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In Vitro Assessment for Anti-Acetylcholinesterase and Antioxidant Activities of Methanolic Extracts of Indian Medicinal Plants and Their Phytochemical Screening

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ABSTRACT
Cognitive impairment in Alzheimer's disease (AD) is consistent with the presence cholinergic deficit due to the degeneration of cholinergic neurons in the basal forebrain. Thus, inhibition of acetylcholinesterase is still deliberated as the "gold standard" therapeutic strategy for the management of AD. In the present study, methanolic extracts of 20 medicinal plants native to India were screened for AChE inhibitory and DPPH radical scavenging activities. Out of 20 extracts, *Barleria prionitis, Cadaba fruticosa, Citrullus colocynthis, Cocculus hirsutus, Dendrophthoe falcata,* Dipteracanthus prostratus**,** *Elytraria acaulis, Eranthemum capense, Ipomoea aquatica, Ipomoea sepiaria, Polygala javana, Smilax zeylanica, Sopubia delphiniifolia, Stachytarpheta jamaicensis, Taxillus tomentosus displayed strong* AChE inhibitory activity with IC_{50} values < 100 µg/mL. *Among the tested plants, P. javana, S. delphinifolia, C. fruticosa, E. acaulis, E. capense and I. sepiaria displayed strong DPPH radical scavenging activities with IC_{50} values <40 µg/mL. However, extracts of E. acaulis***,** *S. zeylanica***,** *S. jamaicensis***,** *T. tomentosus***,** *E. capense, P. javana,* D. falcata and C. colocynthis found to have potential for further analysis as they disclosed strong activities against both targets AChE and DPPH. Phytochemical screening of the methanolic extracts of these plants suggested that flavonoids, tannins and alkaloids alone or in combination might be the major contributors for the AChE enzyme inhibitory and antioxidant activities. All together, the present study demonstrates that **t***hese plant extracts will be safer and better candidates for the future disease modifying therapies against this devastating degenerative disorder. Thus, further works on the isolation of phytochemicals that contribute to the biological properties are strongly recommended.*
Keywords: Alzheimer's disease, Acetylcholinesterase, DPPH, Antioxidant, Phytochemical screening, Medicinal plants.

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INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disorder among the elderly, responsible for two-thirds of all dementia cases [1]. It is characterized clinically by progressive memory deficits, impaired cognitive function, behavioral disturbances and a decreasing ability to perform basic activities of daily living [2, 3]. The incidence of AD is set to increase exponentially with the increase in life expectancy and rising population of the elderly and also due to lack of potential remedy. Currently, 50 million people afflicted worldwide with AD and expected to cross 152 million by 2050. Recent reports revealed that, about 8.8 million Indians older than 60 years live with dementia [4]. The estimated dementia prevalence for adults aged over 60 years in India is 7.4% [5].

Although the etiology of AD remains elusive, several factors, such as low levels of acetylcholine (ACh), oxidative stress, dyshomeostasis of biometals and amyloid-β (Aβ) deposits that promote profound neural damage have been considered to play definitive roles in the pathophysiology of AD [6-9]. Since, AD is because of malfunctioning of different biochemical pathways several hypotheses have been proposed, out of which the most successful so far is the "cholinergic hypothesis" [10]. According to this theory progressive decline in the levels of ACh associated with memory and cognition is the biochemical hallmark of the AD. After being delivered in the cholinergic synapses, acetylcholinesterase (AChE) acts primarily as a regulatory enzyme of cholinergic neurotransmission by hydrolyzing ACh to choline and acetate. Therefore, inhibition of AChE enhances the ACh concentration thereby restoration of brain cholinergic activity provides a possible symptomatic treatment option for AD [11, 12]. Accordingly, four of the five U.S. Federal Drug Administration (FDA) approved anti-AD drugs are AChE inhibitors. However, these drugs are known

to have only modest effectiveness on the cholinergic system is more of a symptomatic and none appears to affect progression or prevention of AD [13]. In addition, these drugs have unfavorable side effects due to their short-half-lives. Taking into account these observations, the search for new sources of safe, effective and selective anti-acetylcholinesterase agents with fewer side effects is imperative. In this juncture, the World Health Organization has also recommended the development of improved and safer herbal medicines in this concern [14].

