Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
NLM (National Library of Medicine): ID: (101671502)
Index Copernicus Value 2022: 83.058
Volume 13, Issue 3; 2024, 1-5

ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

Original Research Article

An Assessment of Invitro Anti-Alzheimer Activity of *Ammania Bacciferra Linn.*

Rahul Maskwade*[1], Kratika Daniel[2], Sachin K Jain[2], Sudha Vengurlekar[2]

[1] Research Scholar, Oriental College of Pharmacy & Research, Oriental university, Indore, MP

[2] Professor, Oriental College of Pharmacy & Research, Oriental university, Indore, MP

Article Info: Received: 17-03-2024 / Revised: 19-04-2024 / Accepted: 24-05-2024
Address for correspondence: Rahul Maskwade
DOI: https://doi.org/10.32553/jbpr.v13i3.1085
Conflict of interest statement: No conflict of interest

Abstract:
Neurodegenerative disorders are characterized by the progressive and irreversible loss of neurons from specific brain areas. Alzheimer's disease (AD), where loss of hippocampal and cortical neurons impairs memory and cognitive function. From the literature review it was revealed that *Ammania bacciferra linn.* possess good antioxidant activity because of its active constituents and anti-oxidant may treat some neurodegenerative disorders. The main objective of this work is to identify the Alzheimer activity of *Ammania bacciferra linn.* Invitro activity was performed by Ellman method and IC50 Value was found to be 315 µg/mL Further extract was subjected for *in vivo* activity.
Keywords: Neurodegenerative disorders, neurons, Alzheimer's diseases. Memory enhancer.

Introduction

Neurodegenerative disorders are characterized by the progressive and irreversible loss of neurons from specific brain areas. Prototypical neurodegenerative diseases include Lou Gehrig's disease, Amyotrophic Lateral Sclerosis (ALS), where degeneration of spinal, bulbar, and cortical motor neurons causes muscular weakness; Alzheimer's disease (AD), where loss of hippocampal and cortical neurons impairs memory and cognitive function; and Parkinson's disease (PD) and Huntington's disease (HD), where abnormalities in movement control result from basal ganglia structure loss. Current treatments for neurodegenerative diseases only alleviate the symptoms; they do not alter the underlying neurodegenerative process. [Goodman, L. S.. 1996]. Cognition enhancers encompass both pharmaceutical drugs and natural supplements, which are employed to boost the performance of several cognitive capacities in humans, including cognition, memory, intelligence, motivation, attention, and concentration, particularly in cases when these abilities have seen impairment [Melnick, 2000)]. *Ammania bacciferra linn.* (Family Lythraceae) commonly known as Aginbuti, Ban mirich, Dadmari, Jungli mehendi etc. It was used for the management of urinary calculi and the creation of blisters, as well as for the treatment of rheumatic aches, herpes, ringworm, and various other dermatological conditions. Herpetic eruptions can be treated by applying a mixture of plant leaves or ashes with oil. The utilization of fresh, bruised leaves has been observed in the treatment of skin ailments, serving as a

rubefacient and an external cure for ringworm and parasitic skin conditions.

According to reports, the plant is found to possess hentriacontine, dotriacontanol, betulinic acid, lupeol, ellagic acid, quercetin, and lawsone. The root exhibited the presence of flavonoids, phenols, and carbohydrates, with a total tannin content of 0.42% and polyphenols accounting for 4.04%. Various components of Ammannia baccifera were found to contain Vitamin C, steroids, triterpenes, coumarines, flavanol, and tannin. In their study, Jain et al. (year) observed the presence of tannin, flavonoids, phenols, and carbohydrates in the stem and leaves of Ammannia baccifera. The total tannin content was determined to be 4.141%, whereas the total phenol content was discovered to be 3.53%. The compounds betulinic acid, 4-hydroxy-a-tetralone, tetralone-4-O-B-D-glucopyranoside, and ellagic acid were obtained from the methanolic extract of Ammannia baccifera by Upadhyay et al. The presence of tetralone derivatives, specifically (-)-(4R), has been documented in the plant.Hydroxy-1-tetralone, also known as (-)-(4S)The compound (-)-(4S) is acetoxy-1-tetralone.This study focuses on the compounds hydroxy-1-tetralone-4-O-β-D-glucoside, β-sitosterol, and β-sitosterol-β-D-glucoside.

