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In-Vitro assessment of Anti-Alzheimer And Antioxidant Potentials of *Jatropha integerrima* Leaves

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Abstract
The present study investigates the phytochemical composition and bioactive potential of *Jatropha integerrima* leaf extract with a focus on its anti-Alzheimer and antioxidant activities. Preliminary phytochemical screening of the methanolic extract revealed the presence of flavonoids, alkaloids, phenolic compounds, tannins, terpenoids, and saponins, which are known to contribute to neuroprotective and free-radical scavenging properties. The antioxidant activity was evaluated using in-*vitro* assays such as DPPH and hydrogen peroxide radical scavenging, demonstrating significant dose-dependent inhibition comparable to standard antioxidants. The anti-Alzheimer potential was assessed through acetylcholinesterase (AChE) and studies, indicating notable enzyme inhibitory activity suggestive of cholinergic pathway modulation. These findings highlight *J. integerrima* leaves as a promising source of natural compounds with potential therapeutic applications in managing oxidative stress and neurodegenerative disorders such as Alzheimer's disease.

Keywords: *Jatropha integerrima*, phytochemical screening, antioxidant activity, anti-alzheimer's potential, acetylcholinesterase inhibition, neuroprotection.

Introduction

Plants are an integral part of our lives and have been so since the time immemorial, due to their omnipresence.

"Medicine heals doubts as well as diseases" - Karl Marx[1]

Throughout history, the pursuit of holistic well-being and optimal health has remained a central focus of human societies. When practiced beyond its cultural origins, traditional medicine is commonly termed "complementary and alternative medicine". For thousands of years, medicinal and aromatic plants—particularly those with ethnopharmacological significance—have been harnessed for their therapeutic properties, forming the foundation of natural healthcare systems. Historically, Hippocrates—often referred to as the "Father of Medicine"—was among the earliest known herbalists, emphasizing the role of nature in the healing process. Even today, medicinal herbs continue to attract global interest, largely due to their natural origins, therapeutic effectiveness, and higher acceptance among patients compared to synthetic drugs. Commonly used herbs include turmeric, soy isoflavones, American ginseng, bee pollen, cat's claw, bladder wrack, chamomile, flaxseed, saw palmetto, and schizandra, among others. Today, modern pharmacopoeias recognize that over 25% of current pharmaceutical drugs are derived from plants, some of which have been modified into synthetic analogues. Herbalism is increasingly becoming mainstream, with growing scientific evidence supporting the therapeutic and preventive value of herbs in managing various diseases. [2]

Disease Profile

Alzheimer's disease (AD): is a neurodegenerative disease and is the most common form of dementia, accounting for around 60–70% of cases. in persons over 65 years of age[3]. Alois Alzheimer, a German psychiatrist (1864–1915), gave the first explanation of it in 1906. Henceforth, the condition was given the term "Alzheimer's disease" by Kraepelin. Dementia is a general term for the symptoms affecting memory, communication and thinking. Alzheimer disease is the most common form of dementia. Types of dementia are

- Alzheimer disease
- Vascular dementia
- Dementia with Lewy bodies
- Temporal lobar dementia(Sabrina Felson)[4]
- Alzheimer's disease is believed to occur when abnormal amounts of amyloid beta (Aβ), accumulating extracellularly as amyloid plaques and tau proteins, or intracellularly as neurofibrillary tangles, form in the brain, affecting neuronal functioning and connectivity[3] AD is focused on two types of proteins
- PLAQUES
- TANGLES.

They lead to destruction of nerve cells in the cerebral cortex of brain.

Beta Amyloid [5]

Beta-amyloid is a fragment that results from the breakdown of APP, a process mediated by the enzyme β-secretase. When beta-amyloid fragments cluster together, they disrupt communication between neurons and trigger a cascade of harmful biochemical events. These include oxidative stress, the formation of free radicals, and ultimately, neuronal death.

Tau Tangles

The accumulation of abnormal tau leads to sticky, thread-like structures called neurofibrillary tangles. This buildup prevents tau from doing its job, damaging the neuron's inner skeleton and impairing communication between cells. These tangles disrupt transport system and are toxic to nerve cells.

