Contents lists available at ThomsonReuters

The American Journal of Science and Medical Research

Journal homepage: http://globalsciencepg.org/ajsmr.html

Research Article

In Vitro Screening of 20 Indian Medicinal Plants for their Anti-Cholinesterase Effects

D.Sripriya[1], T. Suvarna[2] *

[1,2] *Department of Biotechnology, Dravidian University, Kuppam, A.P., India.*

*Corresponding author:
E-mail: sripriyashok13@gmail.com

http://dx.doi.org/10.17812/ajsmr521
Received : 3 March, 2019
Accepted; 7 April, 2019
Available online :21 May, 2019

ISSN: 2377-6196
Published by Global Science Publishing Group. USA

Keywords: Alzheimer's, Graph Pad PRISM, Acetylcholine, Microplate, Medicinal plants

ABSTRACT

The purpose of the present study is to contribute to an understanding of the actual and potential role of 20 traditional medicinal plants based Methanol extracts role in the Enzyme inhibition Assays against Alzheimer's disease. The objective of the study was to investigate the Acetyl cholinesterase Enzyme inhibitory activity of 90% methanol extracts of 20 selected medicinal plants. Quantitative Analysis made with the help of 96 wells Micro plate technique. Readings have been done with the help of Micro plate reader. Calculation of IC-50 Values by interpolation of a RIA (or) ELISA interpolates unknowns from sigmoid curve using Graph Pad PRISM2.8.1.1 (330) Software. Comparison of IC50 Values with standard. In the present study, methanol extracts of 20 plants used in Indian Ayurvedic system of medicine for improving cognitive function were screened for acetyl cholinesterase inhibitory activity by Ell man's micro plate calorimetric method against Alzheimer's disease.

1. Introduction

Alzheimer's disease is a worsening illness that leads brain cells to deteriorate and die. The main reason for this disease is dementia — a continuous deterioration in thinking, behavioral and social skills that damages a person's ability to function independently. Some of the early symptoms of this disease are forgetting recent events or conversations. As the disease advances, a person with Alzheimer's disease will develop severe complications like memory impairment and lose the ability to carry out day to day tasks.

At present, with the available medicines for Alzheimer's disease, we can improve the symptoms or slow the rate of decline. Occasionally these treatments can assist people with Alzheimer's disease maximize function and maintain independence for a time. Different programs and services can help support people with Alzheimer's disease and their caregivers. For the time being there is no complete treatment that cures Alzheimer's disease or revises the disease process in the brain. In advanced stages of the disease, complications from severe loss of brain function like dehydration, malnutrition or infection finally leads to death.

Particular nerve cells are very inactive in people who have advanced Alzheimer's disease. In this case, brain cannot send signals promptly. Certain medications like Cholinesterase inhibitors can increase the communication between the nerve cells and improve the symptoms of Alzheimer's. The approved dosage of these drugs is mild to moderate. At present three different cholinesterase inhibitors are available in Germany via; donepezil, Galant amine and Rivastigmine. They can be administered in the form of tablets. Rivastigmine is also available in a patch and this drug can be absorbed into the body through skin.

In Biochemistry, a **cholinesterase** or **choline esterase** is a family of esterase's that lyses choline-based esters, several of which serve as neurotransmitters. Thus, it is either of two enzymes that catalyze the hydrolysis of these cholinergic neurotransmitters, such as breaking acetylcholine into choline and acetic acid. These reactions are crucial to allow a cholinergic neuron to return to its resting state after activation. For example, in muscle contraction, acetylcholine at a neuromuscular junction provokes a contraction; but for the muscle to relax afterward, rather than remaining locked in a tense state, the acetylcholine should be broken down by a choline esterase. The main type for that purpose is acetyl cholinesterase (also called choline esterase or erythrocyte cholinesterase); it is found mainly in chemical synapses and red blood cell membranes. The other type is Butyrylcholinesterase (also called choline esterase or plasma cholinesterase); it is found mainly in the blood plasma.

2. Material and Methods

2.1 Preparation of samples:

250 Grams of plant leafs fine powder was collected by grinding followed by fine sieving of the plant material. Powder dissolved in 90% of Methanol (1H20:4M), 100ML. After 10 days the solvent with plant constituents were filtered with Whatman

