In Silico and *In Vivo* Evaluation of *Ruellia Tuberosa* Extract For Alzheimer's Disease: A Phytochemical And Pharmacological Analysis

*DR. N. SENTHILKUMAR[1], *DR.A. CHITRA[2], *DR.K. SUMATHI[3], R. PORSELVI[4]

1. The Principal of JKKMMRF'S Annai J.K.K Sampoorani Ammal College of Pharmacy, Department of Pharmaceutical Chemistry

2. Associate professor, JKKMMRF'S Annai J.K.K Sampoorani Ammal College of Pharmacy, Department of Pharmaceutical Chemistry

3. Associate professor, JKKMMRF'S Annai J.K.K Sampoorani Ammal College of Pharmacy, Department of Pharmaceutical Chemistry

4. JKKMMRF'S Annai J.K.K Sampoorani Ammal College of Pharmacy, Department of Pharmaceutical Chemistry, M. Pharm student

Abstract

Cognitive decline, memory loss, and behavioral abnormalities are hallmarks of Alzheimer's disease (AD), a progressive neurological illness. An ethanolic extract of Ruellia tuberosa was examined for phytochemical composition and tested in a model of Alzheimer's disease induced by aluminium chloride in rodents such as wistar rats. Behavioral assessments, biochemical markers (AChE activity, MDA levels), and histopathological analysis were performed in order to investigate the therapeutic potential of Ruellia tuberosa through in silico and in vivo approaches, with a focus on its phytochemical constituents and neuropharmacological effects. Significant neuroprotective efficacy was demonstrated by the results, indicating that Ruellia tuberosa is a viable natural therapeutic option for AD.

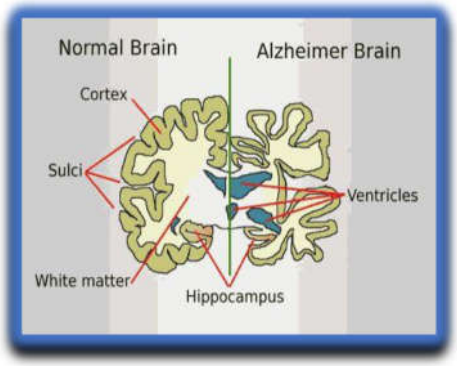
Keywords: In Vivo Study, Molecular Docking, Alzheimer's Disease, *Ruellia tuberosa*, Methodology, and Histopathology

OVERVIEW

ALZHEIMER'S DISEASE

One form of dementia that impacts thinking, behaviour, and memory is Alzheimer's disease. Eventually, the symptoms become severe enough to disrupt everyday activities. A loss of mental capacity linked to the slow death of brain cells is referred to as "dementia." The most frequent cause of dementia in the elderly is undoubtedly Alzheimer's disease (AD), a chronic, gradual degenerative organic brain condition characterized by impairment of many cortical functions, including memory, judgment, orientation, comprehension, learning ability and language

One of the leading causes of dementia in the elderly population is Alzheimer's disease (AD). The pathogenesis includes oxidative stress, tau protein hyperphosphorylation, beta-amyloid aggregation, and cholinergic deficiency. Only symptomatic alleviation is provided by current medicines.



PLANT PROFILE - *RUELLIA TUBEROSA*

The herb *Ruellia tuberosa*, sometimes called Minnie Root or Way-side Tuberose, belongs to the Acantharean family. It is a tropical perennial with striking blue-violet flowers and strong, fusiform tuberous roots. The plant is well-known for its unusual fruit, which is a capsule that releases seeds when it gets wet. It is frequently found in damp, shaded areas and is well-known in folk and Ayurvedic traditions for its many therapeutic applications. A tiny biennial herb with thick, finger-like tuberous roots (other definitions classify it as a perennial). The leaves are opposite and elliptic, and the stem is hairy. Flowers: Violet-coloured, large, funnel-shaped flowers. Fruit: A capsule that releases seeds when it becomes moist. In traditional medicine, the plant is used as an analgesic, diuretic, anti-diabetic, antipyretic, gastroprotective, and neuroprotective. Flavonoids, phenolics, and alkaloids are found in *Ruellia tuberosa*, which is well-known for its neuroprotective, anti-inflammatory, and antioxidant qualities. Its use in AD is still not well understood, though. This work assesses the effectiveness of *R. tuberosa* extract in AD models by combining computational and experimental techniques.

LITERATURE REVIEW

1. M.N.L.C. Harika *et.al* they have evaluated Preliminary phytochemical analysis showed the presence of alkaloids, phenolics, flavonoids, steroids, terpenoids, coumarins, tannins, cardiac glycosides, carbohydrates and amino acids. Further investigation was done using Ultraviolet-Visible spectroscopy, Fourier transform infrared spectroscopy (FTIR) and GC-MS analysis. UV-VIS and IR spectral analysis showed the peaks of corresponding functional groups present in the phytochemical constituents.

