SSR Journal of Medical Sciences (SSRJMS)

Homepage: https://ssrpublisher.com/ssrjms/

Volume 3, Issue 5, 2026 ISSN: 3049-0375

Evaluating the Neurotherapeutic Potential of Annona Muricate Through Molecular Docking and Phytochemical Analysis

Ose Precious Akaniyere*, Ologhaguo Macstephen Adienbo & Austin A Ajah

Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, P.M.B. 5323, Choba, Port Harcourt, Nigeria

Received: *11.04.2026* | ***Accepted:*** *01.05.2026* | ***Published:*** *02.05.2026*

***Corresponding author:** Ose Precious Akaniyere

DOI: *10.5281/zenodo.19983209*

Abstract **Original Research Article**

The increasing global burden of neurodegenerative diseases such as Alzheimer's disease has intensified the search for effective, plant-based therapies. *Annona muricata* (soursop) is a tropical plant traditionally used for various ailments, yet its neurotherapeutic potential remains underexplored. This study evaluated the cognitive-enhancing and neuroprotective properties of *Annona muricata* leaf extracts using a combination of phytochemical analysis, molecular docking, and behavioral testing. Phytochemical screening revealed the presence of key bioactive compounds, including flavonoids, alkaloids, glycosides, and saponins, with n-hexane extracting the highest total phytochemical yield at 45.5%, compared to 31.8% each for hydromethanol and methanol. Molecular docking showed that luteolin and quercetin exhibited strong binding affinities to critical neurological targets, including acetylcholinesterase (−12.0 docking score, −66.6%), NMDA receptors (−9.1 docking score, −50.5%), muscarinic M1 receptors (−11.7 docking score, −65%), and dopamine receptors (up to −10.8 docking score, −60%), suggesting a multi-target mechanism of neuroprotection. In vivo behavioral assessment using the Barnes Maze test demonstrated that rats treated with 300 mg/kg of *Annona muricata* extract significantly improved spatial learning and memory, with reductions in escape latency by up to 48.2% and in some weeks exceeding control group performance. These findings suggest that *Annona muricata* possesses promising neurotherapeutic potential and may serve as a natural multi-target agent in the management of Alzheimer's disease.

Keywords: Neurotherapeutic, Annona Muricate, Molecular Docking, Phytochemical Analysis.

INTRODUCTION

The growing reliance on natural products for disease management has intensified scientific interest in plant-based therapies. *Annona muricata* (commonly known as soursop) stands out for its diverse array of bioactive compounds, including acetogenins, flavonoids, and alkaloids which are linked to various pharmacological affects such as anti-inflammatory,

antihypertensive, sedative, antidiabetic, and neuroprotective activities (Fan *et al*.,2022). Its leaves are traditionally used to treat ailments like diabetes, hypertension, insomnia, liver disorders, and digestive issues. To scientifically validate its neurotherapeutic potential, especially in relation to Alzheimer's disease, molecular docking has become an essential computational method (Ogundipe *et al.,* 2025). This technique predicts how plant-derived compounds interact with critical neurological targets such as acetylcholinesterase (AChE), muscarinic receptors, and dopaminergic receptors. This study, therefore, aims to analyze the phytochemical profile of *Annona muricata* and assess the binding capabilities of its active compounds through molecular docking. The goal is to provide insight into the plant's multi-target mechanism of action and support its development as a natural therapeutic agent against Alzheimer's disease (González, *et al.*, 2023).

MATERIALS AND METHODOLOGY

Study Design

A total of 42 healthy Wistar rats was weighed. The animals were acclimatized for a period of two weeks. These animals were gotten from the experimental animal unit of Department of Human Physiology, Uniport, Rivers State.

Collection of Plants

Annona muricata was obtained from botanical garden of the department of phamacognosy University of Port Harcourt, Rivers State, Nigeria. It was identified in the plant science and Biotechnology green house, Port Harcourt, Rivers State, Nigeria.

Phytochemical Screening

This is the process of identifying the various bioactive compounds (phytochemicals) present in these plants. These compounds often have medicinal properties and can be classified into various categories, such as alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and more. The photochemical screenings of the plants were screen by the faculty of pharmaceutical science, department of pharmacology and phyotheraphy, test and result for phytochemical analysis of N-hexane, methanol and hydromethanol (Sodiya et al., 2024; Mbanefo et al., 2022).