Table-1: List of 20 Medicinal plants and their Uses in Medicine

Sr.No.	Name of the Plant	Family	Uses
1	*Abutilon indica*	Malvaceace	Laxative, Analgesic, Anti-diabetic, Anti-inflammation and blood tonic agent.
2	*Acalypha fruticosa*	Euphorbiaceace	Liver problems, stomach ache best remedy. (Antioxidant activity in vitro and anti-inflammatory activity in rats).
3	*Alangium salvifolium*	Alangiaceace	Treatment of various disease, against, diabetes, antioxidant, anti-arthritis, di-uretic, antifertility, antimicrobial, antioxidant, Anti-epileptic, antifungal properties are present.
4	*Amarthus virudis*	Amaranthaceace	Decoction of the entire plant is used to stop dysentery and inflammation s and also to purify the blood. The root juice is used to treat inflammation during urination .It is also taken to treat constipations. Leaves are diuretic, febrifuge and purgative in nature.
5	*Alternanthera sessile*	Amaranthaceace	The plant has diuretic, cooling, and Useful in the treatment of dysuria and hemorrhoids. (Anti-glucosidase and anti-oxidant activity of solvent fractions of Alternanthera sessile was proven clinically).
6	*Alternanthera tennela*	Amaranthaceace	Fever (tennela), infections and genital inflammation. Inhibit lymphocyte activation. Anti-viral and hepatic protective properties. Ant nociceptive effects and analgesic activity. A, tennela have antibiotic activity in assay using Gram+Ve, Gram-Ve, Bacteria in-vitro.
7	*Boerhaavia diffuse*	Nyctaginaceae	Anti-cancer, Anti-inflammation, pain relief, treating indigestion .Bio-active compounds present in both the leaves and roots.
8	*Calophyllum inophyllum*	Calophyllaceae	Inhibitors of HIV-type 1 .Cancer chemo –preventive agents are present. Skin problems, protective activity on the vascular system. Reduces old scars. Anti-septic and disinfectant. Root decoction is traditionally used to treat ulcers, boils and ophthalmic. Leaves are used in inhalation to treat migraine and vertigo.
9	*Carchorus aestuans*	Malvaceae	Anti-cancer activity against epidermal carcinoma .Anti-leukemic activity in U937 and HL-60 cell lines.
10	*Eincostemma axillare*	Gentianaceae	Diabetis mellitus, rheumatisum, Abdominal ulcers, hernia, swelling, itching and insect poisoning.
11	*Eryrthroxylum monogynum*	Erythroxylaceae	Anti-diabetic, Anti tumour and anti-cytotoxic activity, hepatoprotective activity, anti-obesity activity, Nephroprotective activity, Antihyperlipidemic activity, Ameliorative activity, anti plasmodial activity.
12	*Euphorbia hirta*	Euphorbiaceae	Anti-diabetic, Anti-cancer, diarrhea, dysentery, intestinal parasitosis, asthma, bronchitis, hay fever.
13	*Gmelina asiatica*	Verbenaceae	Rheumatism, yaws and nervous disease, eczema, cough, anti-microbial activity.
14	*Grewia hirsuta*	Tiliaceae	Bleeding disorders, dysuria, aphrodisiac, anti-aging herb, neurological disorders, constipation and gastritis.
15	*Leucophyllum frutescens*	Scrophulariaceae	Leaves decoction used for treating lung congestion, bronchitis, chills and fever associated with colds.
16	*Phyllanthus madraspetensis*	Phyllanthaceae	Hypertension, diabetes, hepatic, urinary and sexual disorders and other common ailments.
17	*Phyllanthus pinnatus*	Phyllanthaceae	Hypertension,diabetes,hepatic,urinary and sexual disorders and other common ailments.
18	*Santalum album*	Santalaceae	Anti-bacterial, anti-cancer, versatile therapeutic and health care uses.
19	*Senna fistula*	Fabaceae	Anti-bilious, burning sensations, leprosy, skin diseases, syphilis , tubercular glands, leaves for erysipelas, malaria, rheumatism and ulcers, buds for biliousness, constipation, fever, heart diseases, inflammation, flower as purgative, fruit as anti-inflammatory, antipyretic, abortifacient, demulcent, purgative, refrigerant.
20	Tibullus terrestris	Zygophyllaceae	Chest pain, Eczema, Enlarged prostate, Infertility, Sexual problems in women's, Cancer, coughs, anemia.

Table: 2. IC50 Values of 20 plants

Sr.No.	Concentrations (µg/mL)	IC50 Values (µg/mL)
Plant-1	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	22.96+/-0.375
Plant-2	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	4.731+/-2.534
Plant-3	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer	28.86+/-9.106
Plant-4	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	14.78+/-1.224
Plant-5	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	11.16+/-0.473
Plant-6	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	47.79+/-2.466
Plant-7	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	44.28+/-2.713
Plant-8	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	47.166+/-3.926
Plant-9	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	23.90+/-2.711
Plant-10	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	35.95+/-0.54
Plant-11	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	17.94+/-1.408
Plant-12	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	25.38+/-3.64
Plant-13	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	53.91+/-4.986
Plant-14	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	41.68+/-0.904
Plant-15	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	25.27+/-0.102
Plant-16	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	9.20+/-5.031
Plant-17	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	10.69+/-11.94
Plant-18	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	11.17+/-7.544
Plant-19	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	59.42+/-0.106
Plant-20	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	31.882+/-90.35
Standard (Galamar4)	10,20,30,40,50,60,70,80.(Plant sample) 990,980,970,960,950,940,930,920. (Phosphate buffer)	79.46+/-1.119

filter paper. Collected within the RB of rot evaporator. 60 RPM/Minute 40^0 of temperature gradually increased to 80 degrees to separate the solvent from the plant constituents .After complete separation of the solvent, crude extract is collected with the help of chloroform and pure Methanol. Now the extract is collected in a collecting vessel. Complete dryness is very important for enzyme inhibitory activity. After 3 months samples have been used for Enzyme assays.