Over production of free radicals and progressive decline of the cellular antioxidant defense with aging, impose oxidative stress. The excess oxidative stress damages biological molecules like cellular lipids, proteins, or DNA thereby causing several chronic diseases, such as cancer, diabetes, aging, atherosclerosis, hypertension, heart attack and neurodegeneration. Since, oxidative stress promotes the appearance of pathological hallmarks it has been implicated in the pathogenesis and progression of AD [15, 16]. Therefore, exogenous intake of antioxidants can help the body to scavenge free radicals effectively and could be considered as potential in both prevention and treatment of AD. Nowadays, there is a noticeable interest in antioxidants, especially from natural rather than from synthetic sources.

With increasing recognition of herbal medicine as an alternative form of health care, exploitation of medicinal plants as alternative AChE inhibitors and antioxidants is of major therapeutic interest in the recent years [17, 18]. Therefore, the objective of this study was to evaluate the AChE inhibitory and antioxidant activities and phytochemical analysis of methanol extracts of 20 selected Indian medicinal plants to explore new pharmacological value for them.

MATERIAL AND METHODS

Chemicals

Acetylthiocholine iodide (ATCI), *Ee* Acetylcholinesterase (EC 3.1.1.7, from Electric eel), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), galantamine and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol and buffer chemicals were purchased from Merck.

Plant materials collection, authentication and sample preparation

Plants investigated in this study were collected in the month of October 2013 from Lankamalla hills located in Kadapa district, Andhra Pradesh, India. Dr. A. Madhusudana Reddy, Professor, Department of Botany, Yogi Vemana University identified the harvested materials employed and specimens were deposited in the herbarium of Yogi Vemana University. The plant materials were cleaned immediately to remove any extraneous material, sliced into small pieces, air-dried for two weeks at room temperature and weighed. The dried materials were ground into fine powder and stored in an air-tight container at ambient temperature until further use.

Plant extraction and sample preparation

Each ground plant material (150 g) was extracted twice with 500mL of 90% methanol by soaking at room temperature for two days. The plant extracts obtained were filtered *in vacuo* through Whatman No.1 filter paper. The combined filtrates were concentrated using rotavapor (Heidolph, Germany) under reduced pressure and then further dried *in vacuo* at ambient temperature for 24h. Yield percentage for each extract was calculated and expressed in terms of air-dried weight of plant material (Table 1). Test solution was prepared by dissolving adequate amount of dried extract in methanol to the desired concentrations.

Assay for inhibition of acetylcholinesterase (AChE)

Acetylcholinesterase inhibition assay was carried out as per the method reported by Ellman et al. with minor modifications [39]. Enzyme activity was first determined in the absence of plant extracts. At least five increasing concentrations of the extracts were assayed to obtain percentage inhibition of the enzymatic activity in the range of 20-80%. In brief, in a 96–well plate, the total volume (250 µL) of reaction mixture consist 150 µL of phosphate buffer (200mM pH 7.7), 10 µL of test sample of various concentrations (15, 30, 90, 150 and 240 µg/mL), 80µL of DTNB (3.96 mg of DTNB and 1.5 mg sodium bicarbonate dissolved in 10 mL phosphate buffer pH 7.7) and 10 µL of enzyme from stock solution (AChE, 2U/mL). The mixture was incubated for 5 min at 25^0C. Then, 15µL of the substrate (acetylthiocholine iodide: 10.85 mg in 5mL of phosphate buffer) was added and incubated for further 5 minutes at 25°C. The rate of reduction in color due to the formation of 5-thio-2-nitrobenzoate anion was measured at 412 nm for 6 min. Galantamine, at 0.12, 0.23, 0.46, 0.92, 1.84, 3.68 and 7.37µg/mL was taken as positive control. The percent of inhibition was determined by comparing the reaction rates for the sample to free enzyme. The IC_{50} values were determined graphically from inhibition curves plotting log inhibitor concentration vs. percent of inhibition.