2. Arokiasamy *et.al* they are proposed that the present study was aimed to evaluate the protective effect of hesperidin (Hes) on aluminium chloride(AlCl3) induced neurobehavioral and pathological changes in Alzheimeric rats

3. Hala *et.al* they are proposed about Alzheimer's disease (AD) is a neurodegenerative disease clinically characterized by progressive cognitive impairment. This work aimed to investigate the role of quercetin

(Q) in the treatment and protection of $AlCl_3$-induced AD in rats through exploring the molecular mechanisms underlying its neuroprotective and therapeutic properties.

4. Gayathri *et.al* they are evaluated the antiparkinsonian activity of the hydroalcoholic extract of *Ruellia tuberosa* leaves in rats using suitable animal models. The phytochemical analysis was conducted using various tests. Catalepsy was induced in rats using Rotenone and HART was administered orally to the treatment group.

5. Nemat *et.al* they are proposed about study aimed to investigate the possible prophylactic and therapeutic effects of aqueous infusions of Boswellia serrata on AD induced in rats. study indicated that Boswellia serrata when was used for treatment of $AlCl_3$ induced AD, its high dose only produced increased activity of rats in the activity cage.

AIM & OBJECTIVE

AIM :

To current work aims to assess anti-Alzheimer's activity of Ruellia tuberosa ethanolic extract in aluminum chloride induced Alzheimer disease in wistar rat using in-vivo studies and phytochemical analysis of plant extract using LC-MS Chromatography & *In silico Docking approaches*

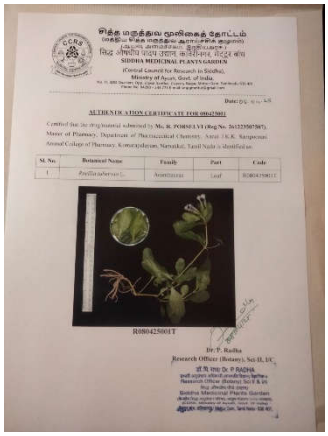
OBJECTIVES:

- Collection and Authentication of Ruellia tuberosa leaves.
- Preparation of Ethanolic extract of Ruellia tuberosa leaves
- Induction of Alzheimer disease in Experimental animals
- To evaluate the Anti-Alzheimer activity of Ruellia tuberosa leaves extract in Aluminium chloride induced Alzheimer in wistar rat using in vivo studies
- Histopathology
- Qualitative phytochemical Analysis (LC-MS)
- In Silico docking
- Statistical analysis by oneway ANOVA using SPPS

MATERIALS AND PROCEDURES

COLLECTION OF PLANTS

The whole *Ruellia tuberosa* plant was gathered, and a specimen was delivered to the Siddha Medicinal Plants Garden in Mettur, Tamilnadu, where it was verified by Dr. P. Radha, the research officer.



GRINDING AND DRYING

Ruellia tuberosa leaves were cleaned, allowed to air dry for a few days, and then ground into a coarse powder. The resulting powder was utilized for more research.

SUCCESSIVE SOXHLET EXTRACTION

Extraction of Solvents in Sequence Using a Soxhlet device, a weighed amount (for example, 100 g) of the dried and powdered plant material was extracted using solvents one after the other. Petroleum ether, chloroform, ethyl acetate, and ethanol (95%), in order of increasing polarity, were used in the extraction process. Every extraction lasted six to eight hours, or until the solvent became clear, signifying thorough extraction. The marc, or leftover plant material, was dried after each solvent extraction before being exposed to the subsequent solvent. After initial extractions with petroleum ether, chloroform, and ethyl acetate, the dried marc was extracted with 95% ethanol in the Soxhlet apparatus. The extraction action continued until the siphoning solvent in the Soxhlet became colorless. The resultant ethanolic extract was filtered through Whatman No. 1 filter paper and concentrated under decreased pressure using a rotary evaporator at a temperature of no more than 40°C. The semi-solid extract was then dried in a desiccator to yield a solid ethanolic extract.

PHYTOCHEMICAL SCREENING

Standard biochemical procedures were used to conduct a qualitative study of alkaloids, flavonoids, tannins, saponins, and phenolic chemicals using HRLCMS chromatography.

IN SILICO DOCKING APPROACHES

The *In silico* research of *Ruellia tuberosa* extract revealed important information on its medicinal potential against Alzheimer's disease. Molecular docking experiments discovered significant phytoconstituents with high binding affinities for vital enzymes such as acetylcholinesterase (AChE), butyryl cholinesterase (BChE), and beta-secretase (BACE1), all of which play important roles in Alzheimer's disease pathogenesis. Key phytocompounds should be discovered using HRLCMS. Molecular docking was to be done with Auto Dock Vina.