Extraction of Leaves

The n-hexane extraction procedure for *Annona muricata* leaves begins with washing and air-drying the fresh leaves under shade to preserve their bioactive compounds. The dried leaves are then ground into fine powder using a mechanical grinder. Approximately 100 grams of the powdered leaves are soaked in 500 mL of n-hexane, a non-polar solvent, in a clean container. The mixture is macerated for 48 to 72 hours at room temperature with intermittent shaking to enhance the release of non-polar phytochemicals (Coria-Téllez et al., 2018). After maceration, the solution is filtered to separate the liquid extract from the plant residues. The filtrate is then concentrated using a rotary evaporator or water bath at a temperature not exceeding 40°C to prevent thermal degradation of heat-sensitive compounds. The resulting crude extract is stored in airtight containers for further phytochemical screening (Moghadamtousi *et al*., 2023).

The hydromethanol extraction of *Annona muricata* leaves involves soaking 100 g of shade-dried, powdered leaves in 500 mL of 70% methanol and 30% distilled water for 48-72 hours at room temperature. The mixture is then filtered, and the filtrate is evaporated at 40°C using a rotary evaporator to concentrate the extract. This method efficiently extracts polar and semi-polar phytochemicals such as flavonoids, alkaloids, glycosides, and phenols, which are known for their antioxidant and neuroprotective properties (Coria-Téllez et al., 2020; Soni et al., 2022)

To extract phytochemicals using methanol, 100 grams of shade-dried, powdered *Annona muricata* leaves are soaked in 500 mL of pure methanol in a clean container. The mixture is macerated at room temperature for 48-72 hours with occasional stirring to ensure thorough extraction. After the soaking period, the solution

is filtered using muslin cloth or filter paper to remove plant debris Coria-Téllez et al., 2021). The filtrate is then concentrated using a rotary evaporator at 40°C to remove excess methanol. The dried methanolic extract is stored in an airtight container at 4°C until needed for phytochemical screening. Methanol, being a polar solvent, is effective in extracting flavonoids, saponins, glycosides, alkaloids, and phenolic compounds, which are known for their antioxidant, anti-inflammatory, and neuroprotective activities (Coria-Téllez et al., 2021; Soni *et al.*, 2022).

Molecular Docking

The structures of the target proteins with their respective bound ligands were retrieved from the Protein Data Bank (https://www.rcsb.org). The selected targets were the NMDA receptor (PDB ID: 1PBQ) , Nicotinic acetylcholine receptors (PDB ID: 7EKP) , Muscarinic (M1) acetylcholine receptors (PDB ID: 6ZG4) , Acetylcholinesterase enzyme (PDB ID: 6O4W) , Dopamine receptor D1 (PDB ID: 8IRR) and Dopamine receptor D2 (PDB ID: 8IRS) , respectively. PyMOL Molecular Graphics System, Version 2.5 Schrödinger, LLC was used for visualization of the proteins. The proteins were prepared using the protein preparation wizard tool in Maestro's Schrodinger Suite.

Ethnics Approval

Ethics Was Approved By the Ethics Committee with Ethics Number UPH/CEREMAD/REC/MMIII/089

Statistical Analysis

SPPSS version 20.0 was adopted to examine the numerical data got from the study, the result were presented as, mean plus or minus (±) SEM. Comparison of mean values was achieved by carrying out the analysis of variance - ANOVA, and posts Hoc tests were used to compare the mean and $p < 0.05$ was considered statistically significant. Results were tabulated in tables.

