

PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF ANNONA SQUAMOSA STEM BARK EXTRACT FOR MEMORY ENHANCING ACTIVITY

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ABSTRACT

The present study was carried out to evaluate the phytochemical, antioxidant, and memory enhancing activity of the stem bark extracts of annona squamosa belonging to the family Annonaceae. Memory impairment and cognitive dysfunction are commonly associated with aging and neurodegenerative disorders. Herbal medicines containing antioxidant phytoconstituents are considered beneficial in improving cognitive functions with minimal side effects. Therefore, the present investigation aimed to scientifically validate the traditional medicinal use of annona squamosa stem bark for memory enhancement. The collected plant material was authenticated and subjected to pharmacognostic and physicochemical evaluation for standardization and quality assessment. Successive solvent extraction of the powdered stem

bark was carried out using petroleum ether, ethyl acetate, and ethanol by soxhlet extraction method. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, phenolic compounds, proteins, and carbohydrates in the extracts. Quantitative estimation of total phenolic content (tpc) and total flavonoid content (tfc) was performed using standard methods. The ethanol extract showed higher phenolic and flavonoid content compared to the ethyl acetate extract. Antioxidant activity of the extracts was evaluated using the dpph free radical scavenging assay, where both extracts exhibited significant concentration dependent antioxidant activity. Among the extracts, the ethanol extract demonstrated comparatively higher antioxidant potential.

KEYWORDS: *Annona squamosa*, Memory enhancing activity, Morris Water Maze, antioxidant activity, Piracetam.

INTRODUCTION

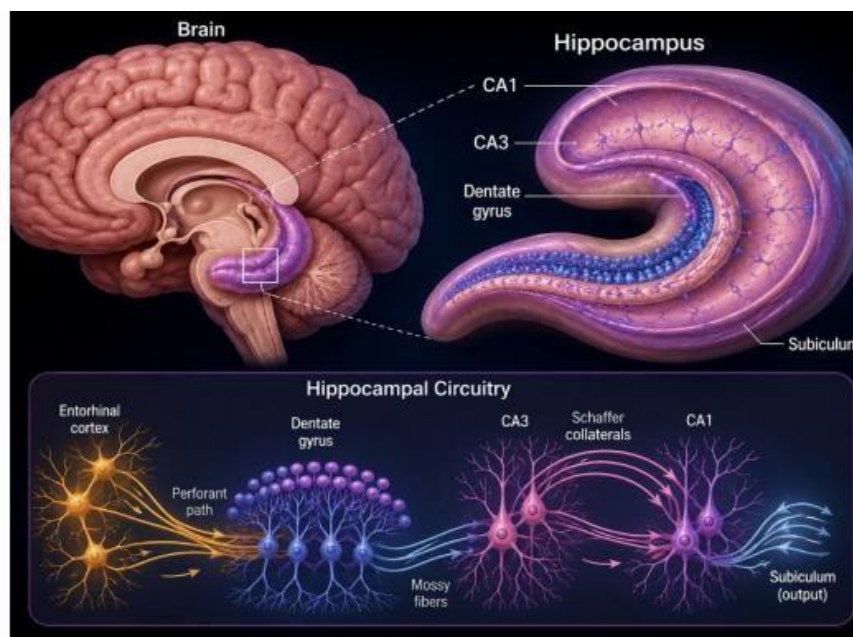
Memory is a fundamental cognitive function that enables individuals to store, retain, and retrieve information. It plays a vital role in learning, reasoning, emotional regulation, and everyday decision-making. Efficient memory function is essential not only for academic and professional achievement but also for maintaining overall quality of life, particularly among the aging population. The human memory system involves complex neural networks and biochemical processes that allow the brain to encode, consolidate, and recall information when needed. Any disruption in these processes can significantly affect cognitive performance and daily functioning.

