Assessment of *in - vitro* Anti-Alzheimer and Anti-Oxidant activity of *Mucuna Pruriens*

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Abstract

Purpose:
The study aims to investigate the potential of Mucuna Pruriens as a source of cholinesterase inhibitors for the treatment and progression slowing of Alzheimer's disease, a neurodegenerative condition characterized by memory loss and cognitive decline.

Methodology:
This research involved an in vitro analysis of various extracts of Mucuna Pruriens to evaluate their anti-oxidant and anti-cholinesterase activities. The DPPH assay and Hydrogen Peroxide radical scavenging effect were utilized to assess anti-oxidant properties, while Ellman's colorimetric method was employed for the estimation of anti-cholinesterase activity.

Results:
The methanolic extract of *Mucuna Pruriens* exhibited significant anti-oxidant activity with IC50 values of 25 µg/ml (DPPH) and 54 µg/ml (Hydrogen Peroxide scavenging), alongside a notable anti-cholinesterase activity with an IC50 value of 115 µg/ml. These results indicate the plant's potential as a therapeutic agent with minimal side effects compared to conventional drugs.

Conclusion:
This study highlights the promising role of *Mucuna Pruriens* in the search for effective Alzheimer's disease treatments, emphasizing the need for further *in vivo* investigations and formulation development to harness its therapeutic properties. The findings contribute to the growing body of evidence supporting the use of plant-derived compounds in neurodegenerative disease management.

1. Introduction

Alzheimer's disease (AD) is a complex and debilitating neurodegenerative disorder that accounts for approximately 60 to 70% of all dementia cases globally (Alzheimer's Association, 2021). Characterized by a progressive decline in cognitive function and memory, AD typically has an insidious onset and worsens over time (Salloway et al., 2014). The disease was first identified by Dr. Alois Alzheimer in 1906, who noted specific pathological features in the cerebral cortex of affected individuals (Alzheimer's Association, 2021). These features include the presence of amyloid plaques and neurofibrillary tangles, which are now recognized as hallmarks of the disease (Selkoe, 2001). The amyloid plaques consist of aggregated beta-amyloid peptides, while neurofibrillary tangles are composed of hyperphosphorylated tau protein (Braak & Braak, 1991). The prevalence of AD increases significantly with age, with a diagnosis typically confirmed in individuals over the age of 65 (Alzheimer's Association, 2021).

The World Health Organization (WHO) estimated that in 2006, approximately 26.6 million people aged over 65 were living with AD, and projections suggest that this number could rise to one in 85 individuals globally by 2050 (World Health Organization, 2012). The symptoms of Alzheimer's disease manifest uniquely in each individual, complicating diagnosis and treatment (Salloway et al., 2014). Diagnosis is typically confirmed through a combination of behavioral assessments, cognitive tests, and neuroimaging techniques (Haeusler et al., 2016). Recent research has highlighted the potential role of activity-dependent neuroprotective protein (ADNP) as a biomarker for assessing Alzheimer's disease risk in older adults (Gozes et al., 2020). Elevated ADNP levels in blood have been correlated with the presence of amyloid plaques in the brain, suggesting a possible avenue for early intervention (Gozes et al., 2020).

The pathophysiology of Alzheimer's disease is multifactorial, involving several biochemical and pathological mechanisms. One prominent theory is the amyloid hypothesis, which posits that an imbalance in the production and clearance of amyloid-beta (Aβ) leads to its accumulation and the formation of senile plaques (Hardy & Higgins, 1992). These plaques are thought to initiate neuroinflammatory processes and increase the production of reactive oxygen species, contributing to neuronal damage (Karran & De Strooper, 2016). Another significant mechanism is the cholinergic hypothesis, which suggests that cognitive impairments in AD are exacerbated by deficits in cholinergic neurotransmission due to the increased activity of acetylcholinesterase and butyrylcholinesterase (Bartus et al., 1982). In light of these hypotheses, various therapeutic strategies have been explored, including the development of anti-amyloid agents and cholinesterase inhibitors (Cummings et al., 2014).

