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Anti-Alzheimer Effect of *Ammannia baccifera* Whole Plants Ethanolic Extract

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Abstract: This study assessed the potential of *Ammannia baccifera* ethanolic extract to prevent Alzheimer's disease in rats. Two dosages of the plant extract were utilized for the treatment. The plant extracts greatly prevented rats from developing Alzheimer's disease in a dose-dependent manner. Rats given an ethanolic extract of *Ammannia baccifera* demonstrated good anti-Alzheimer activity, according to the previously published results. It might be as a result of the extract's active phytoconstituents. Therefore, by using this plant, we will be able to obtain drugs or potentially beneficial active ingredients for the treatment of neurodegenerative conditions at incredibly low cost. Additionally, by bypassing the synthetic way of manufacturing, our research may minimize the immense expenditure and environmental damage involved with the creation of some potential medicines that may be employed to treat neurodegenerative disorders.

Keywords: Alzheimer's disease, Neurological disorder, *Ammannia baccifera*, Ethanolic extract, Anti-Alzheimer, Medicinal plant

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Introduction

Alzheimer's disease (AD) is a neurological problem that gets worse over time and can not be cured. It makes it harder to think, remember, and do even simple things (Al-Snafi, 2020). Alzheimer's disease is the most common reason why older people get dementia. Alzheimer's disease is when a person's thinking, remembering, reasoning, and behavioral skills get worse to the point where it is hard for them to do daily chores and live a normal life (Gauni *et al.*, 2021). Different levels of dementia exist, from mild (when a person's abilities are just starting to be affected) to serious (when they need help for all of their daily tasks). Having different kinds of brain diseases can lead to different kinds of dementia. Lewy body dementia, frontotemporal disorders, and vascular dementia are some other types of dementia. It is not unusual for people to have mixed dementia, which is made up of two or more types of dementia (Surana and Mahajan, 2022).

In people with Alzheimer's disease, neurons and synapses break down in the brain cortex and some subcortical areas. The loss causes the areas affected to get worse quickly, which is called atrophy. There is damage in the temporal and parietal lobes, as well as in some parts of the frontal cortex and cingulate gyrus. A part of the brainstem called the locus coeruleus degenerates (Petchi *et al*., 2015). Alzheimer's disease patients had smaller parts of their brains as their illness got worse, from mild cognitive impairment to Alzheimer's disease. Under a microscope, neurofibrillary tangles and amyloid plaques are easy to see in the brains of people with Alzheimer's disease (Ahire *et al*., 2023). Because it is so complicated, Alzheimer's disease probably can't be cured by a single drug or any other method (Singh *et al*., 2011). The current solutions focus on helping people keep their mental health in good shape, deal with their behavioral issues, and put off certain problems like memory loss (Keservani and Sharma, 2014). Alzheimer's disease is marked by the buildup of abnormal and highly phosphorylated tau protein, which creates neurofibrillary clumps inside cells. There are also amyloid protein plaques that form because the amyloid beta precursor protein is folded and processed incorrectly (Ahire *et al*., 2022).

Neurofibrillary tangles are made when tau protein, which has been overphosphorylated, binds to Fe^{3+} and piles up. The Amyloid-b peptide can connect with transition metal ions and help make H_2O_2 by using transition metals. This can then lead to the production of dangerous OH radicals (Aswathy *et al*., 2021).

A lot of lipid breakdown happens in people with AD, which can kill neurons in a number of ways, such as by stopping ion pumps, glucose transporters, and glutamate transporters from working properly. Ahire *et al.* (2023) state that people who have Alzheimer's disease also have other reactive markers that are linked to protein breakdown, such as 3-nitrotyrosine and protein carbonyls. People with Parkinson's and Alzheimer's are often given a wide range of synthetic drugs to help their symptoms, but many of these drugs have negative side effects. It is still hard for the medical system to control Alzheimer's disease successfully while minimizing any negative effects (Behera *et al*., 2010).