In recent years, memory-related disorders have become increasingly prevalent due to the growing global aging population. Age-associated memory decline is considered a natural aspect of aging; however, it may progress into severe cognitive impairment in neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease, where memory dysfunction represents one of the primary clinical manifestations. These disorders are characterized by progressive neuronal degeneration, synaptic dysfunction, and impaired neurotransmitter activity, ultimately leading to deterioration in cognitive abilities and behavioral changes.

Memory Enhancement

Most research on memory enhancement focuses on psychological events that occur before the actual encoding of information. For example, a large body of literature describes attempts commonly involving chemical agents to increase the speed of learning by modulating arousal and attention. Improvements resulting from such interventions are often referred to as cognitive enhancement, presumably because important components of cognition are being influenced.

Mechanisms of Memory Enhancing



The figure illustrates the major mechanisms involved in memory enhancement, beginning from the encoding of information to the formation of long-term memory. Memory formation starts with the reception of sensory information, which is processed and encoded in the brain, particularly within the hippocampus, a region critically involved in learning and memory. Sensory stimuli activate neuronal networks, initiating communication between neurons through synaptic transmission. A central feature of the figure is synaptic plasticity, especially long-term potentiation (LTP), which is widely considered the cellular basis of learning and memory. During synaptic transmission, neurotransmitters such as glutamate are released from the presynaptic neuron into the synaptic cleft. These neurotransmitters first activate AMPA receptors on the postsynaptic membrane, producing depolarization of the neuron. This depolarization removes the magnesium block from NMDA receptors, allowing calcium ions to enter the postsynaptic neuron. The influx of calcium activates intracellular signalling pathways involving protein kinases such as CaMKII, PKA, and MAPK. These signalling cascades ultimately strengthen synaptic connections through the insertion of additional AMPA receptors and structural modifications at the synapse, thereby producing LTP. The figure also highlights the process of memory consolidation, during which newly formed memories become stabilized and converted into long-term storage. Consolidation involves gene transcription and protein synthesis, which lead to synaptic remodelling and long-lasting structural changes in neuronal connections. These molecular and structural adaptations ensure the persistence and durability of memory traces over time.

Morphological Evaluation Of Stem Bark

Sr.No.	Parameter	Observation
1	Colour	Brown to dark brown
2	Odour	Characteristic, slightly aromatic
3	Taste	Bitter and astringent
4	Texture	Rough and hard
5	Surface	Irregular with cracks and fissures
6	Thickness	Moderately thick
7	Fracture	Fibrous
8	Shape	Curved or flat pieces

Processing of Crude Drugs

The collected fresh Stem Barks of *Annona squamosa* Stem Bark were subjected to shade drying and further crushed to powder, and then the powder is passed through the mesh No. 14, stored in air tight container and subjected to extraction by using Soxhlet extraction method.

Extraction of plant material

The dried and coarsely powdered stem bark of *Annona squamosa* was packed in a thimble and placed in a Soxhlet apparatus. Initially, the material was defatted with petroleum ether. After complete defatting, the marc was extracted successively with ethyl acetate followed by ethanol. The extracts obtained were concentrated using a water bath to remove the solvent and stored in airtight containers for further experimental studies. The prepared extracts were then subjected to preliminary phytochemical screening to identify the presence of various phytoconstituents.

Selection of Solvent

On the basis of nature of phytochemical present in drug and extractive value solvents were selected for the extraction of the stem bark of *Annona squamosa* Stem Bark use of solvent is petroleum ether, Ethanol and Ethyl acetate.

Selection of Extraction method

According to the nature of the phytochemical present in the drug and a literature review, extraction method was selected. The extraction method selected for extraction of stem bark of *Annona squamosa* Stem Bark was continuous hot extraction method using Soxhlet apparatus.



Fig. Soxhlet Assembly.

MATERIALS AND METHODS

Materials

Soxhlet apparatus, heating mantle, powdered drug, petroleum ether, Ethyl acetate and Ethanol.