Despite the advancements in understanding the underlying mechanisms of Alzheimer's disease, effective treatment options remain limited. The reliance on pharmacological interventions has prompted a growing interest in alternative therapies, particularly those derived from natural sources. Approximately 80% of the population in developing countries continues to depend on plant-based medicines for primary healthcare (World Health Organization, 1978). Among the various medicinal plants, Mucuna pruriens Linn., commonly known as Velvet bean, has gained attention for its diverse therapeutic properties. This plant is indigenous to tropical regions, including India and the West Indies, and is a key component of over 200 traditional medicinal

formulations (Mistry, 2009). The seeds of Mucuna pruriens are particularly noted for their high L-Dopa content, a precursor to dopamine, which has implications for neurodegenerative diseases, including Alzheimer's (Mistry, 2009).

Mucuna pruriens has been reported to contain a wide range of phytochemicals, including alkaloids such as mucunadine and mucunine, amino acids, and various fatty acids (Duke, 1992). Recent studies have also identified new lipid derivatives from the seeds, expanding the understanding of the plant's phytochemical profile (Mistry, 2009). The therapeutic potential of Mucuna pruriens is not limited to its dopaminergic effects; it also exhibits antioxidant properties, which may mitigate oxidative stress associated with neurodegenerative diseases (Mistry, 2009). The presence of bioactive compounds in Mucuna pruriens suggests that it could offer a complementary approach to conventional treatments for Alzheimer's disease, particularly in the context of managing symptoms and improving overall quality of life for patients.

In conclusion, Alzheimer's disease represents a significant public health challenge, characterized by complex pathophysiological mechanisms and a growing prevalence among aging populations. While current therapeutic options are limited, the exploration of natural products, such as Mucuna pruriens, offers a promising avenue for developing novel interventions. The integration of traditional medicinal knowledge with modern scientific research may lead to innovative strategies in the prevention and management of Alzheimer's disease, ultimately improving outcomes for individuals affected by this debilitating condition.

2. Method

2.1 *Plant Material*

The plant *Mucuna pruriens* was procured from the Warangal Botanical Garden, where it was identified and authenticated by Dr. K.M. Chetty from the Department of Botany at Sri Venkateshwara University in Tirupati. A Certificate of Analysis was issued to confirm the identity of the plant material used in this study. *Mucuna pruriens*, commonly known as velvet bean, is recognized for its diverse phytochemical constituents and potential therapeutic properties.

2.2 Extraction

To prepare the extracts for subsequent analyses, the plant material was subjected to a drying process in a shaded environment to prevent degradation of sensitive compounds. Once dried, the plant material was finely ground to a powder using a mechanical grinder. The powdered plant material was then subjected to extraction using a Soxhlet apparatus, a method known for its efficiency in extracting bioactive compounds from plant matrices.

Two different solvent systems were employed for the extraction process: methanol and a hydroalcoholic solution. The selection of these solvents was based on their ability to dissolve a wide range of phytochemicals, including phenolics, flavonoids, and alkaloids, which are often responsible for the antioxidant and enzyme inhibitory activities observed in plant extracts. The extraction conditions, including the duration and temperature, were optimized to maximize yield while maintaining the integrity of the bioactive compounds.

2.3 In Vitro Antioxidant Assay

The antioxidant activities of the extracts were evaluated using two well-established in vitro assays: the DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay and the hydrogen peroxide scavenging assay (HPSA). These assays were chosen due to their reliability in assessing the free radical scavenging capacity of various substances, including plant extracts.

2.3.1. DPPH Assay

The DPPH assay was conducted to assess the free radical scavenging ability of the *Mucuna pruriens* extracts. A stock solution of DPPH was prepared by dissolving 0.3 g of DPPH in 100 mL of ethanol to achieve a final concentration of 0.3 mM. Various concentrations of the extracts were prepared (ranging from 10 to 100 µg/mL), and 0.1 mL of each concentration was mixed with 0.4 mL of the DPPH solution in a test tube. The mixture was thoroughly shaken and incubated in the dark at room temperature for 30 minutes to allow for the reaction to occur (Dubey et.al., 2023).