Because of this, natural drugs made from medical plants are becoming more popular because they are cheap, easy to get, and do not have as many bad effects. Sherry *et al.* (2024) suggested that it is very important for medicinal herbs, which have been used for a long time to treat and control Alzheimer's disease, to be backed by science. There are many phytoconstituents in the plant *Ammannia baccifera* L. These include Vitamin C, lawsone, tannins, flavonoidsluteol, quercetin, hentriacontane, elagic acid, betulinic acid, dotriacontanol, ß-sitosterol glucoside, and triacontan-1,30diol. These compounds give the plant its therapeutic and pharmacological properties (Poornima *et al.*, 2014). Researchers have tried to prove that studying the possible anti-Alzheimer effects of *Ammannia baccifera* is a good idea by looking at existing research and proof of the plant's present uses (Patel and Shah, 2013).

This study assessed the potential of *Ammannia baccifera* ethanolic extract to prevent Alzheimer's disease in rats.

Materials and Methods

Selection and Collection of the Plant Material:

The selection of the plant is crucial as it establishes the foundation for the extraction of secondary plant metabolites, making it the most significant aspect of the project. To locate a plant, as was traditionally done in folklore, one must refer to the field of ethanobotany. Ethanobotany, sometimes known as "people's botany," is the scientific discipline that investigates the plants that held importance for early humans. Modern humans can be classified as participants in the discipline of ethanobotany, which is an interdisciplinary area of study focused on botany and encompassing elements of chemistry, pharmacology, and anthropology (Talele *et al*., 2022).

Plants have attracted global attention due to their potent pharmacological effects, low toxicity, and economic feasibility in the face of recent scientific progress. Consequently, researchers have extensively investigated their therapeutic properties. Information regarding the indigenous plants with therapeutic properties in a specific region can be obtained by conducting a search in the library. This will ensure the availability of plants for gathering (Aher *et al.*, 2023).

To select a medicinal plant with strong anti-Parkinson's and anti-Alzheimer's qualities, it is essential to seek guidance from a tribal medical practitioner regarding the plant's traditional and indigenous use for neurological disorders. To verify that the plants on the compliance list have not yet been investigated for their potential in preventing Alzheimer's and Parkinson's disease, it is necessary to undertake a literature review (Upadhyay *et al*., 2013). It is essential to authenticate the unidentified plant components and document the specific plant parts utilized, as well as the cultural methods employed for their preparation. The selection of the plant *Ammannia baccifera* for this study was based on a thorough examination of existing literature and an ethnopharmacological investigation (Kumari and Satyanarayana, 2019).

Extraction of Plant Material:

The newly harvested whole plant of *Ammannia baccifera* were thoroughly cleaned, shade dried and then pulverized into a fine powder using a mechanical grinder. Subsequently, the powder underwent filtration using sieve number 40 and was subsequently stored in a hermetically sealed container for the purpose of extraction. Approximately 500 g of powder was used in the extraction process (Soni *et al.*, 2013). A Soxhlet apparatus was used to evenly fill 500 g of powdered material. Afterwards, it was extracted using various solvents with different polarities, such as petroleum ether, ethanol, and water. The solvents underwent purification before being used. The extraction technique used was continuous hot percolation, which lasted for a duration of 72 h and involved the use of various solvents. The aqueous extraction was conducted using the cold-maceration procedure (Keservani *et al*., 2017).

Preparation of Extracts:

Ethanolic extract of whole plants of Ammannia baccifera Linn:

After extracting with Petroleum ether, the main residue was dried and then subjected to extraction for 72 h using 95% v/v ethanol (at a temperature range of 75–78°C). Distillation was employed to remove the solvent after the completion of the extraction process. A substance with a hue that is predominantly dark green was generated. Subsequently, the extract was stored in a desiccator to eradicate any residual moisture. To further investigate, the dehydrated extract was later stored in a hermetically sealed glass container (Khare and Shukla, 2022).

Biological Activity:

Selection of animals:

The healthy adult Wistar rats (180- 220 g b wt)

were acclimated to the laboratory setting for a period of one week. The animals were housed in well-ventilated polypropylene cages with a 12 h light and 12 h dark schedule, at a temperature of 25°C and a relative humidity of 55-65% (Supalkar *et al.*, 2023). The diet provided to the rats was standard. The rats were provided with unrestricted amounts of commercially available pelleted rat food and water. The Institutional Animal Ethics Committee (IAEC) has obtained authority to perform the experimental inquiry in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Rane *et al*., 2023).