Preparation of Petroleum ether extract

Dried Stem Bark powder was successively extracted with petroleum ether by Soxhlet extractor apparatus according to the standard method till colourless solution was observed in siphon tube. 250 gm of the powdered Stem Bark powder and 1000 ml petroleum ether were used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container. Percentage yield of extract was calculated.

Preparation of Ethyl acetate extract

Dried Stem Barks powder was successively extracted with Ethyl acetate by Soxhlet extractor apparatus according to the standard method till colourless solution was observed in siphon tube. 250 gm of the powdered plant and 1000 ml Ethyl acetate was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container and Percentage yield of the extract was calculated.

Preparation of Ethanol extracts

Dried Stem Barks powder was successively extracted with Ethanol by Soxhlet extractor apparatus according to the standard method till colourless solution was observed in siphon tube. 250 gm of the powdered plant and 1000 ml Ethanol was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container. Percentage yield of extract was calculated.

Animals used

Male and female Wister rats weight 180-230 gm were selected. Route of administration:

Standard- Oral Test- Oral

Grouping of Animals

Group	Treatment	Dose	Route	Description
Group I	Normal Control	-	Oral	Animals received normal saline only
Group II	Standard Drug	2 mg/kg	Oral	Animals received Piracetam before behavioural testing
Group III	ASEA	100 mg/kg	Oral	Ethyl acetate extract of <i>Annona squamosa</i> stem bark (ASEA100mg/kg)
Group IV	ASEA	200 mg/kg	Oral	Ethyl acetate extract of <i>Annona squamosa</i> stem bark (ASEA200mg/kg)
Group V	ASEE	100 mg/kg	Oral	Ethanol extract of <i>Annona squamosa</i> stem bark (ASEE 100 mg / kg)
Group VI	ASEE	200 mg/kg	Oral	Ethanol extract of <i>Annona squamosa</i> stem bark (ASEE 200 mg / kg)

Memory Enhancing Activity

Procedure: The Morris Water Maze test is a widely used experimental method for evaluating spatial learning and memory in rodents. The apparatus consists of a large circular pool filled with water made opaque using non-toxic white paint or milk powder. A small escape platform is submerged approximately 1-2 cm below the water surface in one quadrant of the pool. Visual cues are placed around the testing room to assist the animals in locating the hidden platform. Before the experiment, the animals are acclimatized to the laboratory conditions. During the acquisition or training phase, each animal is individually placed into the pool from different starting positions and allowed to swim freely to locate the hidden platform within a specified period, usually 60-120 seconds. Once the animal finds the platform, it is permitted to remain there for about 15-30 seconds. If the animal fails to locate the platform within the allotted time, it is gently guided to the platform and allowed to stay there for a short duration. The training trials are repeated for several consecutive days to assess learning ability.

OBSERVATION AND RESULTS**Observations For Phytochemical Qualitative Analysis**

Sr. No.	Phytochemical Test	Pet. Ether	Ethyl Acetate	Ethanol
1	Test for Alkaloids			
a	Dragendorff's test	+	+	+
b	Mayer's test	+	-	+
c	Wagner's test	-	+	+

d	Hager's test	-	-	-
2	Test for Flavonoids			
a	Shinoda test	+	+	+
b	Sulphuric acid test	-	+	+
c	Lead acetate test	+	+	+
3	Test for Glycosides			
a	Keller-Killiani test	+	+	+
b	Legal test	+	+	-
c	Foam test	-	+	+
4	Test for Steroids			
a	Salkowski test	+	+	+
b	Liebermann-Burchard test	-	-	+
5	Test for Proteins			
a	Biuret test	+	+	+
b	Millon's test	+	-	-
6	Test for Carbohydrates			
a	Molisch test	+	+	+
b	Fehling's test	+	-	+
c	Benedict's test	+	+	+
d	Barfoed's test	+	+	+
7	Tannins & Phenolic Compounds			
a	Lead acetate test	+	+	+
b	Dil. HNO ₃ test	+	+	+
c	Dil iodine solution	-	+	+