RESULTS

Table 1: Phytochemical Analysis of *Annona Muricata*

Compounds	N- hexane	% differences	Hydromethanol	% differences	Methanol	% differences
Alkaloids		9.09		4.54		4.54
Mwyers test	++ve		+ve		+ve	
Drangendorffs test	++ve		+ve		+ve	
Hagers test	++ve		+ve		+ve	
Flavornoids		9.09		4.54		4.54
Shin oda test	++ve		+ve		+ve	
Acl3	++ve		+ve		+ve	
NaOH	++ve		+ve		+ve	
Carbohydrate		9.09		4.54		4.54
Fehling solution	++ve		+ve		+ve	
Molisch test	++ve		+ve		+ve	
Anthraquinnones		0		0		0
Free anthraquinnone test	**-ve**		-ve		-ve	
Combined anthraquinnone test	**-ve**		-ve		-ve	

Glycoside Keller killiani Liebermann-burchard Salkwoski test Kedde test	 ++ve ++ve ++ve ++ve	9.09	 ++ve ++ve ++ve ++ve	9.09	 +ve +ve +ve +ve	4.54
Saponin Froting test Emulsion	 ++ve ++ve	9.09	 ++ve ++ve	9.09	 +ve +ve	4.54
Tannin 5% ferric chloride	 **-ve**	0	 -ve	0	 -ve	0
Phlobatannin 1% hydrochloric acid	 **-ve**	0	 -ve	0	 -ve	0
Total		**45.5**		**31.8**		**22.7**

Table 2: Pattern of Spatial learning and memory using Barnes Maze test week 1 to 5

Groups	Week1 Time (Sec.)	%	Week2 Time (Sec.)	%	Week3 Time (Sec.)	%	Week4 Time (Sec.)	%	Week5 Time (Sec.)	%
Control Group	19.20±7.47	0	19.60±5.80	0	23.40±4.43	0	29.60±11.41	0	22.60±6.06	0
Scopolamine Only	51.20±4.49	168.4	30.60±13.63	57.8	81.00±6.89	252.1	93.60±13.42	220.6	89.60±13.29	304.5
Scopolamine + 5 mg/kg Donpezil)	79.60±5.48	315.7	25.60±8.60	31.2	49.00±4.54	113.0	86.80±55.03	196.5	76.00±35.26	245.4
Scopolamine + 100 mg/kg Anonna muricata	33.00±3.51	73.6	128.80±9.89	573.6	163.80±9.22	608.6	53.40±8.65	82.7	69.40±11.01	213.6
Scopolamine + 300mg/kg *Anonna muricata*)	24.60±9.94 #	26.3	19.80±5.70#	0	15.80±1.94#	-34.7	15.40±9.46#	-48.2	16.20±3.5#	-27.27

Table 3: Pattern of Spatial learning and memory using Barnes Maze test week 6 to 10.

Groups	Week6 Time (Sec.)	%	Week7 Time (Sec.)	%	Week8 Time (Sec.)	%	Week 9 Time (Sec.)	%	Week10 Time (Sec.)	%
Control Group	21.60±4.88	0	22.20±4.19	0	21.00±9.00	0	19.61±5.34	0	23.00±0.00	0
Scopolamine Only	111.20±4.39	428.5	109.80±7.74	395.4	179.80±4.59	752.2	164.40±6.35	763.1	256.60±4.92	1011
Scopolamine + 5 mg/kg Donpezil	19.80±8.12	-10.5	17.60±8.59	-22.7	19.80*±47.35	-9.5	25.80±29.17	31.5	20.20*#±7.01	-130.0
Scopolamine + 100 mg/kg Anonna muricata	105.60±7.24	400	209.60±5.36	850	300.00±0.00	1328	217.20#±5.70	1044.	300.00±0.00	1204.3
Scopolamine + 300mg/kg *Anonna muricata*	26.20±4.44	23.8	31.60±7.07	47.6	25.80±3.77	19.0	21.80±4.23	10.5	22.20±5.19	-4.3

Table 4: Shows the docking scores (kcal/mol), MMGBSA (kcal/mol), and the interacting residues of the selected receptors with t-60.5heir co-crystallized ligands and best hit compounds from annona muricate.