Anti-Alzheimer's Activity:

Object recognition test:

The apparatus had a grid floor that could be efficiently sanitized with hydrogen peroxide following each experiment. Additionally, it was constructed from plywood with dimensions of 70 × 60 × 30 cm. A 40 W bulb, positioned 50 cm above the box, illuminated the equipment. The components that were to be differentiated were also separated into two separate plywood structures, each with a height of 8 cm and painted in black.

Prior to the trial, the rats were given a 2 min opportunity to investigate the empty box. During the first trial (T1) on test day, two identical items were positioned in two opposite corners of the box. The duration of each rat's investigation of the object for 20 sec was recorded. The phrases "touching" and "exploration" were employed to delineate the procedure of aligning the nose towards an object and thereafter moving towards it within a proximity of less than 2 mm. In trial T1, one of the objects was substituted with a distinct object during the subsequent trial (T2), which occurred 90 min later. The rats remained within the box for a duration of 5 min. Distinct time records were created for the familiar item (F) and the new object (N). Subsequently, the formula N-F/N+F was employed to compute the discrimination index (D). During T2, the placements and functions of the two objects were randomly interchanged to prevent any preferences for certain locations or cues based on smell. The device was also cleansed using hydrogen peroxide. After being chosen, six male and six female Wistar rats were divided into four groups and provided with the necessary care (Satyanarayana, 2019; Sonawane *et al.*, 2023).

The rats were injected either a vehicle, extract (at dosages of 200 and 400 mg/kg, administered intraperitoneally), or piracetam (at a dose of 100 mg/kg, administered intraperitoneally), 30 min before the first session (Table 1). It had been 90 min since the first trial. There were six animals in each group (Keservani *et al*., 2016).

Y–Maze Test:

A set of Wistar rats, including both males and females, was selected and divided into four groups, each including six animals. Subsequently, the rats had the treatments as mentioned in Table 2.

The experiment was conducted on Wistar rats, with measurements taken at 60 and 120 min after treatment. Each rat was individually placed in a symmetrical Y-shaped runway of 33 × 38 × 13 cm for duration of 3 min. An entry was recorded each time a rat utilized all four of its feet to enter the maze (Rane *et al*., 2023).

Results

Extraction of Whole Plant of Ammannia baccifera:

Using various solvents with increasing polarity, entire *Ammannia baccifera* plants were extracted after being dried in the shade and ground into a fine powder. This was accomplished by means of a Soxhlet device and a continuous hot percolation process. In addition, a cold maceration method was used to get aqueous extracts. Table 3 shows the values that were retrieved.

Anti-Alzheimer's Activity:

Object recognition test:

Table 1: Distribution of animal for Object recognition test

Group	Administered	Dose	Type of group
Group I	Propylene glycol	5 ml/kg body weight	Vehicle group
Group II	Extract	200 mg/kg body weight	Intraperitonially
Group III	Extract	400 mg/kg body weight	Intraperitonially
Group IV	Piracetam	100 mg/kg body weight	Standard

Table 2: Distribution of animal for Y – Maze Test

Group	Administered	Dose	Type of group
Group I	Propylene glycol	5 ml/kg body weight	Vehicle group
Group II	Extract	200 mg/kg body weight	Intraperitonially
Group III	Extract	400 mg/kg body weight	Intraperitonially
Group IV	Diazepam	10 mg/kg body weight	Standard

Table 3: Extraction of whole plants of *Ammannia baccifera*

Plant name	Partsused	Method ofextraction	Yield in percentage Ethanolic extract
Ammannia baccifera Linn.	Whole Plant	Continuous Hot Percolation	8.9
Ammannia baccifera Linn.	Whole Plant	Cold Maceration	9.1

Table 4: The impact of the ethanolic extract of *Ammannia baccifera* (EEAB) on the object recognition test in rats

S. No.	Group	Treatment with dosing	Object recognition time in seconds		Discrimination index
			Familiar object	Novel object	
1.	Group I	Propylene glycol 5 ml/kg	81.52±0.98	96.21±0.90	0.096±0.23
2.	Group II	EEAB 200 mg/kg	50.59±1.26	84.69±1.10	0.360±0.52
3.	Group III	EEAB 400 mg/kg	44.54±1.22	82.11±0.96	0.296±0.42
4.	Group IV	Piracetam 100 mg/kg	35.31±0.63	77.52±1.10	0.410±0.15

The animals in the object identification test took longer to investigate the objects during the first trial (T1 session). When a new object took the place of a familiar one in the second trial (T2 session), the ethanol extract of *Ammannia baccifera* and Piracetam considerably shortened the time needed to investigate the new object compared to the familiar one. Additionally, Ammannia baccifera Linn. ethanol extract demonstrated a noteworthy rise in the discrimination index (Table 4).