Pathophysiology

Acetylcholine, a neurotransmitter released by nerve cells acts as a signal intermediate between two nerve cells. Pathological character of acetylcholine depletion in the brain is due to increased levels of Acetycholinesterase (AChE), an enzyme that catalyzes acetylcholine hydrolysis. Another therapeutic target of AD *viz.* the Butyrylcholinesterase (BChE) also shows significantly more than 50% sequence similarity with AChE [8]. Moreover, the function and location of BChE is very similar to Acetycholinesterase. These two therapeutic targets of AD are essential for termination of nerve impulse transmissions at cholinergic synapses by rapid hydrolysis of acetylcholine and this can be counteracted by using therapeutically and biologically active compounds.(molecular docking) AChEIs have been shown to promote an increased concentration of ACh and enhance the duration of ACh action at the synapse. Therefore, cholinesterase inhibitors are considered one of the effective medications for the symptomatic treatment of AD. In this context, Tacrine, donepezil, and rivastigmine are a few AChE inhibitors approved by the U.S. Food and Drug Administration (FDA). [6]

However, these drugs were found to have many restrictions due to their shorter half-lives and adverse side effects such as vomiting, nausea, anorexia, and fatigue, with some even exhibiting hepatotoxicity. More importantly, most synthetic anticholinesterase drugs have originated from plant-based molecules, including major bioactive substances such as indole, steroids, alkaloids, glycosides, coumarins, phenylpropanoids, and terpenoids. [6]

Plant Profile

Jatropha integerrima (Euphorbiaceae) commonly known as peregrina and native to the West Indies, particularly Cuba and Hispaniola. It has since been introduced and naturalized in various tropical and subtropical region. Jatropha integerrima has been cultivated in: United States, India, Bangladesh, Southeast Asia, Caribbean, Central and South America, Australia. In India, it is commonly found in states like Kerala, Maharashtra, Odisha, Delhi, and Uttar Pradesh. [7] It is a medium sized drought-tolerant perennial belonging to the family Euphorbiaceae and it flowers through the entire year making it a significant decorative species planted in tropical and subtropical regions for its magnificent crimson blooms. Flowers are 1 inch wide fivepetaled deep red with yellow stamens are held in branched clusters on 4 inch long stalks at the branch tips.[9]

Materials and method

Plant Collection and Authentication

Jatropha integerrima leaves were collected from Madurai Medical College campus, Madurai, Tamil Nadu during the month of April 2025. The species for the proposed study was identified and authenticated by Dr. Stephen, Professor, Department of Botany, American College, Madurai - 625 002.

Preparation of Plant material

The leaves of the plant washed thoroughly in tap water to remove soil particles and other adhered debris and finally rinsed with sterile distilled water. The leaves were shade dried for extraction at room temperature and coarsely powdered in a mixer. The powdered material was stored and taken up for extraction process.

Extraction of plant material

The powdered leaves were subjected to methanolic extraction using a Soxhlet apparatus. Specifically, 5 grams of the leaf powder were extracted with 70 mL of methanol at a temperature of 60°C. The extract was collected in the upper chamber of the Soxhlet unit. After the extraction process, the filtrate was concentrated at room temperature to ensure complete evaporation of the methanol, yielding the final plant extract [10]

Quantitative estimation

Estimation of total phenolic content of *jatropha integerrima* leaves extract

A series of calibrated 10ml volumetric flask is taken and standard solution (gallic acid) and solution of various concentrations (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml) is taken. To each of this solution add 5ml of distilled water and 0.5ml of Folin's Ciocalteu's reagent is added, mixed and shaken. After 5 minutes, 1ml of 10% sodium carbonate solution is added and the volume is made up to 10 ml with distilled water. It is allowed to incubate for 2 hours at room temperature. Intense blue colour is developed. The reaction mixture without sample is used as blank. After incubation, absorbance is measured at 725nm using UV spectrophotometer and the mean values will be recorded. The calibration curve will be plotted using standard gallic acid. Total phenolic content of extract is expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g) [11].

Estimation of total flavanoid content of *jatropha integerrima* leaves extract

Total flavonoid content was measured with the aluminium chloride colorimetric assay. A series of calibrated 10ml volumetric flask were taken and standard solution (Rutin) and HAEMN solution of various concentrations (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml) were taken. To each of these solutions add 4ml of water and 0.3ml of 5% sodium nitrite solution is added. After 5 minutes, 0.3ml of 10% aluminium chloride is added. At 6th minute, 2ml of 1M sodium hydroxide is added. Finally, volume is made up to 10ml with distilled water and mix well. Orange yellowish colour is developed. The absorbance is measured at 510 nm spectrophotometer using UV-visible spectrophotometer and the mean values will be noted. The blank is performed using distilled water. The calibration curve is plotted using standard Rutin. The total flavonoid content in the extract is expressed as milligrams of Rutin equivalent per gram of extract[12].

