylcholinesterase (AChE) inhibition of the plant extracts and also standard drug physostigmine was determined at different concentrations. The percentage inhibition increases with an increase in concentration of doses in a dose dependent manner for all the plant extracts and also the standard drug. The maximum percentage AChE inhibition (65.48%) was observed the plant extract of *Commiphora berryi* at 3 mg/ml concentration when compared with physostigmine (99.72%) at 0.100 mg/ml concentration. The moderate AChE inhibition 52.61% and 62.60% were observed for the plant extracts of *Commiphora caudata* and *Commiphora pubescens*, respectively. AChE inhibition of the plant extracts to 50% i.e. IC50, for physostigmine was 2.1 μg/mL. However, the IC50 values at 2.29, 2.92, and 2.59 mg/mL were obtained for the plant extract of *Commiphora berryi*, *Commiphora caudata* and *Commiphora pubescens*, and respectively (Table 2).

**B-Amyloid Modulation - Optimization of the dose of Aβ<sub>25-35</sub> to induce toxicity**

In order to evaluate the neuroprotective activity of plant extracts, Aβ<sub>25-35</sub> dose was optimized to induce toxicity in SH-SY5Y cell lines. Dose level such as 2.5, 5, 10 and 20 μM was tested, decrease in cell viability was observed while increasing the concentration of Aβ and the maximum reduction (16.21%) was observed at 20 μM. Hence, in the present study, 20 μM of Aβ<sub>25-35</sub> concentration was selected to induce the toxicity in SH-SY5Y cell (Figures 2 & 3).

**Neuroprotective Effect of Plant Crude Extracts Against Aβ<sub>25-35</sub> Induced cell Death**

The in vitro neuroprotective effect of the plant extracts were examined in neurodegenerative cells induced by Aβ. Three dose levels such as 6.25, 12.5 and 25 μg/mL concentrations were used in the study. Based on the dose optimization these doses have been selected for the further evaluation of plant extracts. Significant improvement in cell viability was observed as 63.42 ± 2.02% (25μg/mL) for *Commiphora berryi*, 56.49 ± 1.88% (25μg/mL) for *Commiphora caudata* and 59.62 ± 1.24% (12.5μg/mL) for *Commiphora pubescens* when compared to Aβ control group. Therefore, the present study result reveals that optimum dose may play an important factor for the pharmacological efficacy of these extracts. Among the plant extracts tested *Commiphora berryi* shows the highest percentage of SH-SY5Y (Figure 4).

![Figure 2: Graded dose response of Aβ<sub>25-35</sub> induced toxicity in SH-SY5Y cell viability by MTT assay.](image-url)

**Table 2: Acetylcholinesterase inhibitory activity of the plant extracts.**

| Concentration (mg/mL) | EECB | Percentage Inhibition (%) | Std. Drug |
|-----------------------|------|---------------------------|-----------|
| 0.5 (0.005)           | 7.24 ± 0.06 | 1.86 ± 0.07 | 5.27 ± 0.03 | 60.76 ± 1.17 |
| 1.0 (0.010)           | 15.12 ± 0.04 | 5.26 ± 0.07 | 11.68 ± 0.05 | 66.35 ± 1.18 |
| 1.5 (0.025)           | 34.54 ± 0.11 | 9.30 ± 0.06 | 30.23 ± 0.05 | 74.89 ± 0.29 |
| 2.0 (0.050)           | 38.41 ± 0.08 | 27.36 ± 0.07 | 38.44 ± 0.11 | 79.67 ± 0.55 |
| 2.5 (0.075)           | 56.56 ± 0.12 | 33.78 ± 0.06 | 40.57 ± 0.04 | 84.59 ± 0.96 |
| 3.0 (0.100)           | 65.48 ± 0.10 | 52.61 ± 0.10 | 62.60 ± 0.12 | 99.72 ± 0.15 |
| IC₅₀ ± SEM (mg/mL)    | 2.29 ± 0.11 | 2.92 ± 0.07 | 2.59 ± 0.31 | 0.0021 ± 0.09 mg/mL |

Values in the parenthesis indicate as concentration of standard drug, Std. Drug, Physostigmine; n=6, each value represents the mean percentage inhibition ± SE.