2.2 Preparation of Dilutions:

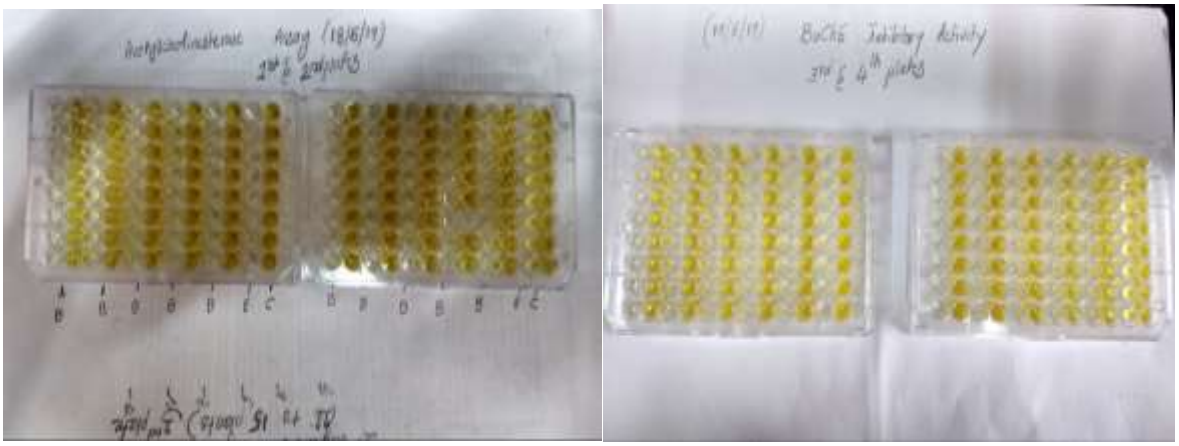
Analysis of 5 plant extracts with total 40 different concentrations for each plant 8 concentractions, 1H,1G,1F,1E,1D,1C,1B,1A. 1 is made up of with 20µg of plant sample dissolved with 1 ML of phosphate buffer. One 96 well micro plate can be used for 5 plants, along with Control. Total 4 Microplates used to proceed the complete assay.

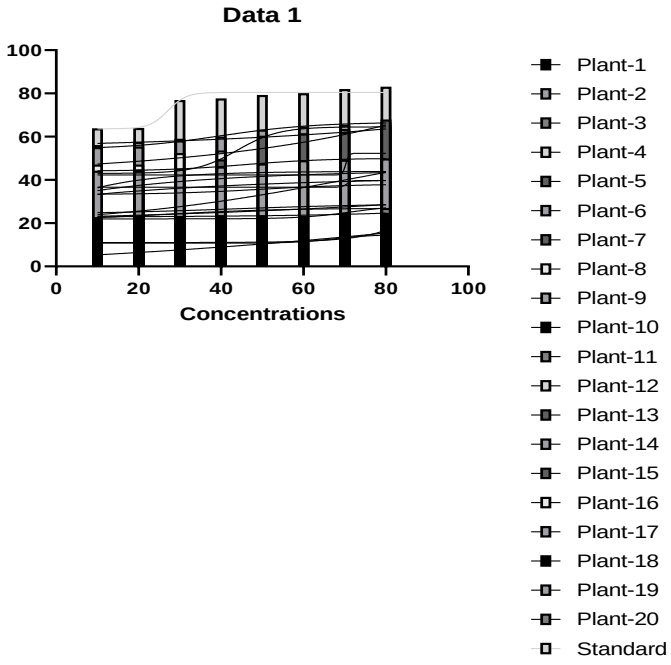
Standard for Cholinesterase: AChE Galamar-4 is the standard.
Note: 990,980,970,960,950,940,930,920 ml of Phosphate buffer. 10, 20,30,40,50,60,70,80, ml of Plant extracts.

2.3 AChE Assay Principle:

The DTNB-Thiols assay measures sulfhydryl groups with the Thiol reagent 5-5dithiobis [2nitrobenzoic acid] (DTNB), which forms the 5-thionitrobenzoic acid and a mixed disulfide. Under conditions of oxidative stress, free Sulfhydryl's decrease and Disulfides increase. Determination of the free Thiol concentration in biological samples reflects the ability to detoxify lipid and other peroxides.