Pharmacological Studies

Measurement Of Dpph Radical Scavenging Activity: [13]

- prepare 0.1 mM of DPPH solution in methanol and add 100 µl of this solution to 300 µl of the solution of Sample Jatropha integerrima at different concentration (100, 80, 60, 40, and 20µg/ml).
- The mixtures have to be shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance has to be measured at 517 nm using a UV-VIS spectrophotometer. (Ascorbic acid can be used as the reference).
- Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical can be calculated by using the following formula.

$$\text{DPPH scavenging effect \% inhibition} = \frac{\text{A 517 Control} - \text{A 517 Sample}}{\text{A 517 Control}} \times 100$$

Determination Of Ache Enzyme Activity: [14]

Acetylcholinesterase (AChE) activity was assayed following an adaptation of the spectrophotometric method. The cuvette used as a blank to control for the non enzymatic hydrolysis of acetylcholine contained a mixture of 500 µl of 3 mM DTNB solution (in 0.1 M potassium phosphate pH 8), 100 µL of 15 mM Acetylcholine (in water), 275 µL of 0.1 M potassium phosphate pH 8, and 100 µL of sample solutions at the different concentrations evaluated (0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL and 1.0 mg/mL). In the reaction cuvette, 25 µL of buffer was replaced by AChE solution 0.16 U/ml. The resulting solutions were placed in a spectrophotometer. The thiocholine formed during the hydrolysis of acetylcholine reacts rapidly with DTNB and a yellow compound is formed. The reaction was monitored for 5 min at 405 nm and the absorbance registered every minute. Enzyme activity was calculated as a percentage of the velocities compared to that of the assay using buffer solution instead of inhibitor (sample).(Ellman *et al*.)

$$\text{\% inhibition} = \frac{\text{A405Control} - \text{A405 Sample}}{\text{A405 Control}} \times 100$$

Result and discusssion

Preparation of methanolic extract of jatropha integerrima leaves

Extraction was done by soxhlet technique using methanol as a solvent.

Table 1: Organoleptic studies of leaf extract of *Jatropha integerrima*

S.NO	Characters	Leaf Extract Of *Jatropha integerrima*
1.	Color	Dark green
2.	Odor	Characteristic odor
3.	Taste	Bitter
4.	Consistency	Sticky
5.	Solubility	Soluble in methanol

Estimation of Total Flavonoid Content

Quantitative estimation of flavonoid was done by aluminum colorimetric assay method using rutin as a standard. The results were reported in the table

Table 2 : Estimation of total flavanoid content

S.no	Concentration µg/ML.	Absorbance of rutin	absorbance of sample
1.	10	0.532	0.457
2.	20	0.985	0.962
3.	30	1.542	1.496
4.	40	2.096	1.992
5.	50	2.698	2.594

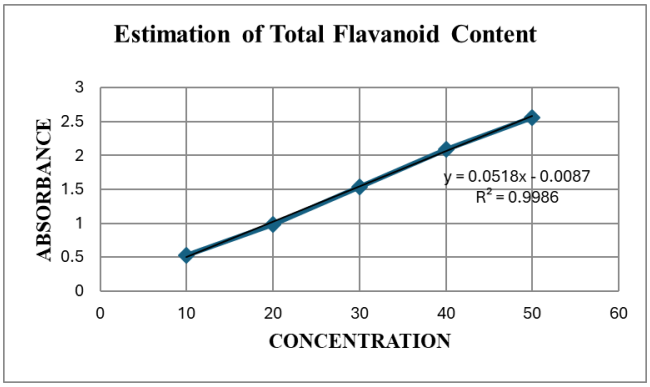

Fig 1 : standard Calibration Curve for Rutin

Result: The quantitative estimation of flavonoids was conducted using the aluminum chloride colorimetric assay method, employing Rutin as a standard. The total flavonoid content was determined using a linear equation derived from the calibration curve of standard Rutin, which was y = 0.051x - 0.008 (where y represents absorbance, x represents concentration). The coefficient of determination (R^2) was found to be 0.998. The total flavonoid content in the extract of Jatropha integerrima(MEJI) was measured at 59.149 mg/g.