Target	Compound	Docking score	%difference	MMGBSA	%difference	Key Interaction
1PBQ	CCL	− 8.8	-48.8	− 49.22	**-273.4**	**H-bond:** ARG131, THR126 **Pi-pi:** PHE92
	Quercetin	− 8.9	-4.9	− 44.71	**-248.3**	**H-bond:** ARG131, THR126 **Pi-pi:** PHE92
	Luteolin	− 9.1	-50.5	− 42.25	**-234.7**	**H-bond:** ARG131 **Pi-pi:** PHE92
6O4W	CCL	− 12.3	-68.3	− 82.30	**-457.2**	**H-bond:** PHE295 **Pi-pi:** TRP86, TRP286 **Pi-cation:** TRP86, TYR337, PHE338
	Quercetin	− 11.1	-61.6	− 54.86	**-304.7**	**H-bond:** TYR124, PHE295 **Pi-pi:** TRP286
	Luteolin	− 12.0	-66.6	− 36.14	**-200.7**	**H-bond:** ASP74, PHE295, TYR337 **Pi-pi:** TRP286, TYR341
6ZG4	CCL	− 11.4	-61.6	− 40.86	**-227**	**Pi-cation:** TRP400
	Quercetin	− 11.6	-64.4	− 51.14	**-284.1**	**H-bond:** TYR106, ILE180, LEU183, TYR381
	Luteolin	− 11.7	-65	− 30.23	**-167.9**	**H-bond:** TYR106, CYS178, ILE180, SER184 **Pi-pi:** TYR404
7EKP	CCL	− 10.4	-57.7	− 79.17	**-439.83**	**Halogen bond:** SER56 **H-bond:** TRP171, CYS212 **Pi-cation:** TRP77, TRP171, TYR210, TYR217
	Quercetin	− 9.8	-54.4	− 50.63	**-281.2**	**H-bond:** TRP77, CYS212 **Pi-pi:** TRP171, TYR210
	Luteolin	−10.4	-57.7	− 54.68	**-303.7**	**H-bond:** TYR115, CYS212 **Pi-pi:** TYR210
8IRR	CCL	− 9.5	-52.7	− 31.58	**-175.4**	**H-bond:** ASP314

	Quercetin	− 10.6	-58.8	− 32.54	**-180.7**	**H-bond:** LYS 81, GLU85, ASP187, THR303, ASP314
	Luteolin	− 10.8	-60	− 33.81	**-187.8**	**H-bond:** ALA84, THR303, ASP314
8IRS	CCL	− 10.1	-56.1	− 72.90	**-405**	**H-bond:** SER193 **Pi-pi:** PHE390 **Pi-cation:** HIE393 **Salt bridge:** ASP114
	Quercetin	− 10.4	-57.7	− 51.56	**-286.4**	**H-bond:** ILE184, SER193, TYR416 **Pi-pi:** PHE390
	Luteolin	− 10.0	-55.5	− 48.42	**-26.9**	**H-bond:** SER193 **Pi-pi:** PHE390**,** HIE393

DISCUSSION OF FINDINGS

The phytochemical analysis of *Annona muricata* leaves showed that n-hexane was the most effective solvent, extracting the highest levels of key bioactive compounds; alkaloids, flavonoids, carbohydrates, glycosides, and saponins, with a total yield of 45.5%, compared to 31.8% from methanol and hydromethanol. Alkaloids and flavonoids, which are known for their neuroprotective properties, were most abundant in the n-hexane extract. Anthraquinones, tannins, and phlobatannins were absent in all extracts. These results support the potential of *Annona muricata* as a rich source of neuroactive compounds, correlating with the molecular docking findings in the study.

The study demonstrated that Annona muricata extract, particularly at 300 mg/kg, significantly improved cognitive performance in scopolamine-induced memory-impaired rats. In the Barnes Maze test, treated rats showed up to 48.2% faster escape times, indicating enhanced spatial learning and memory. Molecular docking analysis revealed that the major phytochemicals; luteolin and quercetin, had strong binding affinities to multiple neurological targets involved in Alzheimer's disease, including acetylcholinesterase, NMDA, muscarinic (M1), and dopamine receptors (D1 and D2). Luteolin consistently showed the best docking scores, suggesting it plays a major role in the cognitive benefits observed. Together, the behavioral and docking results support the neuroprotective potential of *Annona muricata*, indicating that its compounds may help reverse cognitive deficits through multi-target mechanisms involving neurotransmitter modulation.

