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ACETYLCHOLINESTERASE INHIBITORY ACTIVITY AND ANTIOXIDANT ACTIVITY OF SELECTED MEDICINAL PLANT EXTRACTS USED AGAINST COGNITIVE DYSFUNCTION AND MEMORY LOSS IN SRI LANKAN TRADITIONAL MEDICINE

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ABSTRACT

The objectives of the study were to determine the acetylcholinesterase (AChE) inhibitory activity and antioxidant activity in the selected Sri Lankan medicinal plants. AChE inhibitory activity of the selected medicinal plant extracts was determined using the Ellman's method. The antioxidant activities were determined by four *in vitro* methods namely DPPH assay, FRAP assay, NO assay and TBA acid method. The contents of total polyphenols and flavonoids were determined quantitatively. Out of the ten selected medicinal plant extracts *Abrus precatorius, Centella asiatica, Strychnos nux-vomica* and *Ricinus communis* showed IC_{50} values < 200 µg/mL for AChE inhibitory activity. A high antioxidant activity was shown in the extracts of *Cardiosprmum halicacabum*, *Centella asiatica* and *Ricinus communis* in the four selected antioxidant assays. The total polyphenol content and total flavonoid content was in the range of 0.55-7.30 mg/g DW and 19.08±0.29-1283.08± 0.09 µg/gDW. The extracts of *A. precatorius*, *C. asiatica*, *S. nux-vomica* and *R. communis* are deserved to be as potent sources of AChE inhibitors as well as natural antioxidants. Considering the complex multifactorial etiology of Alzheimer's diseases, these plant extracts would be apt candidates for the development of novel pharmaceutical agents in the management of Alzheimer's diseases.

Keywords: Acetylcholinesterase inhibitory activity, Ellman's method, DPPH assay, FRAP assay, NO assay, TBA acid method

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INTRODUCTION

Alzheimer's disease (AD), a multifactorial neurodegenerative disease, is one of the major causes of dementia among persons over age 85. According to epidemiological surveys, an estimated percentage of 7–10% individuals over the age of 65 and 50–60% over the age of 85 suffer from AD, reaching to a number approximately 35 million people worldwide [1,2]. Specifically, AD is expected to become a more of a serious health and social burden in developing countries than in developed countries. This is because unprecedented declines in mortality have resulted in a rapid aging process in most developing countries, which is exacerbated by poor health care facilities. AD patients develop a gradual and an insidious cognitive deficit that becomes incapacitating in the advanced stages of the disease. These devastating symptoms significantly compromise the patients' quality of life, leading to absolute dependence, hospitalization and unavoidably, death occurring on average of nine years after diagnosis [3, 4]. Although the specific cause of AD is unknown, risk factors as age and family history in a first-degree are arguably important for developing dementia among patients [5].

Classic features found in AD include neuronal loss in regions associated with memory and cognition, particularly of cholinergic neurons, neurotransmitter depletion mainly acetylcholine and synaptic dysfunction [6]. The most common microscopic findings are the presence of abnormal protein deposits, including senile neuritic plaques and neuro fibrillary tangles. Senile plaques are result of the extracellular accumulation of insoluble aggregates of β-amyloid protein (Aβ) while neuro fibrillary tangles are composed of paired helical filaments of hyperphosphorylated tau protein [7,8]. These abnormalities lead to activation of neurotoxic cascades that eventually cause synaptic dysfunction and neuronal death [9]. Protein misfolding and abnormal aggregation both play critical roles in AD pathology, leading to the formation of insoluble pathological conformers that cause neuronal degeneration, interneuron dysfunction, oxidative stress and cellular death [3, 10, 8]. A deficiency of acetylcholine and degeneration of cholinergic neurons in the cortex and hippocampus, nucleus basalis of Meynert are considered to play a vital role in learning and memory deterioration of AD patients. Acetylcholine (ACh) is an organic molecule liberated at nerve endings as a neurotransmitter. During neurotransmission, ACh is released from the presynaptic neuron into the synaptic cleft and binds to ACh receptors on the post-synaptic membrane, relaying the signal from the nerve. AChE is located on the post-synaptic membrane, terminates the signal transmission by hydrolyzing ACh. However, neurotransmitter disturbances and insufficient cholinergic functions are identified among the pathological features in central nervous system disorders [11].