The main objective of this work is to assess the invitro anti-Alzheimer activity.

Material & Method

Collection and Authentication of Plant **-** *Ammannia baccifera Linn*. plants were gathered from the paddy fields of nearby Indore Madhya Pradesh, India, in November 2023. The plant was taxonomically recognized and verified by Dr. Bhawna Tomar, agriculturist & Assistant Professor at Oriental University, Indore MP. Herbarium. Was submitted to Pharmacognosy Department of OCPR (Pharmcog/2024/008).

Extraction of Plant **-** The newly harvested plants were dried in a location with little sunlight and then crushed into a rough powder using a mechanical grinding machine. Afterwards, the powder was sifted using a no.40 sieve and stored in an airtight container for extraction. Approximately 500 grams of powder were used in the extraction process.

The continuous hot percolation process is employed for the extraction of extracts. The purified and crushed material of whole *Ammannia baccifera Linn*. plants were used for extraction. A quantity of roughly 500 grams of powdered material was evenly dispersed over a Soxhlet apparatus. The sample was subsequently extracted using a series of solvents, starting with non-polar solvents, and advancing to polar solvents. The solvents used included petroleum ether, and a mixture of water and alcohol in a 70:30 ratio. The solvents were purified before being used. All the extractive values mentioned in Table 1.

Phytochemical Screening –

The extract was subjected for phytochemical screening for alkaloids, glycosides, fats & oils, carbohydrates, proteins all results discussed in Table 2.

Invitro screening for Acetylcholinesterase Enzyme Inhibition

Enzymatic hydrolysis of acetylcholine (ACH) is a crucial stage in neuronal transmission, and its impairment may be a factor in the development of Alzheimer's disease. Ellman's method (Ellman et al., 1961) is a widely used technique for measuring cholinesterase activity and monitoring the hydrolysis of acetylcholinesterase (ACHE) or butyrylcholinesterase (BCHE) in vitro.

Standard - Galantamine was employed as the benchmark or reference. A gift sample of the standard medicine weighing 1 gram was received from Sun Pharma Pvt Ltd.

Test solution – The sample was mixed with a buffer solution (50mM tris-HCl buffer with a pH of 8.0), and the concentration of the sample ranged from 100 to 500µg/mL (Komersova, A 2007).

Procedure- In a test tube, combine 1.71mL of 50mM tris-HCl buffer (pH 8.0) with 0.25mL of plant extracts at varying concentrations of 100 –

500µg/mL. Then, add 10µL of 6.67UmL AChE and 20µL of 10mM DTNB in buffer. The standard solutions were made using the same method as the test solution, which involved dissolving 50mM tris buffer at a pH of 8.0. The mixture was subjected to incubation for a duration of 15 minutes at a temperature of 37 degrees Celsius. Next, 10µL of acetylthiocholine iodide (200mM) in buffer solution was introduced, and the absorbance was recorded at a wavelength of 412nm for a duration of 3 minutes.

Control - The reaction mixture of control does not include drug.

Observation - The percentage of enzyme inhibition was calculated from the rate of absorbance change with time, the calculation as follow: -

$$\%\ of\ inhibition = 100 - \frac{Absorbsnce\ of\ Test\ Compounds}{Absorbance\ of\ Control} \times 100$$

The experiment was conducted three times and the IC50 value was determined. The test extracts that hinder the hydrolysis of acetylcholine were acquired by regression analysis, correlating the percentage of inhibition with the precise concentration, utilizing the Ms-Excel program.