2.5. DPPH radical scavenging activity assay

DPPH radical scavenging activity was measured based on the method described by Sarikurkcu et al. [40]. DPPH radical solution was deep in purple color. While treating these radical solutions with test sample contains an antioxidant this color disappears. A methanol solution (1.5 mL) of each sample at different

concentrations (25, 50, 100, and 200 µg/mL) were added to 9mL of DPPH (in methanol, 60 mM). These samples were vortexed and incubated for 30min in the dark. The absorbance was measured at 517 nm. For each dilution of the extracts, the DPPH scavenging activity (*I*, %) was determined according to the following expression I(%) = 100 × [(A_{blank}− A_{sample})]/A_{blank}, where A_{blank} is the absorbance of the DPPH (control reaction, containing all reagents except the test compound) and A_{sample} is the absorbance of the tested samples. Ascorbic acid was used as positive control. Results are expressed as the mean ± SD from experiments performed in triplicate.

Phytochemical Tests

Phytochemical analysis of secondary metabolites such as alkaloids, flavonoids, saponins, tannins and terpenoids was performed using standard protocols as described previously [41].

Test for Alkaloids

For the confirmation of alkaloids in the titled plants, 5mL of methanolic test solution was taken into a test tube; 1.5mL of 10% HCl was poured into it and heated the mixture for 20min, cooled and filtered. The filtrate was divided into two portions. To the first portion, 1mL of Dragendorff's reagent was added [41, 42]. Formation of a reddish or orange colored precipitate indicates the presence of alkaloids. To the second portion, Mayer's reagent was added. Formation of turbid or white precipitate indicates the presence of alkaloids.

Test for Flavonoids

A 5 mL of each methanolic test solution of plant extract was taken into a test tube and 5 mL of dilute ammonia solution was added followed by addition of 1 mL conc. H_2SO_4. A change in color to yellow indicating the presence of flavonoids in the samples [42].

Test for Tannins

A 5 mL of test solution was added with few drops of 0.1% ferric chloride solution. Indication of a brownish-green or bluish black coloration shows the presence of tannins [42, 43].

Test for Terpenoids

A 5 mL of methanolic test solution of selected plant samples was taken in a test tube, then 2 mL of chloroform were mixed, then 3 mL of sulphuric acid were added. Formation of reddish-brown color indicates the presence of terpenoids in the selected plants [42, 43].

Test for Saponins

In a test tube, 5mL of test solution of each plant extract under investigation was boiled with 10 mL water for few minutes and filtered. The filtrate was vigorously shaken. The persistent froth was observed. Then 3 drops of olive oil were mixed with the froth and shaken vigorously. The formation of the emulsion indicates the presence of saponins in the samples [42, 43].

RESULTS

The present study is an attempt to identify and compare the potential of the plants for their antioxidant and acetylcholinesterase inhibitory activities. For this purpose, methanolic extracts of 20 different plants were prepared, evaluated for their anti-acetylcholinesterase and antioxidant effects and screened for phytochemicals.

Acetylcholinesterase inhibitory activity

The *acetylcholinesterase* inhibitory activity of the methanolic extracts was measured using Ellman's colorimetric method in 96-well microplate [39]. Acetylthiocholine iodide and galantamine were used as substrate and positive control, respectively. In the present study, 20 medicinal plants belonging to 14 families were evaluated for their AChE inhibitory potential. The percentage of inhibition data and IC_{50} values were presented in Table 2. All the tested plant species showed some level of inhibitory activity against AChE in dose-dependent way (Figure 1). At the highest concentration (150 mg/mL), seventeen plants showed good (>50% inhibition), and three showed moderate (30-50% inhibition) AChE inhibition. The tested plants displayed better inhibitory potency with IC_{50} value range from 19.99 to 221.22µg/mL. Among the extracts tested *S. zeylanica*, *S. jamaicensis, E. acaulis, T. tomentosus, E. capense, P. javana, D. falcata* and *C. colocynthis* were found to be most potent AChE inhibitors as they had IC_{50} values 19.99, 23.67, 23.86, 32.07, 40.16, 45.16, 45.55 and 48.55 µg/mL, respectively.