Y - Maze test:

The rats treated with the extract at the tested levels showed a notable reduction in exploratory behavior in the Y-maze test, as compared to the control group (Table 5). Consequently, the ethanol extract of *Ammannia baccifera* effectively decreased exploratory behavior, indicating a positive impact on memory and learning.

The Y-maze test, also referred to as the object recognition test, is a precise and sensitive method for evaluating an experimental animal's spatial identification memory. The Y-maze test results demonstrated a substantial decrease in transfer

Table 5: Y-Maze test using rat: Impact of *Ammannia baccifera* (EEAB) ethanolic extract

S. No.	Group	Treatment with dosing	Exploratory Time	
			1 h	2 h
1.	Group I	Propylene glycol 5 ml/kg	11.29±0.96	12.10±0.85
2.	Group II	EEAB 200 mg/kg	8.39±0.89	8.05±0.89
3.	Group III	EEAB 400 mg/kg	7.26±0.85	4.96±1.1
4.	Group IV	Diazepam 10 mg/kg	5.22±1.1	5.01±0.75

latency, while the object recognition test revealed an elevation in discriminating index. These findings indicate that the rats treated with *Ammannia baccifera* ethanol extract exhibited significant cognitive enhancement. The ethanol extract of *Ammannia baccifera* has been found to possess a neuroprotective effect, perhaps aiding in cognition. The anti-Alzheimer's activity of *Ammannia baccifera* is attributed to its high concentration of antioxidants, specifically flavonoids and polyphenol components.

Discussion

Oxidative damage arises from heightened metabolic activity in cerebral tissues associated with brain lipids. Moreover, the catecholamines in the brain exhibited heightened susceptibility to the production of free radicals. Adrenaline, noradrenaline, and dopamine are catecholamines that have the ability to undergo auto-oxidation, which is the spontaneous breakdown into free radicals. Alternatively, these catecholamines can also be transformed into radicals by endogenous enzymes such as monoamine oxidase. Anti-oxidants, which encompass compounds containing flavonoids, protect neural tissue from harm caused by oxidative stress. Consequently, clinical research indicates that the development of Alzheimer's disease is facilitated by the promotion of free radicals and the reduction of antioxidant levels. Reactive oxygen species are the main contributing component to the pathophysiology of Alzheimer's disease. Multiple studies have demonstrated that plants with high levels of antioxidants had neuroprotective properties, hence potentially aiding in the treatment of memory impairment and neurological problems associated with aging. The Y-maze test results demonstrated a substantial reduction in transfer latency, while the object recognition test revealed an elevation in the discriminating index. These findings indicate that the rat treated with *Ammannia baccifera* ethanol extract exhibited noteworthy cognitive enhancement. Consequently, the ethanol extract of *Ammannia baccifera* exhibits a neuroprotective impact and may aids in cognition.

Conclusion

This study evaluated the efficacy of an ethanolic extract of *Ammannia baccifera* in preventing Alzheimer's disease in rats. The treatment involved the use of two different quantities of the plant extract, specifically 200 mg/kg and 400 mg/kg. The plant extracts effectively inhibited the development of Alzheimer's disease in rats in a manner that was dependent on the dosage. Previous research has shown that rats treated with an ethanolic extract of *Ammannia baccifera* had significant anti-Alzheimer action. It could be due to the active phytoconstituents present in the extract. Thus, through the utilization of this plant, we will have the capacity to acquire pharmaceuticals or potentially advantageous active ingredients (API) for the management of neurodegenerative disorders at remarkably

affordable prices. In addition, our research aims to reduce the significant costs and environmental impact associated with the production of some drugs or active pharmaceutical ingredients (APIs) used to treat neurodegenerative illnesses by avoiding synthetic manufacturing methods.

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