(+)Present, (-) Absent

Total flavonoid content

Values represent mean \pm SEM (n=3)

Sr. No.	Extract	Concentration ($\mu\text{g/ml}$)	Absorbance (Mean \pm SEM)	TFC (%) (Mean \pm SEM)
1	Ethyl acetate Extract	50	0.078 \pm 0.0046	23.8 \pm 0.50
2	Ethanol Extract	50	0.094 \pm 0.0017	36.5 \pm 0.63

Total phenolic Extract

Values represent mean \pm SEM (n=3)

Sr. No.	Extract	Concentration ($\mu\text{g/ml}$)	Absorbance (Mean \pm SEM)	TFC (%) (Mean \pm SEM)
1	Ethyl acetate Extract	100	0.612 \pm 0.0045	25.6 \pm 0.58
2	Ethanol Extract	100	0.902 \pm 0.0053	36.4 \pm 0.70

Pharmacological Screening of *Annona squamosa* stem bark extracts

Wistar rats of either sex weighing between 180–250 g were used in the present study. The experimental animals were maintained under standard laboratory conditions in the animal

house of Systemic Life Sciences & Research Pvt. Ltd., approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Animals were housed under standard environmental conditions with a 12 h light/dark cycle, temperature ($22 \pm 2^\circ\text{C}$), and relative humidity of $55 \pm 5\%$, and were provided with standard pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory conditions for at least one week before the commencement of the experiment. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC). The study titled “Phytochemical and Pharmacological Investigation of *Annona squamosa* Stem Bark Extract for Memory Enhancing Activity” was approved with IAEC approval number 18/IAEC-II/SLSRPL/2025. All experimental procedures were carried out in accordance with CPCSEA guidelines for the care and use of laboratory animals.

Safe Dose Calculation

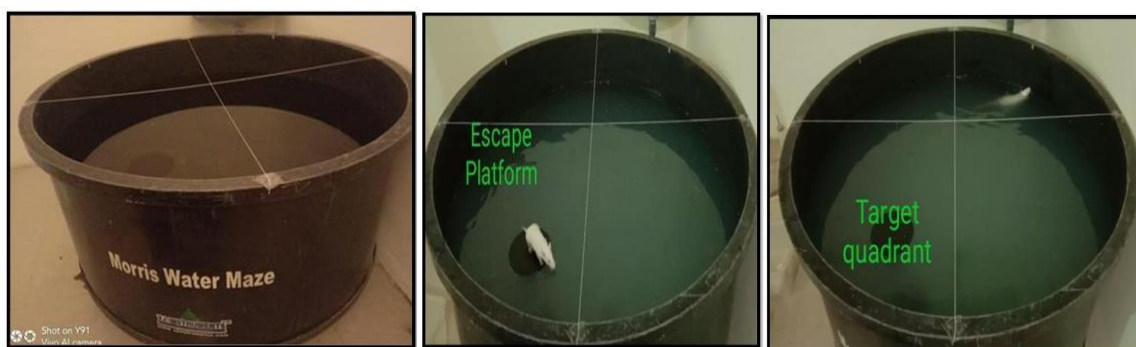
Acute toxicity studies conducted by various researchers for *Annona squamosa* stem bark extracts, as per standard references, revealed that the administration of graded doses of extracts (up to a dose of 2000 mg/kg) did not produce any significant changes in behaviours such as alertness, motor activity, breathing, restlessness, diarrhoea, convulsions, coma, and general appearance of the animals. No mortality was recorded up to the dose of 2000 mg/kg body weight, and the animal remained physically active. The results of these studies indicated that, at a single dose, the plant extract did not show any adverse effects, suggesting that the median lethal dose (LD_{50}) may be greater than 2000 mg/kg body weight in experimental animals such as rats and mice. Accordingly, the safe experimental dose for the present investigation was considered as ≤ 200 mg/kg. Acute oral toxicity studies are performed for the estimation of LD_{50} . As per reported references and guidelines, the safe dose of *Annona squamosa* stem bark extract was found to be 2000 mg/kg, carried out according to OECD Guidelines for Acute Oral Toxicity (423: Acute Toxic Class Method). These studies demonstrated that administration of extract up to the limit dose did not produce significant toxicological changes in experimental animals. Based on this, the therapeutic experimental doses were selected as 100 mg/kg and 200 mg/kg body weight, which are considered safe for pharmacological studies.