Approaches to enhance cholinergic function in AD have included stimulation of cholinergic receptors or prolonging the availability of acetylcholine (ACh) released into the neuronal synaptic cleft by inhibiting ACh hydrolysis via AChE inhibitors [8]. In fact, the elevation of ACh level seems an essential therapeutic target in the management of AD disease. Most of the drugs currently available for the treatment of AD are AChE inhibitors such as tacrine, donepezil, rivastigmine and galanthamine, all of which have limited effectiveness and side effects [12].

Tacrine and donepezil, both from synthetic origin, were the first drugs approved for the treatment of cognitive loss in patients with AD. Rivastigmine was designed from the lead compound physostigmine, a natural AChE inhibitory alkaloid. Galanthamine and huperzine A are natural alkaloids that have been isolated firstly by natural extracts [13]. However, the discovery of plant-derived compounds such as physostigmine, galantamine and huperzine A has been proven the fact that AChE inhibitors could be demanded from natural extracts [14, 15, 16]. Therefore, search for new inhibitors of AChE, particularly from natural sources, with higher efficacy has been continued to the present era. Oxidative stress refers to the physiological condition in which the capacity of endogenous antioxidant system fails to cope with the damaging effects of free radicals. Strong experimental evidences have shown that the oxidative damage plays a major role in neurological degeneration in the pathogenesis of AD [17, 18]. It is linked to neuronal protein misfolding, membrane dysfunction and cell death [18]. Moreover, targeting the inhibition of AChE inhibitory activity and suppression of oxidative stress would be beneficial against memory loss and cognitive dysfunction in AD. Medicinal plants are being recognized as promising sources of lead compounds for the development of new drugs in the management of AD [19]. Sri Lanka constitutes an apt example where medicinal plants have been enacted a prominent role in a variety of therapeutic applications. It's a country blessed with a rich repository of indigenous knowledge developed and practiced over a long period of time that spans over two and half millennia. A large number of Sri Lankan medicinal plants have been used for treating central nervous system disorders as well as for improving the memory and cognitive function [20].

Medicinal plants consist of an array of secondary metabolites such as polyphenols and flavonoids with broad-spectrum of therapeutic effects via antiamyloid production, antiapoptotic, antioxidant and anti-inflammatory activities, in addition to anticholinergic effects [21]. In fact, therapeutic potential of most of the herbal extracts have not been scientifically scrutinized to date. Therefore, it will be appropriate to screen them in search of new AChE inhibitors. The current study is an attempt to determine the AChE inhibitory activity and antioxidant activity *in vitro*, to determine the phytochemical profile of selected medicinal plant extracts. The selection of medicinal plants was based on their traditional use against symptoms commonly observed in Alzheimer's disease, such as loss of cognitive function and memory disorders. Medicinal plants selected for the investigation are listed in Table 1.

MATERIALS AND METHODS

Chemicals and instruments

L-Ascorbic acid, acetyl cholinesterase (AChE: EC 3.1.1.7) Type VI-S, acetylcholine iodide (ATCI), 2, 2′-diphenyl-2-picrylhydrazyl hydrate (DPPH), 5,5′-dithiobis 2-nitrobenzoic acid (DTNB), Folin- Ciaocalteu reagent, gallic acid, N-(1-Naphthyl)ethylediaminedihydrochloride, orthophosphoric acid (H_3PO_4), naphthylethylediamine dihydrochloride, sodium nitroprusside (SNP), sulfanilamide, 2,4,6-tripyridyl-1,3,5-triazine (TPTZ), thiobabituric acid (TBA) and trichloroacetic acid (TAA) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Apart from the above key chemicals, all other chemicals and solvents were of analytical grade and used without any purification. Donepezil was purchased from Alembic Pharmaceuticals Limited, India. A Sanyo Gallenkamp (model SP65) spectrophotometer was used for spectrophotometric measurements.

Collection and identification of medicinal plants

A total of ten plant samples, representing ten Sri Lankan medicinal plant species from eight families were collected during Sep-Dec 2016 from one location in the Southern region of Sri Lanka (Table 1). Botanical identity was determined according to the descriptions given by Jayaweera [20]. Voucher specimens were preserved at the Department of Biochemistry, Faculty of Medicine, and University of Ruhuna, Sri Lanka.

Preparation of plant extract

Selected parts of the medicinal plants were cut into small pieces and oven dried (40 ºC). Then, 5 g of each ground

plant material was refluxed with distilled water (60 mL) and the final volume was reduced to 50 mL after refluxing for 4 h. The final concentration of the refluxed extract was 0.05 g/mL. A graded series of dilutions was prepared for respective bio assays.