Figure-1: In-Vitro 96 well microplate





Exocell's DTNB-Thiols assay can be used with a spectrum of biological samples including body fluids, tissue, and cell specimens. An Acetylcholinesterase Assay is based on an improved Ellman method, in which Thiocholine produced by the action of acetyl cholinesterase forms a yellow color with 5.5'-dithiobis (2-nitrobenzoic acid). The intensity of the product color, measured at 412 nm, is proportionate to the enzyme activity in the sample. AChE catalyzes the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation.

2.4 Acetyl cholinesterase Assay Procudure:

Add 100 µL of buffer +10µL of plant extract +10 µL of AChE +50µL DTNB one by one. Pre incubation period is 5 Minutes. Add 15µL of Acetylcholine Iodide (substrate). Read the absorbance at 412 nm after 5 Minutes of incubation.

3. Results and Discussion

Percent of inhibition is calculated with the help of formula.

% of Inhibition= T-C÷C×100
C=Control
T= Test (Blank-Test).

Based on average of 8 values (Control) and according to the IC50 Values, Comparing with the Galamar-4 (Standards) 20 plant sample values are less. Cholinesterase activity was assessed by plotting percentage of inhibition against a range of concentrations followed by determining the IC50 values by interpolation of a RIA (or) ELISA Interpolate unknowns from sigmoidal curve using Graph Pad PRISM2.8.1.1 (330) Software.

4. Conclusion:

The methanolic extract s of 20 selected medicinal plants were investigated using in-vitro assay. The IC50 (Haifa maximal inhibitory Concentration) of the extracts on Cholinesterase were significantly nearer to some plants (19,13 (59.42+/-0.106,53.91+/-3.64) plants), not higher than the reference (Standard-Galamar-4). (79.46+/-1.119). 7,8,6 Plants possess moderate capacity (44.28+/-2.713,47.166+/-3.926,47.79+/-2.466). 10 and 20 plants have normal range of Inhibitory capacity (35.950+/-0.54, 31.882+/-90.35).Plants 3,12,15,9,1 ranges from (28.86+/-9.106,25.38+/-3.64,25.27+/-0.102,23.906+/-2.7,22.96+/-0.375). Low level or without activity plants also detected in this regard. 2, 16, 17, 5, 18. (4.73+/-2.534, 9.209+/-5.031, 10.69+/-11.94, 11.16+/-0.473, 11.17+/-7.544).

.

Competing Interests

The authors have declared that no competing interests exist.

References

1. Ellman G. L., Courtney K. D., Andres V., Jr., Featherstone R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology. 1961;7(2):88–95. doi: 10.1016/0006-2952(61)90145-9. [PubMed] [CrossRef] [Google Scholar]
2. Stedman, E., Stedman, E., Easson, L.H.: Choline-esterase. An enzyme present in the blood-serum of the horse. Biochem. J. 26(6), 2056 (1932) Cross Ref.
3. (https://doi.org/10.1042/bj0262056) Google Scholar
4. (http://scholar.google.com/scholar_lookup?title=Choline esterase.%20An%20enzyme%20present%20in%20the%20bloodserum%20of%20the%20horse&author=E.%20Stedman & author=E.%20Stedman&author=LH.%20Easson&journal=Biochem.%20J.& volume=26&issue=6&pages=2056&publication_year=1932) .
5. Silver, A.: The Biology of Cholinesterases. American Elsevier Pub. Co., New York (1974) Google Scholar (https://scholar.google.com/scholar?q=Silver%2C%20A.%3A%20The%20Biology%20of%20Cholinesterases.%20American%20Elsevier% 20Pub.%20Co.%2C%20New%20York%20%281974%29).
6. Kovarik, Z., et al. (2003) Biochem. J. 373: 33-40.
7. Magnottl, R.A., et al. (1987) Clin. Chem. 33/10: 1731-1735.
8. Vizi, E.S., et al. (1985) J. Pharmacol. Methods 13: 201-211.
9. http://www.actabp.pl/pdf/3_2013/401.pdf[Research Gate].
10. Cuartero M., García M. S., García-Cánovas F., Ortuño J. Á. New approach for the potentiometric-enzymatic assay of reversible- competitive enzyme inhibitors. Application to acetylcholinesterase inhibitor galantamine and its determination in pharmaceuticals and human urine. *Talanta*. 2013;110:8–14. doi: 10.1016/j.talanta.2013.03.022.
11. da Silva J. I., de Moraes M. C., Vieira L. C. C., Corrêa A. G., Cass Q. B., Cardoso C. L. Acetylcholinesterase capillary enzyme reactor for screening and characterization of selective inhibitors. *Journal of Pharmaceutical and Biomedical Analysis*. 2013;73:44–52. doi: 10.1016/j.jpba.2012.01.026.