In-vitro antioxidant activity-DPPH (2, 2-diphenyl 1, 1-picrylhydrazyl) radical scavenging activity

Table Antioxidant Activity Values represent mean \pm SEM (n = 3).

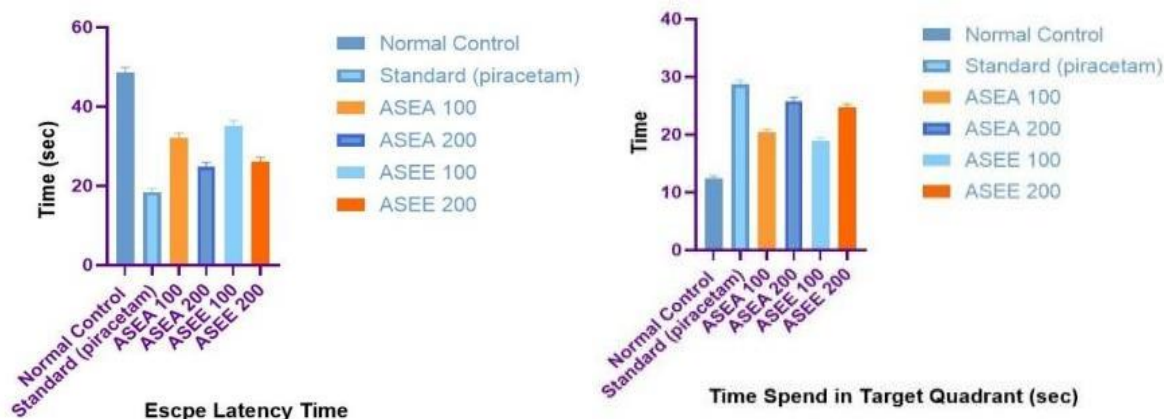
Conc (mug/ml)	Rutin (% inhibition)	Gallic acid (%inhibition)	Ascorbic acid (%inhibition)	Ethyl acetate (%inhibition)	Ethanol (%inhibition)
25	89.12 \pm 0.36	80.1 \pm 0.44	85.02 \pm 0.4	79.85 \pm 0.32	85.9 \pm 0.28
50	92.45 \pm 0.32	83.05 \pm 0.37	87.1 \pm 0.35	85.1 \pm 0.39	87.92 \pm 0.26
75	94.30 \pm 0.28	86.20 \pm 0.30	89.25 \pm 0.31	88.20 \pm 0.34	89.85 \pm 0.29
100	96.50 \pm 0.25	90.75 \pm 0.27	91.80 \pm 0.26	91.65 \pm 0.28	92.95 \pm 0.32
125	98.10 \pm 0.20	94.80 \pm 0.22	94.25 \pm 0.24	93.95 \pm 0.25	94.90 \pm 0.23

Water Maze Test



Group	Escape Latency Time (sec)	Time Spent in Target Quadrant (sec)
Normal Control	48.62 \pm 1.28	12.36 \pm 0.54
Standard Drug (Piracetam 2mg / kg)	18.42 \pm 0.96 [^] **	28.74 \pm 0.68 [^] *
ASEA 100 mg/kg	32.18 \pm 1.14 [^] **	20.36 \pm 0.59 [^] *
ASEA 200 mg/kg	24.86 \pm 1.02 [^] **	25.82 \pm 0.63 [^] **
ASEE 100 mg/kg	35.24 \pm 1.21 [^] **	18.92 \pm 0.56 [^] **
ASEE 200 mg/kg	26.14 \pm 1.08 [^] **	24.68 \pm 0.61 **

Values are expressed as mean \pm standard deviation (SD) for six animals in each group (n = 6). Statistical analysis was performed by comparing treated groups with the negative control group. A value of * $p < 0.05$ was considered statistically significant, while ** $p < 0.01$ was considered highly significant.