Acetylcholinesterase (ache) inhibitory activity

Inhibition of AChE activity was determined using Ellman's colorimetric method by Ellman et al. [22] with some modifications by Dzoyem and Eloff [23]. A volume of 250 µL of 15 mmol/L ATCI in water, 120 µL of 3 mmol/L DTNB in Buffer A (50 mmol/L Tris-HCl, pH 8, containing 0.1 mol/L NaCl and 0.02 mol/L $MgCl_2.6H_2O$), 500 µL of Buffer B (50 mmol/L, pH 8, containing 0.1% bovine serum albumin) and 250 µL of plant extract at a graded dose of 0.001-0.025 mg/mL were mixed.

Then, 100 µL AChE enzyme (EC 3.1.1.7) (0.2 U/mL) was added to the reaction mixture. The absorbance of the inhibition of AChE inhibitory activity of the resultant solution was measured spectrophotometrically at 405 nm. Donepezil was used as the reference compound. The AChE inhibitory activity is expressed in terms of IC_{50} (concentration of the extract

/reference compound required to inhibit AChE activity by 50%). Percentage of AChE inhibitory activity was calculated using the following formula.

% Inhibition of AChE inhibitory activity = (A $_{control}$-A $_{sample}$)/A $_{control}$ x 100%

where, A $_{control}$ represents the absorbance of the control without the plant extract/reference compound, A $_{sample}$ represents the absorbance of the plant extract/reference compound.

2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) radical scavenging assay

A previously reported DPPH radical scavenging assay method was used [24]. The radical scavenging activity of the plant extract against stable DPPH radical (DPPH*) was determined. Briefly, 1.0 mL of each extract at different concentrations (1-500 µg/mL) was added to 3.0 mL of 0.004% ethanol DPPH solution.

The mixture was shaken vigorously, allowed to stand at 25°C in the dark for 30 min. The decrease in absorbance of the resultant solution was measured spectrophotometrically at 517 nm against an ethanol blank. L-Ascorbic acid was used as the reference compound. The antioxidant activity is expressed in terms of IC_{50} (concentration of the extract /reference compound required to inhibit DPPH radical formation by 50%).

% DPPH radical scavenging activity= (A $_{control}$-A $_{sample}$)/A $_{control}$ x 100%

where, A $_{control}$ represents the absorbance of the control without the plant extract/reference compound, A $_{sample}$ represents the absorbance of the plant extract/reference compound.

Ferric reducing antioxidant potential (FRAP) assay

The FRAP assay was performed according to the method of Benzie and Strain [25]. The assay is based on the reducing power of a compound (antioxidant). A potential antioxidant reduces the ferric ion (Fe^{3+}) to ferrous (Fe^{2+}) ion; the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. The FRAP working reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ at 10:1:1 (v/v/v).

The FRAP reagent (3.0 mL) and sample solution (100 µL) were mixed and the absorbance of the resultant solution was measured (t=0) at 593 nm spectrophotometrically (A sample t=o). Thereafter, the sample was placed in a water bath (37°C) and the absorption was measured at the same wave length after 4 min. (A$_{sample}$ t=4). L-ascorbic acid (100 µM) was used as the standard compound and proceeded as in the same way.

FRAP value of the plant extract (µM) = (A $_{sample\ t=0-4}$)/A $_{standard\ t=0-4}$ x FRAP value of 1000 µM ascorbic acid

where, (A $_{sample\ t=0-4}$) represents the change in absorbance in a sample from 0 to 4 min,

(A $_{standard\ t=0-4}$) is the change in absorbance in the standard from 0-4 min. FRAP value of ascorbic acid is 2.

Nitric oxide (NO) radical scavenging assay

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with the Griess reagent and the absorbance of the chromophore formed was measured spectrophotometrically [26]. L-Ascorbic acid was used as the reference compound. 1.0 mL of SNP (5 mM) was mixed with 4.0 mL of the plant extract at different concentrations (1-500 µg/mL) and incubated the resultant solution at 29°C for 2 h. Incubated solution (2.0 mL) was mixed with 1.2 mL of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% N-(1-Naphthyl) ethylediamine dihydrochloride and measured the absorbance at 550 nm (A $_{sample}$) against a distilled water blank.

The absorbance of the control without the plant extract/reference compound was also measured at the same wave length. The antioxidant activity is expressed in terms of IC_{50} (micromolar concentration required to inhibit NO radical formation by 50%).