Estimation of total phenolic content

Total phenolic content was estimated by Folin-Ciocalteu method using gallic acid as a standard. The results were reported in the table

Table 3 : Estimation of total phenol content

S.no	Concentration	Absorbance of gallic acid	Absorbance of sample
1.	10	0.114	0.094
2.	20	0.224	0.187
3.	30	0.327	0.284
4.	40	0.432	0.395
5.	50	0.535	0.487

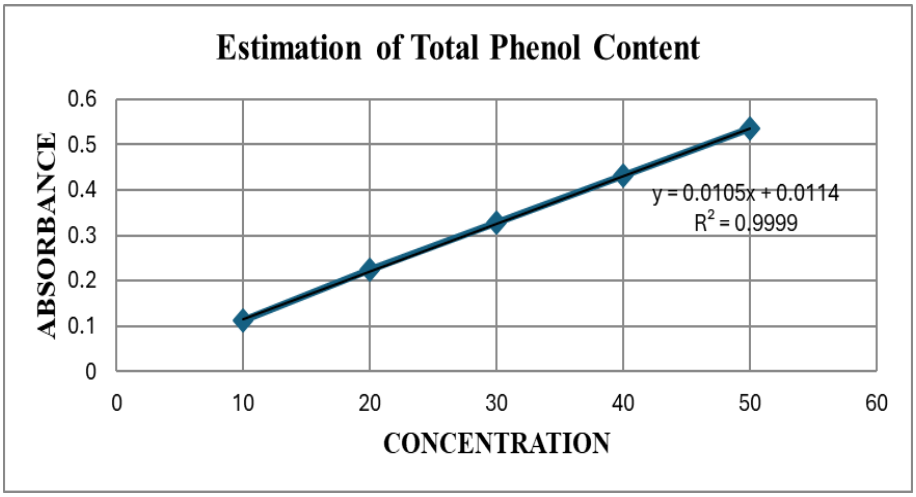

Fig 2 : Standard Calibration Curve for Gallic acid

Results

The quantitative estimation of phenols was performed using the Folin-Ciocalteu method, utilizing gallic acid as a standard. The total phenol content was determined using the linear equation derived from the calibration curve of standard gallic acid: y = 0.010x+0.011 (where y represents absorbance, x represents concentration).The coefficient of determination (R^2) was found to be 0.999. The total phenolic content in the extract of Jatropha integerrima (MEJI) was measured at 55.68 mg/g, expressed in terms of gallic acid equivalents (GAE).

In-*Vitro* Antioxidant Activity Of *Jatropha Integerrima* By Dpph Radical Scavenging Assay

Methanolic extract of Jatropha integerrima was subjected to in-*vitro* antioxidant activity

Table 4 : Determination of DPPH radical scavenging assay of Methanolic extract of *Jatropha integerrima* (MEJI) leaves

S.no	Tested sample concentration (µg/ml)	OD Value at 517 nm	Percentage of inhibition (%)
1	Control	0.948	0.00
2	20	0.904	4.641
3	40	0.788	16.877
4	60	0.596	37.130
5	80	0.456	51.898
6	100	0.213	77.531
7	Ascorbic acid	0.044	95.29

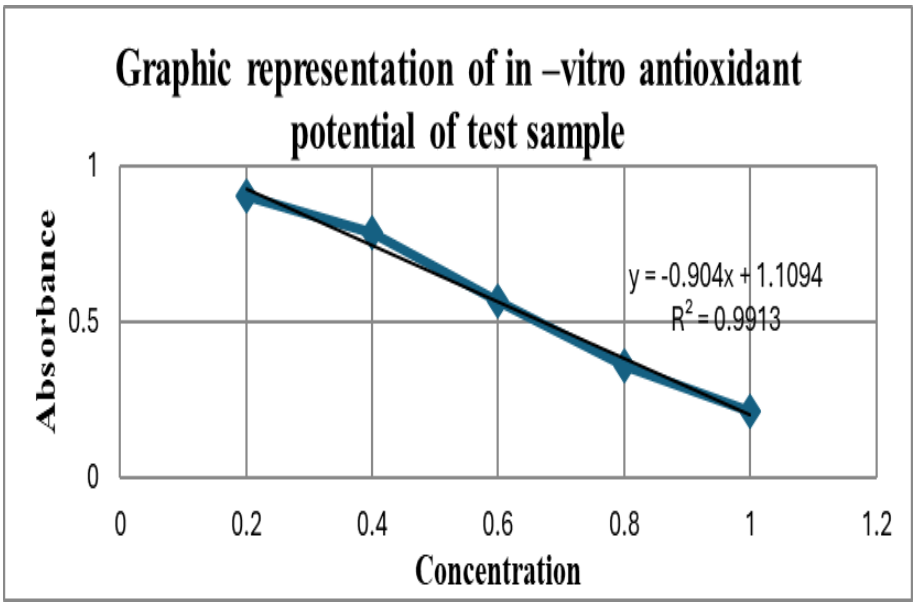

Fig 3 : Graphical representation of in –*vitro* antioxidant potential of test sample