Antioxidant activity

To control the deleterious role of free radicals in disorders like AD, radical scavenging activities (RSA) are very important. The antioxidant activity of methanolic extract of plants was determined using the DPPH free radical by the addition of various concentrations of extracts. Concerning DPPH assay, the degree of color change of purple-colored DPPH radical solution [40] by hydrogen donation ability of fractions was measured at spectrophotometrically at 517 nm and the results are presented in Table 2. Figure 2 depicts a dose-response curve with respect to an increase of extract concentration. In DPPH assay, all the extracts showed strong RSA with IC_{50} values in the range of 19.18-326.87µg/mL. The highest activity was recorded

in *P. javana* and the lowest activity was registered by *Cassia angustifolia***.** The higher activity against DPPH indicating that the phyto constituents of tested extracts have capacity to donate hydrogen to a free radical and prevent the potential damage.

Phytochemical analysis

Plant methanolic extracts with cholinesterase inhibitory and DPPH scavenging activities were preliminarily screened for the existence of alkaloids, steroids, terpenoids, saponins, tannins and flavonoids [41-43]. Plants which possess plenty of these secondary metabolites may have more priority in drug discovery. The present study has revealed the presence of phytochemicals such as flavonoids, alkaloids, terpenoids, tannins and saponins in the samples which were usually considered as bioactive constituents. The results of the phytochemical screening showed that 3 plants are rich in alkaloids, 7 extracts are rich in flavonoids, 2 samples are rich in tannins, 2 extracts are rich in terpenoids and 3 extracts showed to contain much saponins (Table 3).

DISCUSSION

It is estimated that 70 to 80% of the people worldwide rely chiefly on traditional health care system and largely on herbal medicines. Out of 20,000 medicinal plants of the world, India harbors about 15 percent (3000–3500) medicinal plants. About 90 percent of these are found growing wild in different climatic regions of the country. Large sections of the rural population in India still rely on medicinal plants and herbal medicines as primary health care, however, to date, only a small percentage of the medicinal plants of the Southern India have been investigated phytochemically, and the fraction that has been subjected to biological and pharmacological screening is even smaller [44, 45]. Hence, it will be appropriate to screen more medicinal plants scientifically and methodically in search of new pharmacological values for known plants.

Methanolic extract of aerial parts of *S. zeylanica* showed highest AChE inhibitory activity with IC_{50} value of 19.99 µg/mL and found to contain flavonoids, tannins and saponins. *Smilax* species have been shown to have flavonoids, phenolics and phenyl propanoids and found to display anti-infective, anti-cancer, anti-inflammatory, antioxidant, cardiovascular protection, hepatoprotective activities [46, 47]. Steroidal saponins have been isolated from *Smilax* species and reported to possess anti-inflammatory, neuroprotective and hepatoprotective activities [48]. As these effects were implicated in AD treatment, similar compounds present in the *S. zeylanica* may contribute to the strong AChE inhibitory and antioxidant properties. Pyrazole alkaloids were reported from *E. acaulis* and anti-inflammatory, antioxidant, antihyperglycemic and antifertility activities were also documented for this plant extract [49]. The methanolic extract of *E. acaulis* showed an IC_{50} value of 23.86 µg/mL for AChE inhibition, which may be ascribed to the presence of alkaloids, flavonoids and tannins in this plant.

S. jamaicensis has been reported to exhibit anti-inflammatory, antioxidant and neuroprotective activities which were found to be significant in the management of AD. Phytochemical studies on this plant species reported several steroidal glucosides, flavonoids and tannins [37, 50]. In the present study, methanolic extract of *S. jamaicensis* showed strong AChE inhibitory activity with IC_{50} value of 23.67 µg/mL, which may be due to the presence of flavonoids and tannins. *C. colocynthis* and *D. falcata* were found to be strong AChE inhibitors as they had IC_{50} values 48.55 and 45.55 µg/mL, respectively. The anticonvulsant, anti-inflammatory and antioxidant activities have been reported for the hydroalcoholic fruit extract of *C. colocynthis* and ethanolic extract of stems of *D. falcata* [24, 26]. Several cucurbitacins, polyphenols, flavonoids have been reported from the fruits of *C. colocynthis* [51]. Phytochemical investigation on *D. falcata* revealed several pentacyclic triterpenes and flavonoids with anticonvulsant, anti-inflammatory and antioxidant activities [52]. As convulsion is a neurologic disorder, compounds present in the *C. colocynthis* and *D. falcata* may be responsible for their activity. The medicinal plant *T. tomentosus* had anti-infammatory, antioxidant, anticancer, antimicrobial, antiviral, diuretic, antihypertensive, antihyperglycemic properties [38, 53, 54]. Phytochemical studies on this plant species reported several flavonoids, phenolics, phenylpropanoids and tannins as major constituents. Thus, the flavonoids and tannins could be responsible for AChE inhibitory and DPPH radical scavenging activities of *T. tomentosus* in this study.

