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IN-VITRO ANTICHOLINESTERASE ACTIVITY OF *PHYLLANTHUS EMBLICA* AND *TAMARINDUS INDICA*

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Keywords:

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ABSTRACT: Alzheimer's disease (AD) is a major type of dementia. AD is associated with memory impairment and cognitive deficit. It is characterized by low levels of acetylcholine in the brain of AD patients. The inhibition of acetylcholinesterase (AChE) has been one of the most used strategies for the management of mild to moderate AD. In the present study, n-hexane, ethyl acetate, and hydroalcoholic leaf extracts of two medicinal plants *Phyllanthus emblica* (Euphorbiaceae) and *Tamarindus indica* (Fabaceae) were screened for anticholinesterase activity and compared with standard acetyl-cholinesterase inhibitor (galantamine) by Ellman's spectrophotometric method. The results obtained from the three extracts of *P. emblica* and *T. indica* against AChE enzyme was evaluated for percentage inhibition and IC_{50} values. Among the extracts of *P. emblica*, ethyl acetate extract (96.62 ± 0.165) at a concentration of 200 µg/ml was found to be potent. Ethyl acetate extract of *T. indica* showed very potent inhibition (97.55 ± 0.087) at the concentration of 200 µg/ml and found to be equally potent with that of galantamine (98.63 ± 0.046) at the concentration of 200 µg/ml. Out of three extracts of *P. emblica* and *T. indica*, inhibitory concentration (IC_{50}) data reveals that strongest AChE inhibition activity was exhibited by hydroalcoholic extract of *P. emblica* with (IC_{50} = 0.033 µg/ml) followed by ethyl acetate extract of *T. indica* with (IC_{50} = 0.046 µg/ml) when compared to galantamine (IC_{50} = 0.837 µg/ml). The most active extracts showed the presence of flavonoids, alkaloids, and tannins, which may be related to strong AChE inhibition.

INTRODUCTION: In 1906, Alois Alzheimer, a German psychiatrist, for the first time described a new neurodegenerative disease containing distinctive plaques and neurofibrillary tangles in the brain histology of a 50-year-old woman, Auguste D, which was further named as Alzheimer's Disease (AD), a major type of dementia [1].

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AD is associated with memory impairment and cognitive deficit. It is characterized by low levels of acetylcholine in the brain of AD patients. According to the cholinergic hypothesis, the inhibition of acetylcholinesterase (AChE), an enzyme that hydrolyzes acetylcholine to acetyl and choline, increases the levels of acetylcholine in the brain, thus improving cholinergic functions in AD patients [2].

The inhibition of acetylcholinesterase (AChE) has been one of the most used strategies for the management of mild to moderate AD. Out of five, FDA approved drugs, tacrine, rivastigmine, donepezil, and galantamine are acetylcholinesterase inhibitor (AChEI), while memantine is an N-

methyl-D-aspartate (NMDA) receptor antagonist [3]. As AD is a multifactorial disease, [4] the current propensity in drug design and discovery is the rational design of new drug entities challenging multiple targets in AD. Multiple targeted drugs often have their origin in natural sources. Since two of the currently approved drugs for AD are based on natural products - galantamine and the physostigmine-derivative rivastigmine, many plants are now under investigation as a potential source of new drugs.

In an endeavor to disclose new sources that can potentially be used in the treatment of AD, various extracts of *Phyllanthus emblica* and *Tamarindus indica* were evaluated for their anticholinesterase activity.

MATERIALS AND METHODS:

Chemicals: Acetylthiocholine iodide (ATCI), AChE from electric eel (type VI-S lyophilized powder), 5, 5′-dithiobis [2-nitrobenzoic acid] (DTNB), galantamine were purchased from Sigma Aldrich (Bangalore). All other reagents used were of analytical grade and obtained locally.