% NO radical scavenging activity= (A $_{control}$ – A $_{sample}$)/A $_{control}$ x 100%

where, A $_{control}$ represents the absorbance of the control without the plant extract/reference compound, A $_{sample}$ is the absorbance of the plant extract/reference compound.

Thiobabituric acid (TBA) assay

TBA assay was performed as described by Ottolenghi, [27]. A solution of 20% trichloroacetic acid (2.0 mL) was mixed with 0.67% thiobabituric acid (2.0 mL) and sample solution (1.0 mL) was added to the above acidic media. This mixture was boiled (100º C) in a water bath for10 min. It was allowed to cool and filtered. The absorbance

of the sample was measured at 552 nm using water blank. L-ascorbic acid was used as the reference compound. The antioxidant activity is expressed in terms of IC_{50} (micromolar concentration required to inhibit peroxy radical formation by 50%).

% Peroxy radical scavenging activity= $(A_{control} - A_{sample})/A_{control} \times 100\%$

Where, $A_{control}$ represents the absorbance of the control without the plant extract/reference compound, A_{sample} is the absorbance of the plant extract/reference compound.

Total polyphenol content

Total polyphenol content was measured using Folin-Ciocalteu colourimetric method [28]. Plant extract (1.0 mL) was mixed with 1.0 mL of 95% ethanol, 5.0 mL of distilled water and 0.5 mL of 50% Folin-Ciocalteu reagent. The mixture was allowed to react for 5 min and 1.0 mL of 5% Na_2CO_3 was added.

Thereafter, it was thoroughly mixed and placed in dark at 25°C for 1 h and the absorbance was measured spectrophotometrically at 725 nm. Quantification was done with respect to the standard curve of gallic acid (0-50 μg/mL). The results were expressed in gallic acid equivalents mgGAE/g of the dry weight.

Total flavonoid content

The flavonoid content of the plant extracts was determined using aluminum chloride colorimetric method [29, 30]. The plant extract (0.50 mL) was mixed with ethanol 95% (1.5 mL) followed by $AlCl_3$ 10% (0.10 mL), 1M potassium acetate (0.10 mL) and distilled water (2.8 mL). The resultant mixture was incubated at 27 °C for 30 minutes. The absorbance of the reaction mixture was measured spectrophotometrically at 415 nm. The results are expressed in terms of micrograms of quecertin equivalent μgQE/g of the dry weight of plant material.

Qualitative screening of phytochemical constituents

Qualitative phytochemical screening for the presence of saponin glycosides, anthracene glycosides, cardenolide glycosides, cyanogenic glycosides, polyphenol compounds, alkaloids, flavonoids, tannins, reducing sugars, and proteins was carried out by the reported protocol [31].

Statistical analysis

The triplicates of each sample were used for statistical analysis and the values were expressed as mean ± standard deviation. Pearson's correlation and regression analysis was carried out using Minitab software to establish a relationship between total polyphenol content, total flavonoid content vs antioxidant activities and AChE activity of selected medicinal plant extracts. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

A wide range of medicinal plants and herbs has been used in the management of AD in Sri Lankan traditional medicine. Such medicinal plants are important as adjuncts to existing therapies and as drug leads for the development of novel pharmaceutical agents against

memory loss and cognitive dysfunction in AD. AChE is a key enzyme in the cholinergic nervous system. AChE inhibitors have proven to be the most viable therapeutic target for symptomatic improvement of AD which enhances cholinergic transmission with modest and transient therapeutic effects [32]. In addition, antioxidant therapy has also been proven to be successful in improving cognitive function and behavioral deficits in patients with AD [33, 34].

However, it has been speculated that this duality could lead to improve clinical outcomes, particularly in respect to the management of AD. It has been hypothesized that natural antioxidants would provide a better therapeutic paradigm in the management of AD [35]. The modern therapies utilized for the treatment AD have many adverse effects, driving the quest for more safe and effective medications [36].

In the present study, we evaluated the AChE inhibitory activity and *in vitro* antioxidant activity of ten selected medicinal plant extracts used in Sri Lankan traditional medicine. Aqueous extracts were selected for the study considering the relevancy of them in traditional medicine applications. In addition, it is reported that flavonoids are extracted into water than any other organic solvent which is generally in use [37]. The AChE inhibitory activity of the selected medicinal plant extracts was determined using Ellman's method spectrophotometrically. The results are shown in Table 2.