Polygala is considered as a powerful tonic herb in traditional medicine that can help to develop the mind and aid in creative thinking. The observed potent AChE inhibitory and antioxidant activities of the methanol extract of *P. javana* may be due to the presence of saponins, phenolics and flavonoids as principle phytochemicals in it [55]. Indeed, many of saponin components from *Polygala* species are reported to have neuroprotective potential against Aβ-induced neurotoxicity in PC 12 cells suggesting a potential therapeutic value for *Polygala* in neurodegenerative disorders [56, 57]. Limited available data revealed that *E. capense* has been ethnopharmacologically used as anti-inflammatory and antioxidant agents [29]. These activities have key role in developing anti-AD agents. Its phytochemistry remains to be investigated.

However, the present screening revealed the presence of flavonoids and terpenoids as phytoconstituents of *E. capense*, hence may be responsible for its anti-AChE activity.

CONCLUSIONS

The present *in vitro* study of the methanolic extracts of the twenty selected plants revealed significant antioxidant, and acetylcholinesterase inhibitory activities. Amongst, *S. zeylanica*, *S. jamaicensis, E. acaulis, T. tomentosus, E. capense, P. javana, D. falcata* and *C. colocynthis* are particularly promising in the context of this study because of their potent bioactivities against AChE and DPPH and have potential for development as therapeutic agents against AD. Strong activities can be related with the presence of antioxidant constituents such as tannins and flavonoids in most of the cases. The study corroborates traditional claims of these Indian medicinal plants to enhance cognition or to correct cognitive decline. However, safety will have to be examined in more detail. This warrant further investigation to isolate and characterize the active substances in these plants and to explore their potential in combating neurodegenerative diseases like AD.

Table 1: Indian medicinal plants used in the present study and their ethnomedicinal uses.

Plant	Family	Local name	Voucher no	Traditional uses
Alternanthera pungenes	Amaranthaceae	Machi ponnaganti	YVU 05 AGD	Whole plant: Gonorrhoea, diuretic, galactagogue, febrifuge, cholagogue [19].
Argemone mexicana	Papaveraceae	Bramhadandi	YVU 11 AGD	Whole plant: Leprosy, eczema, leucorrhoea, dental problems, eye problems, constipation, anaemia. Leaves: Menorhoea, leucorrhoea, indolent ulcers and skin diseases Leaves-Diabetes [20].
Barleria prionitis	Acanthaceae	Gorantachettu	YVU 13 AGD	Whole plant: Antidontalgic Leaves: Stomach disorders, urinary infection, gout, pruritus, poisoning, leprosy, diabetes. Root: Diaphoric, expectorant, indigestion, fever, dyspnoea, cough [21].
Cadaba fruticosa	Capparidaceae	Aadamorinika	YVU 17 AGD	Leaves: Eczema, swelling, constipation, purgative, rheumatism Root: Deobstruent, emmenagogue, uterine obstructionsa, antiphlogistic [22].
Cassia angustifolia	Caesalpiniaceae	Nelathangedu	YVU 21 AGD	Leaves: Laxative, purgative, constipation, febrifuge, gout, worm infections, skin diseases, abdominal and blood disorders. Root: Skin disease and asthma Flowers: Diabetes, urinary disorders [23].
Citrullus colocynthis	Cucurbitaceae	Chittipapara	YVU 24 AGD	Fruit: Hydragogue, cathartic, gastrointestinal irritant, rheumatism, jaundice, diabetes, varicose veins and piles. Root: Diuretic, febrifuge, purgative, jaundice, rheumatism, urinary troubles, inflammation, swelling. Leaves: Sprains [24].
Cocculus hirsutus	Menispermaceae	Cheepuruteega	YVU 26 AGD	Root: Dyspepsia, laxative, demulcent, sudorific, alterative, antirheumatic Leaves- Refrigerant, eczema, abdominal disorders, menorrhagia, syphilis, polyuria, diabetes, prurigo and impetigo [25].