Result

Ic 50 value of MEJI against DPPH was found to be 0.98 µg/ml and the percentage of inhibition of standard ascorbic acid is 95.29% to that of MEJI is 77.531 %. The percentage of inhibition occurs in dose-dependent manner.

In-*vitro* acetylcholinesterase inhibitor assay of methanolic extract of Jatropha integerrima

OD Value at 410 nm

Control Mean OD value: 1.156

Table 5 : Percentage inhibition of acetylcholine esterase assay

S.NO	Tested sample concentration (µg/ml)	OD Value at 410 nm (in triplicates)	Percentage of inhibition (%)
1	0.2	1.023	11.49
2	0.4	0.938	18.83
3	0.6	0.812	29.74
4	0.8	0.692	40.14
5	1	0.528	54.36

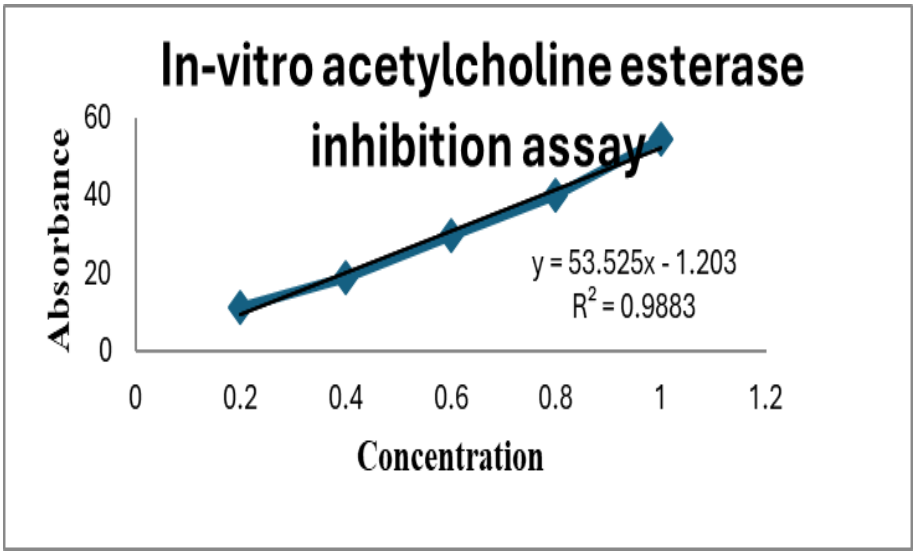

Fig 4 : Graphic representation of in-*vitro* acetylcholine esterase inhibition assay

Result

IC50 value of MEJI against Anti-Alzheimer was found to be 0.98 µg/ml and the percentage of inhibition of acetylcholine esterase of standard Physostigmine is 100 % to that of is 54.36%. The percentage of inhibition of the sample occurs in a dose-dependent manner.

Conclusion

The methanolic leaf extract of *Jatropha integerrima* (MEJI) exhibited dark green color, characteristic odor, and bitter taste, indicating the presence of chlorophylls, flavonoids, phenols, and alkaloids. Its sticky consistency and good solubility in methanol confirm the extract's richness in polar bioactive compounds suitable for further evaluation. The total phenolic and flavonoid contents were found to be 55.68 mg/g and 59.149 mg/g respectively, suggesting strong antioxidant potential. In the DPPH assay, MEJI showed 77.53% inhibition with an IC_{50} value of 0.98 µg/mL, demonstrating dose-dependent radical scavenging activity. These findings support the extract's potential in mitigating oxidative stress, a key factor in Alzheimer's disease progression. The acetylcholinesterase inhibition assay further indicated MEJI's role in preserving acetylcholine levels, comparable to the standard physostigmine, highlighting its promise as a natural anti-Alzheimer's agent.

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