The extracts of *A. precatorius*, *C. asiatica, S. nux-vomica* and *R. communis* showed IC_{50} values < 200 μg/mL for AChE inhibitory activity. The AChE inhibitory activity was highest (lowest IC_{50}: 84.42±0.98) in the extract of *C. asiatica* and lowest (highest IC_{50}: 299.00±2.33 μg/mL) in the extract of *C. fistula*. Donepezil was used as the reference compound which showed the IC_{50} value of 8.40 ± 0.89 μg/mL. Donepezil showed a very significant AChE inhibitory activity and the IC_{50} of donepezil was not comparable with any of the tested plant extracts in the present study. However, the IC_{50} value of donepezil is comparable to the previously published report by Uddin et al. [38].

AChE exerts a prominent role in enhancing cholinergic neurotransmission as well as in reducing the aggregation of Aβ peptide, the key pathogenic step in the pathogenesis of AD. From the results, it is clear that the extracts *A. precatorius*, *C. asiatica, S. nux-vomica* and *R. communis* would be promising sources of drug leads of AChE inhibitors that would be useful in the management of AD. The IC_{50} value of the aqueous extract of *C. asiatica* is in accordance to the report by Mukherjee et al. [39] and Orhan et al. [40].

However, this is the first report on AChE inhibitory activity of selected medicinal plant extracts of Sri Lankan origin. A wide variety of methods have been used to determine the antioxidant activity of plant extracts and no single assay provides an accurate method to determine the capacity to scavenge free radicals, particularly in a mixed or complex system such as plant extracts. Therefore, it is essential to determine the antioxidant activity via diverse antioxidant assays in order to assess different aspects of the oxidation process. Furthermore, different compounds may act as antioxidants through

different mechanisms. Therefore, it has been recommended that at least three different assays should be considered in evaluating the antioxidant activity of plant extracts [41]. In the present study, DPPH assay, FRAP assay, NO assay and TBA method were employed to determine the antioxidative activity of selected aqueous medicinal plant extracts. All extracts exhibited concentration dependent radical scavenging activities in DPPH, NO, TBA assays as shown in Table 2.

The radical scavenging activity was expressed in terms of IC_{50} values in three assays to obtain a precise single value for comparisons. A high antioxidant potential was observed in the extracts of *C. asiatica, R. communis, M. pudica, A. precatorius, C. halicacabum, S. nux-vomica* with an evidence of high radical scavenging activity in DPPH, NO and TBA assays.

The highest and lowest FRAP values were obtained in the extracts of *C. asiatica,* (27.19±1.00) and *W. somnifera* (2.45 ±0.78) respectively. The results obtained from the DPPH assay was found in correlation with other aqueous medicinal plant extracts grown in South East countries [42-44]. Among the ten plants species investigated, five medicinal plants were identified as potentially rich sources of natural antioxidants. They are the whole plant extract of *C. asiatica*, leaf extract of *R. communis,* root extract of *M. pudica*, leaf extract of *A. precatorius* and fruit extract of *S. nux-vomica*.

The high antioxidant activity of the whole plant extract of *C. asiatica* was comparable with the findings of several other authors [39, 45]. Discrepancy in the antioxidant activity values in FRAP values with the IC_{50} values of other three radical scavenging assays was noticed. This may be due to different aspects of the antioxidant capacity. In fact, different radicals exert the oxido-reduction reactions via single electron transfer reactions and hydrogen atom transfer methods while oxido reductions in FRAP assay involves two electron transfers between two species.

The AChE inhibitory and antioxidant activities may due to the presence of polyphenols, flavonoids, tannin, alkaloids and saponins (Table 3). In addition to the qualitative phytochemical analysis, the total polyphenol content and the total flavonoid content of the ten aqueous medicinal plant extracts were determined by regression analysis of gallic acid calibration curve and quecertin calibration curve respectively. As shown in Fig. 1, the total polyphenol content varied widely in a range of 0.55 ± 0 - 7.30±0.07 mg/GAE g DW whereas total flavonoid content varied in a range of 19.08±0.29 -1283.08± 0.09 µg/ QE g DW.

It was evident that the extracts of *C. asiatica*, *C. fistula*, *R. communis* have high total polyphenol and flavonoid contents in both assays. High polyphenol content or/and flavonoid content is not always coinciding with the antioxidant activity of the selected medicinal plant extracts. Any polyphenolic compound including aromatic amines, ascorbic acid and sugar reduce the Folin-Ciocalteu reagent thereby over estimation of total polyphenol content by this method could have affected the results of this assay [46].