Result and Discussion

Steroids, phenolic compounds, terpenoids, and flavonoids are examples of naturally occurring chemicals generated from plants that have garnered a lot of attention recently due to their many pharmacological properties, including antioxidant function. An increasing number of people are interested in studying particular flavonoids, triterpenoids, and steroids because of research on their potential health benefits. One of their main advantages in this regard is its capacity to halt the course of neurodegenerative diseases. Free radicals can be avoided and eliminated with the use of antioxidants, helping to protect against degenerative diseases. Considering this, the purpose of this study was to evaluate the anti-alzheimer effects of extracts of Ammannia baccifera Linn. Ammannia Baccifera Linn. was chosen for this investigation to demonstrate its potency as an anti-Alzheimer medication in vitro.

Identification of extractive values of whole plant of *Ammannia Baccifera Linn*.

The shade dried coarsely powdered whole plant of *A. baccifera Linn* was first defatted with petroleum ether and then subjected to hydroalcoholic extract in the ration 70:30 of alcohol and water using Soxhlet's apparatus. Extractive values, physical appearance and Percentage yield of hydroalcoholic extract was summarised in Table 1.

Table 1: Extractive Values of Hydroalcoholic extract of *Ammannia Baccifera Linn.*

S. No	Particulars	Observation
1	Plant used	***Ammannia Baccifera Linn***.
2	Part	Whole plant
3	Yield	15.6%
4	Colour of extract	Dark brown
5	Texture of extract	Semi Solid

Phytochemical Screening

Table 2 presents the results of a preliminary phytochemical investigation that revealed the presence of flavonoids, proteins, sugars, steroids, glycosides, and tannins as phytoconstituents in ***Ammannia Baccifera Linn*** Hydroalcoholic extracts.

Table 2: Phytochemical screening of *Ammannia Baccifera Linn* Hydroalcoholic extracts

S. No	Phytoconstituents	Tests	Result
	Alkaloids	Mayer's Test	-
		Dragondroff's test	-
		Hager's test	-
		Wager's test	-
	Sterols	Liebermann's Burchard test	+
		Salkowski's test	+
	Carbohydrates	Anthrone test	+
		Fehling's Test	+
		Benedict Test	+
	Flavonoids	Shinoda's test	+
		Sulphuric acid test	+
	Glycosides	Molisch's test	+
	Proteins & amino Acids	Millon's reagent	-
		Ninhydrin reagent	-
		Biuret Test	-
	Gums & Mucilage's	Precipitation with alcohol	+
	Saponin	Foam Test	+
	Fixed oils	Spot test	-
.	Phenolic compounds and Tannins	Dilute ferric chloride solution	+
		1% solution of Gelatin & NaOH	+
		10% lead acetate solution	+

Invitro screening for Acetylcholinesterase Enzyme Inhibition

One of the defining changes of Alzheimer's disease (AD) is increased activity of the enzyme acetyl cholinesterase (AChE), which hydrolyzes acetylcholine in both cholinergic and non-cholinergic brain neurons (Curcio et al., 1984). The percentage of inhibition is directly correlated with concentration, as shown by Table 3 and Figure 1.

Table 3: Percentage Inhibition of extracts of AChE Enzyme activity

S. No.	Concentration (µg/mL)	% Inhibition of extracts	IC50 Value
1.	100	26.63 ± 0.82	315 µg/mL
2.	200	38.36 ± 0.83	
3.	300	49.43 ± 0.86	
4.	400	52.56 ± 0.23	
5.	500	63.66 ± 1.02	
6.	Standard Galantamine (10 µg/mL)	78.92 ± 0.86	

Data are given as mean± SEM (n = 3)

On the other hand, it has been demonstrated that elevated AChE activity occurs inside and surrounding amyloid plaques, helping to facilitate the fibril formation of amyloid beta-peptides and boost their cytotoxicity (Dhanasekaran, 2015). At 500 µg/ml, hydroalcoholic extract shown very strong inhibition of 63.66±1.02 in comparison to standard galantamine (78.92±0.86). IC50 (50% Inhibition) was calculated by using Ms-Excel Program and found at 315µg/mL.