Plant Material: The plant materials of *Phyllanthus emblica* and *Tamarindus indica* were collected from the Aswini medicinal garden of Hindu College of Pharmacy, Guntur, Andhra Pradesh, India, in September 2018. The plant species was authenticated by Dr. M. Ramaiah, Department of Pharmacognosy, Hindu College of Pharmacy, Guntur, Andhra Pradesh, India. A voucher specimen with the number HCOP/Phcog/2018/PE and HCOP/Phcog/2018/TI was deposited in our laboratory for future reference.

Extraction Process: The freshly collected leaves of the plants were washed with water, shade dried, and powdered. The powdered material of leaves was taken and allowed to contact with, n-hexane, ethyl acetate, and a mixture of water: ethanol (30:70) (hydro-alcoholic extract) solvents and then subjected to cold maceration process.

Cold Extraction (Maceration): The dried powdered plant material was allowed to contact with solvents n-hexane, ethyl acetate, and hydro-alcohol (30:70) in a closed vessel and then allowed to macerate with occasional shaking for 3 days. Strain the liquid, press the marc, mix the liquids, and finally clarifying by filtration. Then the extracts obtained such as n-hexane extract of *Phyllanthus emblica* (HEPE), ethyl acetate extract of *Phyllanthus emblica* (EAEPE), hydroalcoholic extract of *Phyllanthus emblica* (HAEPE), n-hexane extract of *Tamarindus indica* (HETI), ethyl acetate extract of *Tamarindus indica* (EAETI), hydroalcoholic extract of *Tamarindus indica* (HAETI) was concentrated under vacuum (40 °C) by using rotary evaporator and water bath, dried completely using desiccator and weighed.

Phytochemical Screening: The phytochemical screening of various extracts was tested for all the major groups by standard methods [5-9].

***In-vitro* Acetylcholinesterase Inhibition Assay and Determination of IC_{50}:** AChE inhibition activity was measured by using spectrophotometer based on Ellman's method [10]. The principle of the assay was that the enzyme AchE hydrolyses the substrate Acetylthiocholine iodide resulting in the product thiocholine which in turn reacts with Ellman's reagent DTNB(5,5'-dithio-bis[2-nitrobenzoic acid]) and produces 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate a yellow colored substance detected at 412 nm. In test tube 1710 µL of 50 mM Tris–HCl buffer pH 8.0 and 250 µL of plant extracts at the concentrations of 25 - 200 µg/mL, 10 µL 6.67 U/ml AChE and 20 µL of 10 mM of DTNB in buffer were added. Positive control namely galanthamine were prepared in serial concentration as same as test extract by dissolving in 50 mM Tris-HCl buffer pH 8.0. The mixture was incubated for 15 min at 37 °C. Then, 10 µL of acetylthiocholine iodide (200 mM) in buffer were added to the mixture and the absorbance was measured at 412 nm. The enzyme inhibition (%) was calculated as follows:

$$\% \text{ Inhibition} = (A_0 - A_1) / (A_0) \times 100$$

Where, A_0 = Absorbance of control; A_1 = Absorbance of extract/ standard.

The experiment was done in triplicate and concentrations of the test extract that inhibit the hydrolysis of the substrate (acetylcholine) by 50% (IC_{50}) were determined by probit analysis between the percentage inhibition versus concentration of the extract by using the excel program.

RESULTS AND DISCUSSION:

Extractive Values: The extractive values of all the extracts were calculated and given in **Table 1**.

TABLE 1: PERCENTAGE YIELD OF PLANT EXTRACTS

Plant material	Solvent used	% Yield
Phyllanthus emblica	n-hexane	3.88
	ethyl acetate	5.14
	hydroalcoholic (30:70)	23.58
Tamarindus indica	n-hexane	12.48
	ethyl acetate	27.20
	hydroalcoholic (30:70)	15.58

Phytochemical Analysis: The phytochemical analysis of n-hexane extract of *Phyllanthus emblica* revealed that the tested extract showed the presence of carbohydrates and flavanoids. Ethyl acetate extract of *Phyllanthus emblica* revealed that the tested extracts showed the presence of steroids, alkaloids, and tannins. Hydro-alcoholic extract of *Phyllanthus emblica* revealed that the tested extracts showed the presence of carbohydrates, alkaloids, flavonoids, tannins, and saponins.