Polyphenols are the most abundant antioxidants in the plant kingdom, and the antioxidant activity of the polyphenolic compound is believed to result from their redox properties, which play an important role in adsorbing and neutralizing free radicals. Flavonoids are the most ubiquitous group of secondary metabolites present in the mechanism of action of flavonoids is through the scavenging of free radicals.

The linear regression and correlation analysis was done to establish a correlation between total polyphenol content, total flavonoid content vs AChE inhibitory activity and antioxidant activity of the selected medicinal plant extracts. The correlation analysis is given in Table 3. A linear correlation between the total polyphenol content and antioxidant activity was established ($p < 0.05$). Accordingly, Uddin et al [38] and Boonsong et al [47] reported the fact that polyphenols attributed to both antioxidant and AChE inhibitory activity significantly. In fact, polyphenolic compounds such as gallotannins, condensed tannins and flavonoids contribute directly to the antioxidant activity and AChE inhibitory activity of plant extracts [48].

Indeed, different classes of flavonoid compounds such as pomiferin extracted from *Maclura pomifera* (Family: Moraceae), 5′-geranyl-5,7,2′,4′-tetrahydroxyflavone from *Morus lhou* (Family: Moraceae), *Naringenin* from *Citrus junos* (Family: Rutaceae) showed significant AChE inhibitory activity *in vitro* [19].

The total polyphenol content, total flavonoid content vs DPPH radical scavenging activity were moderately correlated as r = 0.619 (R^2 = 0.717), 0.769(R^2 =0.789) respectively ($p < 0.05$). A strong correlation values of 0.937(R^2=0.878), 0.873(R^2=0.891), 0.789(R^2=0.891) was found between total polyphenol content vs antioxidant activity by FRAP assay, NO assay and TBA assay. Similarly total flavonoid content was positively correlated with the above three *in vitro* antioxidant assays. The positive correlation obtained in the present study is comparable with our previously published reports on antioxidant activity of medicinal plant extracts used in the management of oxidative stress related chronic diseases [49, 50].

The total polyphenol and total flavonoid contents were moderately correlated with the AChE inhibitory activity however, the degree of correlation was lower than that in the correlation observed with the total antioxidant activity by four assays. Therefore, we suggest that mostly polyphenol compounds and flavonoids attribute significantly for the antioxidant activity but not for the AChE inhibitory activity.

On the other hand, the extracts containing non-polar phytochemicals such as alkaloids might be responsible for high inhibitory activity particularly on AChE. In fact, the alkaloids are the main secondary metabolites which are of concern in the synthesis of novel anticholinesterase drugs rather than polyphenolic/flavonoid compounds [48, 51, 52].

It is needed to determine correlation between the alkaloid content and AChE inhibitory activity which is considered as a limitation of the present investigation. Although quantitative estimations were not performed, the presence of alkaloids in some of the medicinal plant extracts that

was confirmed from the results of qualitative phytochemical screening.

However, the contribution of alkaloids to AChE inhibition and antioxidant activity need to be rectified in future. In fact, generally synthetic flavonoids are strong AChE inhibitors at the submicromolar range, though natural flavonoid compounds are less potent against AChE [19]. According to our literature survey, correlation between total polyphenol and total flavonoid contents vs AChE inhibitory activity has not previously been reported elsewhere. Different varieties of natural alkaloids as berberine, tubocurarine, galantamine,

geissospermine etc were identified as potent inhibitors of AChE [14, 53, 54].

Although all the selected medicinal plants are traditionally used against in the treatment of cognitive dysfunction and memory loss in AD , some extracts were not shown potent AChE inhibitory activity in the present study. However, weak activity may be due to the insufficient quantities of the active compound(s) in the crude extracts to show target bioactivities otherwise, they may involve in different mechanism of action which is apart from the antioxidant and AChE activities