Dendrophthoe falcata	Loranthaceae	Jiddu	YVU 33 AGD	Whole plant: Haemorrhage, urinary calculi, worm infections, wounds. Bark: Astringent, narcotic, mania. Leaves: Menstrual disorders, consumption, asthma, wounds [26].
Dipteracanthus prostratus	Acanthaceae	Nelaneelambaramu	YVU 33 AGD	Leaves: Renal infection, gonorrhea, syphilis and other venereal diseases [27]
Elytraria acaulis	Acanthaceae	Eddadugu	YVU 36 AGD	Leaf: Venereal diseases, cough [28]
Eranthemum capense	Acanthaceae	Konkaani	YVU 39 AGD	Whole plant: Anti-inflammatory [29]
Ipomoea aquatica	Convolvulaceae	Thootikoora	YVU 49 AGD	Root: Diabetes, galactogogue, aphrodisiac, general debility Whole plant: Liver complaints Flowers: Ring worm [30]
Ipomoea sepiaria	Convolvulaceae	Mettathuti	YVU 52 AGD	Whole plant: Diuretic, tonic, aphrodisiac, and anti-ulcer , burning sensation, strangury, general debility, and sterility in women [31]
Polygala javana	Polygalaceae	Selanganchedi	YVU 71 AGD	Leaves: Cut, boiles, constipation, bronchitis, asthma, catarrhal infections Root: Bronchitis, fever [32]
Premna tomentosa	Verbenaceae	Pedda narava	YVU 72 AGD	Root: Stomach disorders, diabetes Bark: Diarrhoea, stomach disorders Leaves: Dropsy, indigestion, diuretic, vulnerary, anaemia, jaundice [33]
Punica granatum	Lythraceae	Danimma	YVU 78 AGD	Fruit: Astringent, diarrhea, dysentery, colitis, diabetes, hyperacidity, cardiotonic, haemorrhage, dental disorders, intermittent fever, dysuria, piles, anaemia, sterility, cough, uterine disorders [34]
Smilax zeylanica	Smilacaceae	Kondadanthena	YVU 81 AGD	Root:Venereal diseases, rheumatism, urinary complaints, dysentery, nervous system disorders, epilepsy, psychosis, polyuria, hemiplegia, parkinsonism, congenital diseases, leprosy, rejuvenator [35]
Sopubia delphinifolia	Scrophulariaceae	Edintajada	YVU 85 AGD	Leaves: Sores, astringent Root: Sores on feet [36]
Stachytarpheta jamaicensis	Verbenaceae	Ceemal-nayurur	YVU 86 AGD	Whole plant- Intestinal worms, ulcers, venereal diseases, dropsy, stomach problems, abortifacient, febrifuge, anti-inflammatory, rheumatism Bark: Diarrhea, dysentery Leaves: Cardiac troubles [37]
Taxillus tomentosus	Loranthaceae	Nooguthellanugu	YVU 94 AGD	Whole plant: Skin cancer Flower and fruit: Mental disorders Leaves: Leprosy, skin diseases [38]

Table 2: IC_{50} Values of 90% methanolic extracts of plants in AChE inhibition and DPPH free radical scavenging assays.