After incubation, the absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The absorbance of the control (blank) was recorded using a solution containing all reagents except the test extract. The scavenging effect of the DPPH radical was calculated using the following formula:

$$\% \text{ Scavenging activity} = \text{Abs (control)} - \text{Abs (standard)} / \text{Abs (control)} \times 100$$

The IC50 value, which represents the concentration of the extract required to inhibit 50% of the DPPH radicals, was determined from the dose-response curve generated from the absorbance readings. The tests were conducted in triplicate to ensure the reliability of the data.

2.3.2. Hydrogen Peroxide Scavenging Assay (HPSA)

The hydrogen peroxide scavenging activity of the extracts was assessed according to the method described by Ruch et al. A solution of hydrogen peroxide (H_2O_2) was prepared at a concentration of 2 mM in a phosphate buffer (pH 7.4). Various concentrations of the extracts (10 to 100 µg/mL) were then added to 0.6 mL of the hydrogen peroxide solution in separate test tubes (Dubey et.al., 2023)..

After a 10-minute incubation period, the absorbance of the mixture was measured at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. The scavenging activity was calculated using the same formula as in the DPPH assay:

$$\% \text{ Scavenging activity} = \text{Abs (control)} - \text{Abs (standard)} / \text{Abs (control)} \times 100$$

Ascorbic acid, a well-known antioxidant, was used as a standard for comparison in this assay.

2.3.3Anti-Acetylcholinesterase Assay

The anti-acetylcholinesterase (AChE) activity of the Mucuna pruriens extracts was evaluated using Ellman's method, which is a colorimetric assay developed in 1961. This assay measures

the inhibition of the enzyme acetylcholinesterase, which plays a crucial role in the regulation of neurotransmission (Dubey et.al., 2023).

In this assay, a reaction mixture was prepared containing 150 µL of 0.1 M sodium phosphate buffer (pH 8), 10 µL of the test compound solution at varying concentrations, and 20 µL of the enzyme solution (0.09 units/mL). The mixture was incubated at 25°C for 15 minutes to allow for enzyme-substrate interactions.

Following the incubation, 10 µL of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) solution and 10 µL of acetylthiocholine iodide were added to the mixture. The reaction was allowed to proceed for an additional 10 minutes, during which a yellow-colored complex was formed as a result of the enzymatic reaction. The intensity of the color produced was measured spectrophotometrically at 410 nm.

The percentage of enzyme inhibition was calculated using the following formula:

% Enzyme inhibition = [(Optical density of test-blank) X 100]

Physostigmine, a reversible inhibitor of acetylcholinesterase, was used as a standard for comparison in this assay.

3. Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). The IC50 values for the antioxidant assays were calculated using GraphPad Prism software, which allows for the analysis of dose-response curves. Statistical significance was assessed using appropriate statistical tests, and a p-value of less than 0.05 was considered statistically significant.

In summary, the methodological approach employed in this study involved the careful selection of plant material, extraction techniques, and a series of well-established in vitro assays to evaluate the antioxidant and anti-acetylcholinesterase activities of Mucuna pruriens extracts. The results obtained from these assays contribute to a better understanding of the pharmacological potential of this plant and its bioactive

4. Results

4.1Antioxidant Activities

The present study aimed to evaluate the antioxidant potential of various extracts of *Mucuna pruriens* through two primary assays: the DPPH radical scavenging activity and the hydrogen peroxide scavenging assay. Given the growing body of evidence linking oxidative stress to neurodegenerative diseases, the findings from these assays provide critical insights into the potential protective mechanisms of *Mucuna pruriens* extracts against oxidative damage.

4.2 DPPH Radical Scavenging Activity

The DPPH radical scavenging assay is widely recognized for its ability to assess the free radical scavenging capacity of various substances. The results obtained from this assay are

summarized in Table 1, which presents the percentage inhibition of DPPH radicals by the methanolic and hydroalcoholic extracts of *Mucuna pruriens* at different concentrations, alongside the standard antioxidant, butylated hydroxytoluene (BHT).