The phytochemical analysis of n-hexane, ethylacetate, and hydroalcoholic extracts of *Tamarindus indica* revealed that the tested extracts showed the presence of flavonoids, alkaloids, and tannins.

***In-vitro* Acetylcholinesterase Inhibition Assay:** The results obtained from the three extracts of *Phyllanthus emblica* and *Tamarindus indica* against AChE enzyme was evaluated and tabulated for percentage inhibition and IC_{50} values.

TABLE 2: PERCENTAGE INHIBITION OF VARIOUS EXTRACTS OF *PHYLLANTHUS EMBLICA* AND *TAMARINDUS INDICA* ON AChE ENZYME ACTIVITY

Concentration (µg/ml)	% Inhibition of HEPE	% Inhibition of EAEPE	% Inhibition of HAEPE	% Inhibition of HETI	% Inhibition of EAETI	% Inhibition of HAETI	% Inhibition of Galantamine
25	68.54 ± 1.462	90.56 ± 0.324	89.82 ± 0.301	63.40 ± 0.309	93.08 ± 0.481	64.07 ± 0.715	90.21 ± 1.009
50	75.71 ± 1.215	93.10 ± 0.474	92.17 ± 0.424	71.90 ± 0.905	92.20 ± 4.626	75.81 ± 1.218	94.27 ± 1.14
100	82.80 ± 1.742	94.86 ± 0.592	93.80 ± 0.403	83.07 ± 1.054	96.40 ± 0.070	82.52 ± 0.734	97.52 ± 0.047
200	90.32 ± 0.082	96.62 ± 0.165	95.60 ± 0.633	90.04 ± 0.667	97.55 ± 0.087	90.19 ± 0.793	98.63 ± 0.046

Data are given as Mean ± SEM (n=3)