**Table 3: Effect of selected plant extracts on SH-SY5Y cell viability measured by MTT assay.**

| Conc. (µg/mL) | Viability (Percentage of control) |
|--------------|----------------------------------|
|              | EECB | EECC | EECP |
| 3.15         | 91.92 ± 0.82 | 91.18 ± 1.37 | 87.60 ± 1.05 |
| 6.25         | 89.02 ± 0.83 | 85.69 ± 2.01 | 78.65 ± 0.85 |
| 12.5         | 79.60 ± 0.76 | 72.54 ± 2.74 | 73.25 ± 1.35 |
| 25           | 73.80 ± 2.37 | 62.72 ± 2.53 | 62.91 ± 1.26 |
| 50           | 63.60 ± 1.80 | 54.16 ± 1.65 | 55.33 ± 1.25 |
| 100          | 56.08 ± 1.70 | 44.55 ± 1.24 | 46.09 ± 1.51 |
| IC₅₀ (µg/mL) | 81.06 ± 4.40 | 52.03 ± 3.56 | 51.81 ± 1.48 |

Values are mean percentage inhibition ± SEM, n=6.
Figure 3: Phase contrast photomicrograph (10x) of SH-SY5Y cell viability by MTT assay.
a) SH-SY5Y Cell on day 7
b) SH-SY5Y Cell on day 21
c) SH-SY5Y Cell treated with Aβ showing reduced growth.

Figure 4: Effect of Crude Extracts on Aβ25-35 Induced Toxicity in SH-SY5Y Cell line.
a) Viability of cell treated with Aβ + EECB
b) Viability of cell treated with Aβ + EECC
c) Viability of cell treated with Aβ + EECP.
The viability of untreated control cells was defined as 100 %. Results are the mean ± SEM, n= 6. ***P< 0.001 between treatment versus amyloid beta, *P<0.05 between control versus amyloid beta.
DISCUSSION

The three plant species which are included in our study have been previously reported for its natural antioxidant activities. A large amount of evidence demonstrated that oxidative stress is intimately involved in age-related neurodegenerative disease. There have been a significant number of studies that examined the positive benefits of antioxidants to block the neuronal death occurring in the pathophysiology of neurological disorders.23 Hence the present study was mainly focused on plants with an antioxidant property, which may protect the nervous system against oxidative damage. It may also enhance their utility in the treatment of neurodegenerative diseases. Previous data suggest that treatment with methanolic extracts of Commiphora berryi decreased the level of SOD, catalase, and GPx enzymes against CCl₄-induced oxidative damage in rats, which further confirms the free radical scavenging properties.19 Ethanolic extracts of Commiphora caudata and Commiphora pubescens have been previously reported for its free radical scavenging activity.20

The percentage AChE inhibition of standard drug physostigmine was determined at various doses of 0.005, 0.010, 0.025, 0.050, 0.075, 0.100 mg/mL. The percentage of acetylcholinesterase inhibition of all the selected plant extracts was measured at different concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, and 3 mg/mL. The AChE inhibitory activity is classified according to the percentage of inhibitory activity as described by Vinutha et al.21 as potent inhibitors (greater than 50% inhibition), moderate inhibitors (30-50% inhibition) and weak inhibitors (below 30%). IC₅₀ value refers to the half-maximal inhibitory concentration of plant extracts where the response is reduced by half. It is used to determine the effectiveness of the acetylcholinesterase inhibitory activity of plant extracts. Plants with a higher percentage of inhibition of the enzyme had a lower IC₅₀ value. The percentage inhibition increases with an increase in the concentration of selected plant extracts in a dose-dependent manner. All the plant extracts showed maximum inhibition at the concentration of 3mg/mL. The greatest percentage of inhibition was nearer to or greater than 50%. Plant extracts that have the minimum IC₅₀ value were considered as the most potent plant extracts with acetylcholinesterase inhibitory activity. The percentage AChE inhibition of standard drug physostigmine was determined at various doses. A gradual dose-response was observed from 0.005 to 0.100 mg/mL concentration. Maximum inhibition was observed at 0.100 mg/mL concentration.

Some of the plants with reference to our selected families have been studied for AChE inhibitory properties. Similar to our results, ethanolic extract from the leaves of Canarium patetinnervium, which belongs to the Burseraceae family, also exhibited good acetylcholinesterase inhibition with an IC₅₀ value of 29.53 ± 0.19 μg/mL.22 Additionally, resins obtained from the chloroform extract of Boswellia socotranaco Balf.f., and Boswellia elongata Balf. f., which also belongs to the Burseraceae family, has also been reported for its potent acetylcholinesterase inhibitory activity of 71% and 46% at 0.2mg/mL respectively.23 The active phytoconstituents present in the plants of their respective families would be responsible for the acetylcholinesterase inhibitory activity in the selected plants from those families in this current research.