As shown in Table 1, the methanolic extract exhibited a significant dose-dependent increase in DPPH radical scavenging activity, with an IC50 value of 25 µg/ml. In contrast, the hydroalcoholic extract displayed a much higher IC50 value of 147 µg/ml, indicating a lower radical scavenging capacity compared to the methanolic extract. The standard BHT demonstrated an IC50 value of 10 µg/ml, underscoring its potency as an antioxidant.

At the highest concentration tested (100 µg/ml), the methanolic extract achieved a remarkable 88.87% inhibition of DPPH radicals, which is substantially higher than the hydroalcoholic extract's 37.45%. This stark contrast suggests that the methanolic extract contains more effective antioxidant compounds, possibly due to the extraction efficiency of the solvent used. The ability of the methanolic extract to scavenge DPPH radicals more effectively than the hydroalcoholic extract aligns with previous findings that have indicated the superior antioxidant properties of extracts obtained using non-polar solvents.

Table 1: DPPH Radical Scavenging activity of various extract

S. No.	Concentration (µg/ml)	% Inhibition		
		Standard (Butylated hydroxyl Toulene)	Methanol	Hydroalcoholic Extract
1.	20	53.95	45.94	19.34
2.	40	64.81	59.86	29.34
3.	60	76.62	68.18	30.34
4.	80	85.21	76.95	34.32
5	100	91.45	88.87	37.45
IC_{50} (µg/ml)		10	25	147

4.3 Hydrogen Peroxide Radical Scavenging Activity

The hydrogen peroxide scavenging assay further elucidated the antioxidant potential of the extracts. As presented in Table 2, the methanolic extract again demonstrated a higher percentage of inhibition across all tested concentrations compared to the hydroalcoholic extract. The IC50 value for the methanolic extract was determined to be 54 µg/ml, while the hydroalcoholic extract had a significantly higher IC50 of 131 µg/ml. The standard ascorbic acid, a well-known antioxidant, showed an IC50 value of 47 µg/ml, indicating its strong capability to neutralize hydrogen peroxide.

At 100 µg/ml, the methanolic extract achieved a 65.56% inhibition of hydrogen peroxide, while the hydroalcoholic extract only reached 38.56%. These results reinforce the notion that the methanolic extract possesses a more potent antioxidant activity, which may be attributed to the

presence of phenolic compounds, flavonoids, and other bioactive constituents that are better extracted in methanol.

Table 2: Hydrogen peroxide Radical Scavenging activity of various extract

S. No.	Concentration	% inhibition		
		Standard (Ascorbic acid)	Methanol	Hydroalcoholic Extract
1	20 µg/ml	32.23	40.43	12.34
2	40 µg/ml	38.13	45.65	24.54
3	60 µg/ml	56.34	50.81	28.45
4	80 µg/ml	73.32	57.65	32.54
5	100 µg/ml	88.34	65.56	38.56
IC_{50} (µg/ml)		47	54	131

4.4 Anti-Acetylcholinesterase Assay

The anti-acetylcholinesterase (AChE) assay was conducted to evaluate the potential of the extracts as inhibitors of AChE, an enzyme implicated in neurodegenerative disorders such as Alzheimer's disease. The results of this assay, summarized in Table 3, indicate that both extracts exhibited inhibitory activity against AChE, but the methanolic extract demonstrated a significantly lower IC50 value of 115 µg/ml compared to the hydroalcoholic extract's IC50 value of 950 µg/ml. The Methanolic extract has shown good enzyme inhibition activity. Hence, they were subjected to in vivo Pharmacological studies and result are tabulated in Table 3 and Figure-1.

The efficacy of the methanolic extract in inhibiting AChE can be attributed to its higher concentration of active phytochemicals, which may include alkaloids, flavonoids, and phenolic compounds known for their neuroprotective properties. The lower IC50 value signifies that less of the methanolic extract is required to achieve a comparable level of AChE inhibition, suggesting its potential utility in the development of therapeutic agents aimed at managing or preventing neurodegenerative diseases.