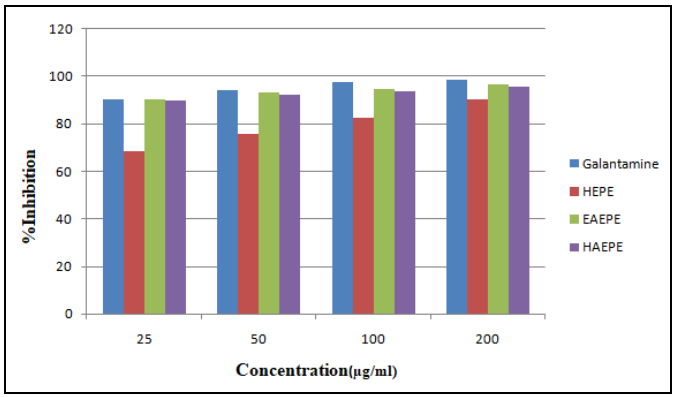

FIG. 1: PERCENTAGE INHIBITION OF VARIOUS EXTRACTS OF *PHYLLANTHUS EMBLICA* ON AChE ACTIVITY

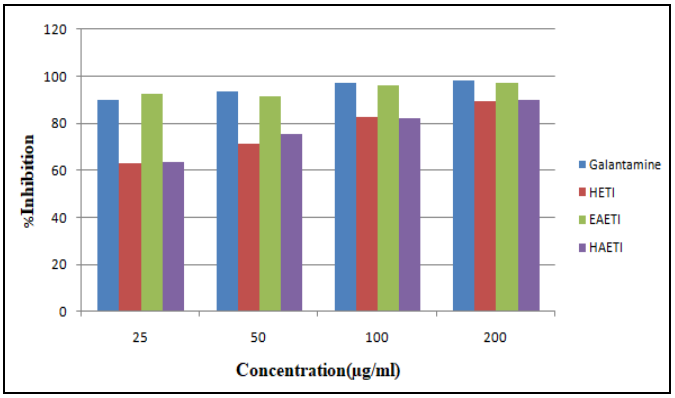

FIG. 2: PERCENTAGE INHIBITION OF VARIOUS EXTRACTS OF *TAMARINDUS INDICA* ON AChE ACTIVITY

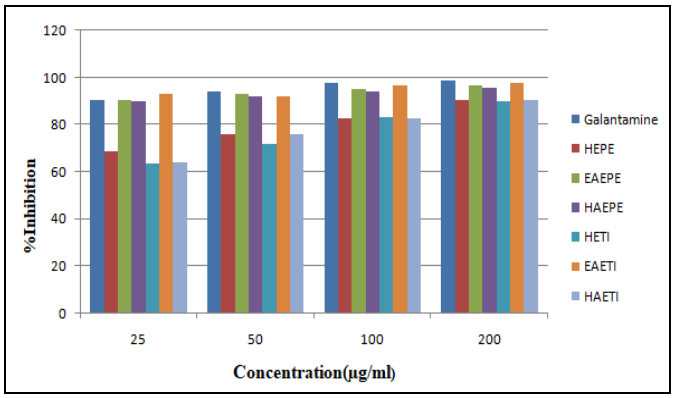

FIG. 3: PERCENTAGE INHIBITION OF VARIOUS EXTRACTS OF *PHYLLANTHUS EMBLICA* AND *TAMARINDUS INDICA* ON AChE ACTIVITY

TABLE: 3 IC_{50} VALUES FOR AChE INHIBITION BY PLANT EXTRACTS AND STANDARD

Extract / Positive control	IC_{50} Value AChE inhibition assay (µg/ml)
HEPE	8.165 ± 1.06
EAEPE	0.058 ± 0.009
HAEPE	0.033 ± 0.005
HETI	13.489 ± 2.32
EAETI	0.046 ± 0.004
HAETI	1.042 ± 0.008
Galantamine	0.837 ± 0.012

From the table and figures, it is observed all the extracts were found to be highly effective in inhibiting AChE enzyme in a dose-dependent manner. Among the extracts of *Phyllanthus emblica*, ethyl acetate extract (96.62 ± 0.165) at a concentration of 200 µg/ml was found to be potent. Ethyl acetate extract of *Tamarindus indica* showed very potent inhibition (97.55 ± 0.087) at the concentration of 200 µg/ml and found to be equally potent with that of galantamine (98.63 ± 0.046) at the concentration of 200 µg/ml.

Out of three extracts of *Phyllanthus emblica* and *Tamarindus indica*, inhibitory concentration (IC_{50}) data reveals that strongest AChE inhibition activity was exhibited by HAEPE with (IC_{50} = 0.033 µg/ml) followed by this EAETI with (IC_{50} = 0.046 µg/ml) when compared to galantamine (IC_{50} = 0.837 µg/ml).

CONCLUSION: In the cholinergic nervous system, acetylcholinesterase (AChE) is a crucial enzyme. For treating AD, therapies are designed to inverse the cholinergic insufficiency, which is mostly based on AChE inhibitors to improve cholinergic transmission with moderate and transient beneficial effects. A number of studies revealed that cholinesterase inhibitors might act on multiple therapeutic targets such as prevention of the formation of β-amyloid plaques, antioxidant activity, and modulation of APP processing.[11] However, there is a prerequisite for novel AChE inhibitor lead compounds with reduced toxicity and extreme central nervous system (CNS) penetration.

The results suggest that ethyl acetate extract of *Phyllanthus emblica* and hydroalcoholic extract of *Tamarindus indica* showed strong AChE inhibitory properties similar to that of galantamine (standard) might be categorized as high potency agonists because they were able to elicit inhibition in smaller quantities than the reference substance.

The preliminary phytochemical examination of selected plants suggested that they have alkaloids, flavonoids, tannins. Therefore, it is assuming that these phytoconstituents are responsible for anticholinesterase activity. It is therefore worth study further to isolate the pure molecules responsible for anticholinesterase activity.

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CONFLICTS OF INTEREST: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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