Table 1: Details of medicinal plants selected for the investigation

Plant name	Common name	Family	Part used	Medicinal uses related to neurodegenerative disorders
Abrus precatorius	Indian liquerice/olinda	Fabaceae	leaves	Paralysis and brain stimulant
Acalypha indica	Indian copper leaf/ Kuppameniya	Euphorbiaceae	whole plant	Nerve diseases
Cardiosprmum halicacabum	Wel penela	Sapindaceae	aerial parts	Nervous system disorders
Cassia fistula	Purgin cassia/ Ehela	Fabaceae	stem bark	Nervous system disorders
Centella asiatica	Pennywort/ Gotukola	Apiaceae	whole plant	Nervous system disorders, paralysis, epilepsy, spasms and improves memory
Mimosa pudica	Sleeping grass/ Nidikumba	Fabaceae	root	Convulsions
Ricinus communis	Castor/ Endaru	Euphorbiaceae	leaves	For nervous system disorders and stimulant and memory enhancer
Sida alnifolia	Heen babila	Malveceae	root	Nervous system disorders
Strychnos nux-vomica	Nux-vomica tree/ Goda, karduru	Loganiaceae	fruit	Nervous system disorders, paralysis, epilepsy,
Withania somnifera	Winter cherry/Amukkara	Solanaceae	root	Nervous system disorders, and memory enhancer

. **Table 2:** Acetylcholine esterase inhibitory activity and antioxidant activity of ten medicinal plant extracts

S.No. Plant name	AChE inhibitory activity IC_{50}^{a} (ug/ml)	Total antioxidant activities			
		DPPH radical scavenging assay IC_{50}^{b}(ug/ml)	FRAP assay FRAP value^{c} (µM)	NO radical scavenging assay IC_{50}^{d} (ug/ml)	TBA assay IC_{50}^{e} (ug/ml)
1 *Abrusprecatorius*	165±2.23	121.23±3.56	7.34±1.00	178.45±3.12	198.23±2.14
2 *Acalypha indica*	233.6±3.32	190.23±2.23	3.12±0.78	390.16±3.90	198.23±2.14
3 *Cardiosprmum halicacabum*	233.6±3.32	156.29±1.18	9±0.33	156.23±1.34	290.45±2.67
4 *Cassia fistula*	299.001±2.33	145.67±1.19	12.34±1.12	179.34±1.12	189.34±1.89
5 *Centella asiatica*	84.42±0.98	8.67 ± 0.89	27.19±1.00	148.45±1.10	170.34±1.56
6 *Mimosa pudica*	242.07±3.56	117.56±0.78	5.67±0.67	167.23±1.90	165.23±3.23
7 *Ricinus communis*	174.89±3.01	100.56±1.10	15.28±1.17	155.23±2.34	177.23±1.19
8 *Sida alnifolia*	241.56±2.56	178.23±2.67	3.45±0.34	399.37±3.37	290.45±2.10
9 *Strychnos nux-vomica*	103.24±1.89	134.56±2.95	5.6±1.12	151.78±1.26	167.23±2.45
10 *Withania somnifera*	283.22±3.67	150.23±1.23	2.45 ±0.78	439.45±3.20	253.11±2.90
11 L- Ascorbic acid	N/A	8.34±0.89	N/A	12.45	48.17±2.12
12 Donepezil	8.4±0.89	N/A	N/A	N/A	N/A

All values are the mean of three measurements and expressed as mean ± SD.

[a] Acetylcholine esterase (AChE) inhibitory activity is expressed is expressed in IC_{50} (Concentration of the extract / reference required to inhibit AChE by 50%)

[b] Radical scavenging activity by DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) assay is expressed in IC_{50} (Concentration of the extract / reference required to inhibit DPPH radical formation by 50%)

[c] FRAP(ferric reducing power). Data are expressed as µM.

[d] Radical scavenging activity by NO (Nitric oxide) inhibition assay is expressed in IC50 (Concentration of the extract / refernce required to inhibit NO radical formation by 50%)

[e] Radical scavenging activity by TBA assay is expressed is expressed in IC_{50} (Concentration of the extract / reference required to inhibit HOO- radical formation by 50%)

Table 3: Correlation analysis between total polyphenol, flavonoid content vs antioxidant and acetylcholine esterase inhibitory activity of ten medicinal plant extracts

	DPPH radical scavenging assay (1/I C_{50}) r (R2)	FRAP assay FRAP value (µM) r (R2)	NO radical scavenging assay (1/I C_{50}) r(R2)	TBA assay (1/I C_{50}) R(R2)	AChE inhibitory activity (1/I C_{50})
Total Polyphenol content	0.619 (0.717)	0.937 (0.878)	0.873 (0.891)	0.789 (0.891)	0.673 (0.771)
Total flavonoid content	0.769* (0.789)	0.971* (0.894)	0.845 * (0.771)	0.760* (0.879)	0.723* (0.7 90)