Table 3: Acetyl cholinesterase assay of various extract

Extract	IC_{50} value
Methanol extract	115 µg/ml
Hydroalcoholic Extract	950µg/ml

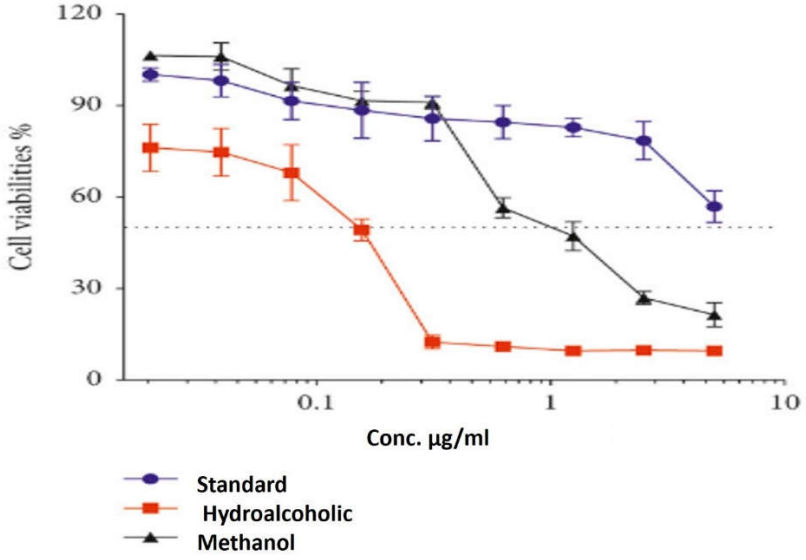

Fig 1: Acetyl cholinesterase assay of various extract

5. Discussion

The findings from the DPPH and hydrogen peroxide scavenging assays clearly indicate that the methanolic extract of *Mucuna pruriens* possesses superior antioxidant capabilities compared to the hydroalcoholic extract. This is consistent with the hypothesis that the choice of solvent plays a crucial role in the extraction of bioactive compounds. Methanol, being a polar aprotic solvent, is known to effectively extract a wide range of phytochemicals, including polyphenols and flavonoids, which are primarily responsible for antioxidant activity.

Acetylcholinesterase (AChE) serves as a biomarker for amnesia. The percentage of enzyme inhibition was quantified for all extracts, revealing an IC50 value of 115 µg/ml for the methanolic extract in comparison to 950 µg/ml for the hydroalcoholic extract. While all extracts exhibited inhibitory activity against AChE, the methanolic extract of Mucuna pruriens demonstrated superior inhibitory efficacy. The utilization of methanol as a solvent facilitated enhanced activity compared to the hydroalcoholic extract as reported in various studies. The elevated efficacy of the methanolic extract may stem from its ability to extract compounds with higher AChE inhibitory potential. Thus, the methanolic extract is deemed suitable for subsequent investigations.

The IC50 values obtained from both antioxidant assays suggest that the methanolic extract can effectively mitigate oxidative stress, which is a significant contributor to neuronal damage in neurodegenerative diseases. The ability to scavenge free radicals and neutralize reactive oxygen species (ROS) is essential for protecting neuronal integrity and function. The results align with existing literature that highlights the protective effects of antioxidants in the context of neurodegeneration, further justifying the exploration of *Mucuna pruriens* as a potential therapeutic agent.

Moreover, the results from the anti-acetylcholinesterase assay indicate that the methanolic extract not only exhibits antioxidant properties but also possesses the ability to inhibit AChE, thereby potentially enhancing cholinergic function. This dual action of antioxidant activity and

AChE inhibition positions *Mucuna pruriens* as a promising candidate for further pharmacological studies aimed at developing treatments for cognitive decline associated with aging and neurodegenerative diseases.

A multitude of studies have established a connection between oxidative stress and neurodegenerative diseases associated with aging. Concurrently, various investigations have highlighted the beneficial effects of antioxidants in mitigating or preventing neuronal death that is central to the pathophysiology of these disorders. Moreover, the antioxidant capacity of a compound can be linked to its ability to scavenge free radicals and demonstrate total antioxidant activity. To assess the potential of plant extracts as antioxidants, evaluations were conducted measuring their capacities to scavenge DPPH and perform hydrogen peroxide scavenging assays.