IN VIVO STUDIES

EXPERIMENTAL ANIMALS

Wistar rats weighing 150-200 g were utilized, with a total of six rats distributed among five groups:

- Group I : Normal saline-0.9% (control)
- Group II : Inducing agent -Aluminium chloride (100 mg/kg, p.o.)
- Group III : Aluminium chloride (100 mg/kg, p.o.) + Donepezil (5 mg/kg, i.p)
- Group IV : Aluminium chloride (100 mg/kg, p.o.) + *R. tuberosa* extract (200 mg/kg)
- Group V : Aluminium chloride (100 mg/kg, p.o.) + *R. tuberosa* extract (400 mg/kg)

BEHAVIORAL STUDIES

- **Y-Maze**: For spatial working memory
- **Morris Water Maze**: To improve memory and learning
- **Board Test**: For exploratory behaviour

BIOCHEMICAL ANALYSIS

- **AChE Activity**: Ellman's method
- **MDA Levels**: The assay known as thiobarbituric acid reactive compounds (TBARS)
- **Total Antioxidant Capacity**

HISTOPATHOLOGY

Histopathological studies of Alzheimer's disease focus on examining brain tissue under a microscope to identify characteristic changes associated with the condition. Key features observed include:

Amyloid plaques: Extracellular deposits of beta-amyloid protein
Neurofibrillary tangles: Accumulations of hyperphosphorylated tau protein inside cells
Neuronal loss: Widespread death of neurons, particularly in the cerebral cortex and hippocampus
Synaptic loss: Reduction in synaptic density and neurotransmitter release
Gliosis: Proliferation and activation of glial cells, especially astrocytes and microglia
Vascular changes: Blood vessel changes, such as cerebral amyloid angiopathy

Histopathological techniques used in Alzheimer's disease research include:

Immunohistochemistry: To detect specific proteins like beta-amyloid and tau
Silver staining: To visualize neurofibrillary tangles
Electron microscopy: For detailed examination of cellular structures
Fluorescence microscopy: To study protein interactions and localization

CONCLUSION

My research study is to *Ruellia tuberosa* extract significant neuroprotective effects against Alzheimer's disease in both computational and experimental models. The activity was to be observed is likely due to phytochemicals acting through cholinesterase inhibition and antioxidative pathways. Further *In vivo* animal study and histopathological methods also to be done for significant AD diseases.

Ruellia tuberosa L. presents a promising, multi-targeted approach to Alzheimer's disease management. Both computational and animal studies advocate its neuroprotective efficacy. With further validation, it may emerge as a lead candidate for phytopharmaceutical development against AD.

REFERENCES

[1] Krasovskii GN, Vasukovich LY, and Chariev OG. Experimental investigation of the biological consequences of lead and aluminum after oral ingestion. Environmental Health Perspectives 1979; 30:47–51.

[2] Howes MJR, Perry NSL, & Houghton PJ. (2003). Plants with traditional uses and activities related to the treatment of Alzheimer's disease and other cognitive disorders. Phytother Research.

[3] Anand R, Gill KD, and Mahdi AA. (2014). Alzheimer's disease therapeutics: Past, Present, and Future. Neuropharmacology.

[4] Dhanasekaran M, Holcomb LA, Hitt AR, Young K, Tharakan B, Porter JW, et al. (2007). Centella asiatica extract reduces amyloid beta levels in an Alzheimer's disease animal model. J Ethnopharmacol.

[5] Kumar D. et al. (2020). Molecular docking and pharmacological testing of bioactive compounds from medicinal plants for Alzheimer's disease. Journal of Biomolecular Structure and Dynamics.

[6] Ellman GL et al. (1961). A simple and rapid colorimetric method for determining acetylcholinesterase activity. Biochem Pharmacia.

[7] Thenmozhi AJ, Raja TR, Janakiraman U, and Manivasagam T. Hesperidin has a neuroprotective effect against aluminum chloride-induced Alzheimer's disease in Wistar rats. Neurochemical Research. 2015 Apr;40:767–76.

[8] Lakshmi B., Sudhakar M., and Prakash K. S., The protective effect of selenium against aluminum chloride-induced Alzheimer's disease: Behavioral and biochemical changes in rats. Biological Trace Element Research, 165(1), 67-74 (2015).

[9] Sharma, V, and Kumar, S. (2011*). Ruellia tuberosa* Linn: a phytochemical and pharmacological profile. Journal of Applied Pharmaceutical Science, 1(9):16-19.

[10] Lee, C. J.; Chen, L. G.; Liang, W. L.; and Wang, C. C. (2011). *Ruellia tuberosa* has anti-inflammatory properties on RAW 264.7 macrophages and adjuvant-induced arthritis in rats. Journal of Ethnopharmacology, 137(2), 891–898