CONCLUSION

The study was designed to scientifically validate the traditional medicinal importance of the plant and to explore its possible role as a natural therapeutic agent for cognitive dysfunction and memory impairment. Pharmacognostic evaluation of the stem bark revealed characteristic organoleptic and morphological features such as brown colour, bitter taste, rough texture, fibrous fracture, and irregular surface. These features are useful in the identification and standardization of the crude drug. Physicochemical parameters including ash value, acid insoluble ash, water soluble ash, and loss on drying were also evaluated and found to be within acceptable limits, indicating good quality and purity of the plant material. The extraction studies demonstrated that ethanol and ethyl acetate were effective solvents for extracting phytoconstituents from the stem bark of *Annona squamosa*.

Preliminary phytochemical screening confirmed the presence of several important bioactive constituents such as alkaloids, flavonoids, glycosides, steroids, tannins, phenolic compounds, carbohydrates, and proteins in different extracts. These phytochemicals are known to possess various pharmacological properties including antioxidant, neuroprotective, anti-inflammatory, and cognition enhancing activities. Quantitative estimation of total phenolic content and total flavonoid content revealed that the ethanol extract contained higher amounts of phenolic and flavonoid compounds compared to the ethyl acetate extract. Phenolic compounds and flavonoids are well known for their ability to scavenge free radicals and protect cells against oxidative stress. Oxidative stress is considered one of the major factors responsible for neuronal degeneration and memory impairment. Therefore, the higher concentration of these phytoconstituents suggests that the extracts possess strong antioxidant and neuroprotective potential.

The antioxidant activity of the extracts was further evaluated using the DPPH free radical scavenging assay. The antioxidant activity observed in the present study supports the role of *Annona squamosa* stem bark in protecting neuronal cells from oxidative damage and improving cognitive function. The pharmacological evaluation of memory enhancing activity was carried out using the Morris Water Maze model in Wistar rats. The results demonstrated that treatment with both ethyl acetate and ethanolic extracts significantly improved learning and memory performance. The extracts reduced escape latency time and increased the time spent in the target quadrant, indicating enhancement of spatial memory and learning ability. Among all treatment groups, the ethyl acetate extract at a dose of 200 mg/kg showed the most significant memory enhancing activity and produced effects comparable to the standard drug piracetam. The possible mechanism involved in the memory enhancing activity may be related to antioxidant and neuroprotective effects of the phytoconstituents. Flavonoids and phenolic compounds are known to improve neuronal function by reducing oxidative stress, enhancing cerebral blood circulation, protecting neurons against free radical induced damage, and improving synaptic plasticity. Alkaloids may also contribute to cognitive enhancement through modulation of neurotransmitter systems, particularly the cholinergic pathway, which plays an important role in learning and memory processes. Overall, the findings of the present study scientifically support the traditional medicinal use of *Annona squamosa* and clearly demonstrate that the stem bark possesses significant antioxidant and memory enhancing activities. The study suggests that the plant may serve as a valuable natural source for the development of safe and effective herbal nootropic agents for the management of memory impairment, cognitive dysfunction, and neurodegenerative disorders required before the plant can be developed into a standardized herbal formulation for therapeutic use.

In conclusion, the present investigation establishes *Annona squamosa* stem bark as a promising medicinal plant with considerable nootropic and antioxidant potential. The encouraging results obtained in the present study provide a strong scientific basis for future research aimed at developing novel herbal therapies for improving memory and cognitive function.

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