SH-SY5Y neuronal-like cells for Alzheimer’s disease have been used in this study. The SH-SY5Y cell line has been extensively used in the drug discovery in experimental neurological studies related to neurodegenerative disease.24 Aβ (25-35) is an undecapeptide that is typically involved in neurotoxic properties, and it leads to neurodegeneration.25 The neuroprotective effect of the ethanolic extracts of EECB, EECC, and EECP has been studied at various doses. The effect of the plant extracts on cell viability has been assayed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. MTT assay is used to measure the metabolic function of cells that affect the mitochondria. Viable cells produce an NADH or NADPH reducing equivalent which passes the electrons to electron transfer reagent that reduces tetrazolium product to formazan. In the case of dead cells, they lose the ability to form a formazan product.

The fact that AChE accelerates the amyloid formation and the effect is sensitive to peripheral anionic site blockers of the enzyme suggest that the AChE inhibitors may well provide an attractive possibility for treating Alzheimer’s disease.26 Human Aβ sequences have been shown to induce toxicity in neuronal cell lines and primary cultures of neuronal origin as well as in vivo.27,28 Commiphora whightii (Guggulu) is a plant contain in guggulsteron that inhibits lipid peroxidation during oxidative stress in neurodegenerative disease, causing a neuroprotective effect.29 Mrryterpenoids K and Mrrrterpenoid N isolate from Commiphora whightii showed a neuroprotective effect against MPP+ induced neuronal cell death in dopaminergic neuroblastoma SH-SY5Y cells.27 The results of the present study indicated that optimum dose might be a vital factor for the pharmacological efficacy of these extracts. Among the plant extracts tested Commiphora berryi, showed the highest percentage viability and greater IC₅₀ value of Aβ (25-35) toxicity in SH-SY5Y cell lines by MTT assay.

CONCLUSION

The outcome of various experimentation leads to the conclusion that the therapeutic potential of all the plant extracts evaluated showed remarkable inhibitory activity against AChE. Furthermore, the plant extracts also reduced the effects of beta-induced neuronal cell death. Since AD needs a multitarget drug strategy for the prevention and symptomatic treatment, it is crucial to identify the active phytoconstituents useful in both assays, and the same has to be compared with the crude extract to reveal their possible synergistic interaction. Thus, further work is in isolation of specific bioactive compounds through bio-assay guided fractionation and their characterization may be necessary for the exploration of these species for potential new therapeutic drug leads.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

This work was funded by Department of Science and Technology, Government of India to Dr.PSelvamani, Assistant Professor, Department of Pharmaceutical Technology, Centre for Excellence in Nanobio Translational Research (CENTRE), Anna University, Bharathidasan Institute of Technology Campus, Tiruchirappalli, Tamil Nadu, India. (DST:Ref. No. NO.SR/FT/LS-41/2010 Dated 04.05.2012.) Authors would like to thank the University Grants Commission for the financial grant provided to Ms. P. S. Dhivy, Senior Research Fellow. UGC Letter No. F1-17.1/2012-13-RGNF-2012-13-SC-TAM-27613/SA-III/Website and dated 06-Jun-2012 posted in the UGC website on 19.02.2013.

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### Graphical Abstract

| Plant Name               | TLC Bioautographic Assay | Microplate Assay (IC50 ± SEM (mg/mL)) | Cell Line Studies |
|--------------------------|--------------------------|---------------------------------------|-------------------|
| Commiphora herveyi (Am.) | Rf value 0.97            | IC50 2.29 ± 0.11 65.48 ± 0.10 (3mg/mL) | Evaluation of AChE inhibition by microplate assay |
| Commiphora caudata (Wight & Arn.) | Rf value 0.93 | IC50 2.92 ± 0.07 52.61 ± 0.10 (3mg/ml) | Effect of Aβ25-35 Induced Toxicity in SH-SY5Y Cell line |
| C. caudata var. petersenii (Wight & Arn.) | Rf value 0.96 | IC50 2.59 ± 0.51 62.60 ± 0.12 (3mg/ml) | |

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Cite this article: Dhivya PS, Selvamani P, Latha S, Mani V, Azahan NSM. In Vitro Evaluation of Acetylcholinesterase Inhibitory and Neuroprotective Activity in Commiphora Species: A Comparative Study. Pharmacogn J. 2020;12(6):1223-31.