In summary, the methanolic extract of *Mucuna pruriens* demonstrates significant antioxidant activity and AChE inhibitory effects, warranting further exploration of its bioactive constituents and their mechanisms of action. Future studies should focus on isolating and characterizing the specific compounds responsible for these effects, as well as conducting *in vivo* studies to validate the therapeutic potential of *Mucuna pruriens* in neurodegenerative conditions. The promising results of this study contribute to the growing body of evidence supporting the use of natural products in the prevention and treatment of age-related neurodegenerative diseases.

5. Conclusion:

This study encompassed the evaluation of *in vitro* antioxidant activity and *in vitro* AChE inhibitory potential of various extracts of *Mucuna pruriens*. The methanolic extract exhibited significant radical scavenging activity in a dose-dependent manner. The AChE inhibition study, performed using a modified Ellman's method, verified that the methanolic extract of *Mucuna pruriens* achieved maximum inhibition relative to the IC50 values of all tested extracts. Consequently, these extracts demonstrate notable antioxidant capability and AChE inhibitory activity, paving the way for potential alternative sources of antioxidants and AChE inhibitors, with further *in vivo* studies warranted based on these findings.

References:

1. Alzheimer's Association. (2021). Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, 17(3), 327-406.
2. Salloway, S., Sperling, R., and Fox, N. C. (2014). **Amyloid imaging in Alzheimer’s disease: a review of the literature**. *Alzheimer's & Dementia*, 10(4), 489-496.
3. Selkoe, D. J. (2001). Alzheimer’s disease: genes, proteins, and therapy. *Physiological Reviews*, 81(2), 741-766.
4. Braak, H., & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathologica*, 82(4), 239-259.
5. World Health Organization. (2012). Dementia: a public health priority. Geneva: WHO.
6. Haeusler, A., (2016). Diagnostic and therapeutic approaches to Alzheimer's disease. *Nature Reviews Neurology*, 12(3), 155-167.

7. Gozes, I., (2020). Activity-dependent neuroprotective protein (ADNP) as a biomarker for Alzheimer's disease. *Frontiers in Molecular Neuroscience*, 13, 78.
8. Hardy, J. and Higgins, G. A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science*, 256(5054), 184-185.
9. Karran, E., and De Strooper, B. (2016). The amyloid cascade hypothesis: are we poised for success? *Nature Reviews Drug Discovery*, 15(1), 43-58.
10. Bartus, R. T., Dean, R. L., Beer, B., and Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science*, 217(4558), 408-414.
11. Cummings, J., (2014). Alzheimer's disease drug development pipeline: 2014. *Alzheimer's & Dementia*, 10(6), 679-688.
12. Mistry, A. (2009). Mucuna pruriens: A review. *International Journal of Pharmaceutical Sciences and Research*, 1(2), 1-6.
13. Duke, J. A. (1992). Handbook of phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, FL: CRC Press.
14. Dubey, A., Ghosh, N. S., and Singh, R. (2023). A Toxicological Study on Seed Extracts of Asparagus Racemosus Linn (Ethanolic and Water) in Experimental Animals. Journal of Advanced Zoology, 44(2), 71–78. https://doi.org/10.17762/jaz.v44i2.194
15. Dubey, A., Ghosh, N. S., and Singh, R.S., (2023). Effects of aqueous and ethanolic seed extract of Asparagus racemosus Linn on neurobehavioral pattern of acrylamide induced experimental Zebra fish. Research Journal of Biotechnology.18(11),81-88. https://doi.org/10.25303/1811rjbt081088.
16. Dubey, A., Ghosh, N. S., and Singh, R.S., (2023). Role of Aqueous and Ethanolic Seed Extract of Asparagus racemosus on Acr- Induced Neurotoxicity in Adult Zebrafish: Emergence of Neuroprotective Results. Egyptian Journal of Aquatic Biology & Fisheries, 27(6), 285-296.DOI: 10.21608/EJABF.2023.329192
17. Adewusi EA, Moodley N, Steenkamp V. (2011). Antioxidant and acetylcholinesterase inhibitory activity of selected southern African medicinal plants. S Afr J of Bot, 3:638–644