r: Pearson correlation
R2 : Coefficient of determination
The correlation (R) is given between total polyphenol content, total flavonoid content and 1/IC50 in DPPH(2, 2-diphenyl-2-picrylhydrazyl hydrate), FRAP (ferric reducing power) value and IC50 in NO (nitric oxide) inhibition assay. (IC 50 : Concentration of the extract required to inhibit DPPH/NO radical formation by 50%)
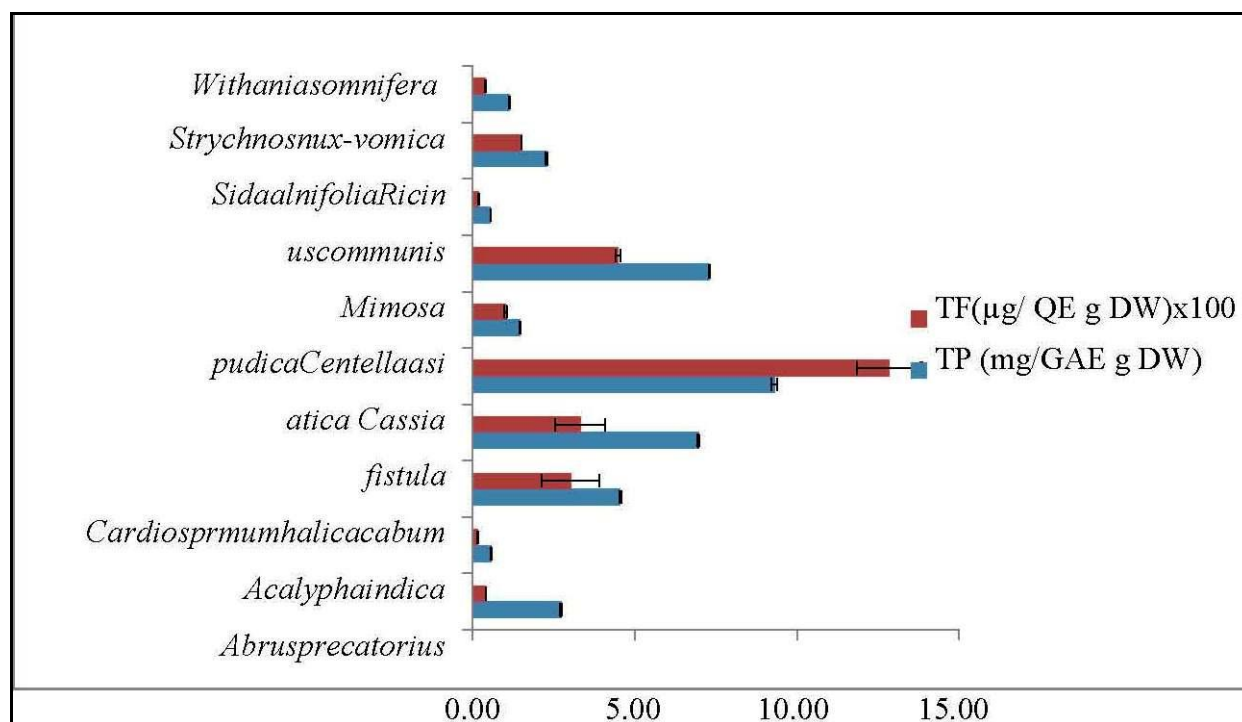*The correlation is statistically significant at $p < 0.05$.

Figure.1. Total polyphenol content and total flavonoid content of ten selected medicinalplant extracts.
Total polyphenol and total flavonoid contents are expressed in gallic acid equivalents of the dry weight (mgGAE/g of DW) and quecertin equivalent (µgQE/g of DW) respectively. All values were the mean of three measurements and expressed as mean ± SD.

CONCLUSIONS

Of the ten medicinal plants selected the extracts of *A. precatorius*, *C. asiatica, S. nux-vomica* and *R. communis* are deserved to be promising sources of AChE inhibitors as well as antioxidants. The results of the correlation analysis revealed that the polyphenol compounds and flavonoids attribute greatly to the antioxidant activity and moderately to the AChE inhibitory activity of the selected medicinal plant extracts. According to our literature survey, AChE inhibitory activity of selected medicinal plant extracts used in Sri Lankan traditional medicine has not previously been reported. Therefore, this is the first report on scrutinizing the AChE inhibitory activity of the selected medicinal plant extracts grown in Sri Lanka. Further studies are warranted on isolation of active metabolites and determination of *in vivo* toxicity of the selected medicinal plant extracts.

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