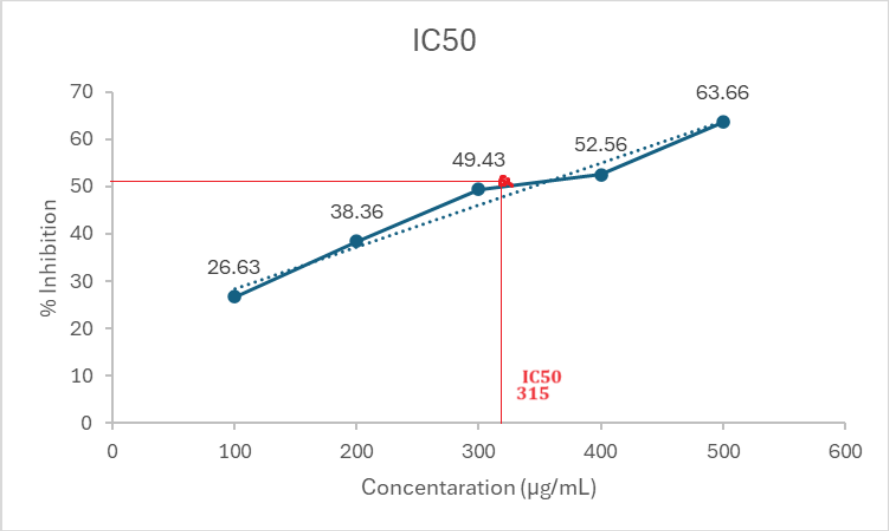

Figure 1 Graph between Concentration and % Inhibition

References

1. Goodman, L. S., & Gilman, A. G. (2001). Goodman & Gilman's the Pharmacological Basis of Therapeutics. McGraw-Hill Professional Publishing.
2. Armand S. Schachter, MD; Kenneth L. Davis, MD. Alzheimer's disease. Dialogues in Clinical Neuroscience. 2000: Vol-2, No 2; 91-100.
3. MacGill, M. (2023, April 21). *What to know about Alzheimer's disease*. https://www.medicalnewstoday.com/articles/159442 retrieved on 16th November 2023
4. *What Is Alzheimer's Disease?* (n.d.). National Institute on Aging. https://www.nia.nih.gov/health/what-alzheimers-disease retrieved on 16th November 2023
5. Prusiner SB. Biology and genetics of prions causing neurodegeneration. *Annu Rev Genet*, **2013**, *47*:601–623.
6. Snezana Agatonovic-Kustrina, Christine Kettleb, David W. Mortonb. A Molecular approach in drug development for Alzheimer's disease. Biomedicine & Pharmacotherapy, 2018; 553–565.
7. Begüm Nurpelin Saglık, Derya Osmaniye , Ulviye Acar evik , Serkan Levent ,Betül Kaya avu soglu, usuf Özkay and Zafer Asım Kaplanci. Design, Synthesis, and Structure–Activity Relationships of Thiazole Analogs as Anticholinesterase Agents for Alzheimer's Disease. Molecules. 2020; 25: 4312.
8. Shubhada R, Satyendra K, Rajput,S.S Sharma, Cognition Enhancers: Current Strategies and Future Perspectives , **2008**, Vol. 9, 42
9. Wollen, K. A. (2010). Alzheimer's disease: the pros and cons of pharmaceutical, nutritional, botanical, and stimulatory therapies, with a discussion of treatment strategies from the perspective of patients and practitioners. *Altern Med Rev*, *15*(3), 223-44.
10. Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology*, *7*(2), 88-95.
11. Dhanasekaran, S., Perumal, P., & Palayan, M. (2015). In-vitro Screening for acetylcholinesterase enzyme inhibition potential and antioxidant activity of extracts of Ipomoea aquatica Forsk: therapeutic lead for Alzheimer's disease. *Journal of Applied Pharmaceutical Science*, *5*(2), 012-016.