Plant	Part used	% of yield	% inhibition		AChE IC_{50} µg/mL	DPPH IC_{50}µg/mL
			Conc. µg/mL	% of inhibition		
A. pungenes	Aerial parts	12.26	*15* *30* *90* *150*	*15.25* *20.59* *36.87* *52.69*	142.47	41.18
A. mexicana	Whole plant	11.25	*30* *90* *150* *240*	*19.68* *36.98* *47.58* *64.98*	149.56	91.71
B. prionitis	Aerial parts	07.29	*30* *90* *150* *240*	*41.59* *50.47* *58.69* *72.25*	91.42	52.27
C. fruticosa	Aerial parts	9.67	*30* *90* *150* *240*	25.28 50.87 64.98 75.36	83.79	21.39
C. angustifolia	Aerial parts	10.49	*30* *90* *150* *240*	*16.36* *27.59* *39.35* *52.36*	221.22	326.87
C. colocynthis	Fruit	16.69	*15* *30* *90* *150*	35.21 46.78 55.41 63.76	48.55	50.09
C. hirsutus	Aerial parts	08.69	30 90 150 240	32.11 51.47 65.48 79.28	72.32	79.44
D. falcata	Whole plant	11.68	*15* *30* *90* *150*	31.25 40.21 61.58 73.28	45.55	43.39
D. prostratus	Aerial parts	16.35	*15* *30* *90* *150*	*21.25* *32.29* *50.29* *69.97*	68.77	80.39
E. acaulis	Whole plant	13.59	*15* *30* *90* *150*	*44.24* *53.18* *65.79* *78.97*	23.86	23.23
E. capense	Whole plant	13.69	15 30 90 150	31.25 47.25 58.59 68.69	40.16	34.17
I. aquatica	Whole plant	9.67	*30* *90* *150* *240*	*21.97* *33.36* *40.11* *52.36*	91.01	87.95
I. sepiaria	Whole plant	7.89	*15* *30* *90* *150*	*25.36* *40.97* *57.27* *73.97*	51.19	39.16
P. javana	Whole plant	14.11	*30* *90* *150* *240*	*16.11* *29.97* *51.28* *66.69*	45.16	19.18
P. tomentosa	Leaves	11.19	*30* *90*	*11.28* *33.25*	122.01	62.9

			150 *240*	*55.87* *74.78*		
P. granatum	Peel	10.16	*30* *90* *150* *240*	*25.56* *40.28* *68.76* *81.97*	104.27	86.09
S. zeylanica	Aerial parts	13.25	*15* *30* *90* *150*	*42.7* *59.29* *68.57* *77.94*	19.99	42.9
S. delphinifolia	Whole plant	10.29	*30* *90* *150* *240*	*35.29* *60.97* *75.19* *84.67*	55.44	21.19
S. jamaicensis	leaves	12.59	*15* *30* *90* *150*	*43.69* *54.29* *65.57* *75.19*	23.67	49.28
T. tomentosus	Whole plant	13.28	*15* *30* *90* *150*	*31.29* *44.16* *59.95* *76.26*	32.07	44.86
Galantamine	—	—	—	—	0.77 ± 0.09	—
Ascorbic acid	—	—	—	—	—	11.35±1.39

Table 3: Qualitative phytochemical screening of methanolic extracts of plants

Plant	Alkaloids	Flavonoids	Tannins	Terpenoids	Saponins
A. pungenes	—	+	—	+++	+
A. mexicana	+++	+	—	+++	—
B. prionitis	—	++	++	+	+
C. fruticosa	++	++	—	++	+
C. angustifolia	+	+++	++	+	+
C. colocynthis	++	+++	+	—	+++
C. hirsutus	+++	+	+	—	+
D. falcata	—	++	++	++	+
D. prostratus	—	+	—	—	+
E. acaulis	+++	+++	++	—	+
E. capense	+	++	—	++	—
I. aquatica	+	+	+	+	_
I. sepiaria	+	+	+	+	+
P. javana	+	+++	++	+	+++
P. tomentosa	+	+	+	+	+
P. granatum	—	++	++	+	—
S. zeylanica	+	+++	+++	+	+++
S. delphinifolia	++	+	++	+	++
S. jamaicensis	+	+++	+++	+	++
T. tomentosus	+	+++	++	+	—

(+++): Rich in quantity, (++): Moderate in quantity, (+): Low in quantity,(—): Absent

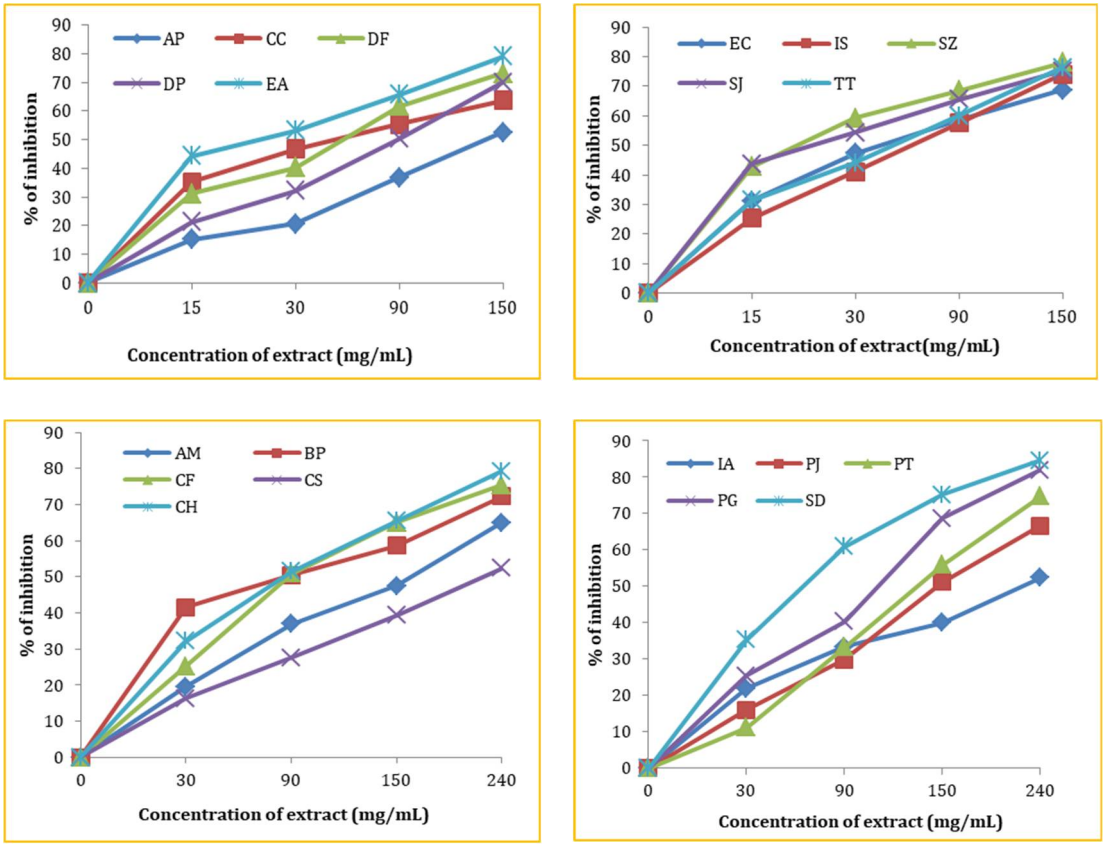

Figure 1: Inhibition of AChE activity by plant extracts. Results with Mean values of 3 independent experiments have been plotted.

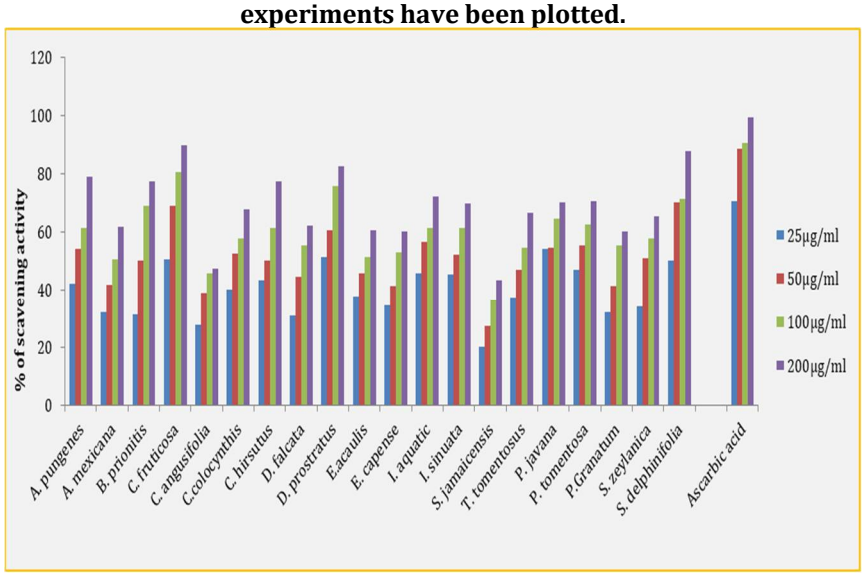

Figure 2. DPPH Free Radical Scavenging Activity of the Extracts.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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