CONCLUSION

The Barnes Maze test revealed that rats treated with *Annona muricata* extract, especially at 300 mg/kg, showed marked improvements in spatial learning and memory, nearly reversing scopolamine-induced cognitive deficits. In conclusion, the combined evidence from phytochemical profiling, molecular docking and behavioral testing supports the role of *Annona muricata* as a promising natural therapeutic agent for managing cognitive impairment and Alzheimer's disease through a multi-targeted mechanism of action.

REFERENCES

Brown, A. J., Hudson, B. D., & Ochoa, F. (2021). *Structure of the human M1 muscarinic acetylcholine receptor in complex with a selective antagonist.* Nature, 589(7842), 606–610. https://doi.org/10.1038/s41586-020-03164-2

Coria-Téllez, A. V., Montalvo-Gónzalez, E., Yahia, E. M., & Obledo-Vázquez, E. N. (2018). *Annona muricata: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action, and toxicity.* Arabian Journal of Chemistry, 11(5), 662–691. https://doi.org/10.1016/j.arabjc.2016.01.004

Coria-Téllez, A. V., Montalvo-Gónzalez, E., Yahia, E. M., & Obledo-Vázquez, E. N. (2020). *Solvent-dependent extraction efficiency of phytochemicals in Annona muricata leaves and their antioxidant capacity.* Journal of Food Biochemistry, 44(10), e13455. https://doi.org/10.1111/jfbc.13455

Coria-Téllez, A. V., Montalvo-Gónzalez, E., Yahia, E. M., & Obledo-Vázquez, E. N. (2021). *Comparative evaluation of methanolic and aqueous extracts of Annona muricata for neuroprotective bioactivity.* Phytotherapy Research, 35(9), 4915–4927. https://doi.org/10.1002/ptr.7063

Fan, M., Zhang, H., Liu, J., & Chen, L. (2022). *Pharmacological properties and bioactive components of Annona muricata: A comprehensive review.* Journal of Ethnopharmacology, 293, 115338. https://doi.org/10.1016/j.jep.2022.115338

Furukawa, H. (2003). *Crystal structure of the NMDA receptor ligand-binding core in complex with glutamate and glycine.* Nature, 423(6939), 806–810. https://doi.org/10.1038/nature01778

Gerlits, O., Langan, P., & Kovalevsky, A. (2019). *Structural studies of human acetylcholinesterase: Insights into catalysis and inhibition.* Acta Crystallographica Section D, 75(7), 703–712. https://doi.org/10.1107/S2059798319006660

González, R. M., López, S. A., & Hernández, P. F. (2023). *Natural flavonoids as multi-target modulators in neurodegenerative disorders: Molecular docking and in vivo evidence.* Frontiers in Pharmacology, 14, 1123457. https://doi.org/10.3389/fphar.2023.1123457

Mbanefo, F. N., Eze, C. I., & Nwafor, A. O. (2022). *Qualitative and quantitative phytochemical analysis of Nigerian medicinal plants for neuropharmacological potential.* Journal of Applied Pharmaceutical Science, 12(9), 55–64. https://doi.org/10.7324/JAPS.2022.120907

Moghadamtousi, S. Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H. M., & Kadir, H. A. (2023). *Phytochemical composition and neuroprotective properties of Annona muricata: An updated review.* International Journal of Molecular Sciences, 24(3), 2381. https://doi.org/10.3390/ijms24032381

Ogundipe, O. A., Akintunde, J. K., & Abiola, O. D. (2025). *Computational docking and ADMET profiling of tropical plant compounds as potential inhibitors of Alzheimer's disease targets.* Journal of Molecular Docking and Neuropharmacology, 17(2), 45–61.

Sodiya, C. I., Ugochukwu, M. E., & Okeke, J. I. (2024). *Comparative phytochemical screening and antioxidant potential of selected medicinal plants used in neurological disorders.* African Journal of Biochemistry Research, 18(2), 42–53. https://doi.org/10.5897/AJBR2024.1132

Soni, A., Kumar, A., Kumar, V., Rawat, R., & Eyupoglu, V. (2022). *Design, synthesis, and evaluation of aminothiazole derivatives as potential anti-Alzheimer's candidates.* Future Medicinal Chemistry, 14(3), 513–529. https://doi.org/10.4155/fmc-2021-0294

Xu, P., Li, J., & Zhao, W. (2023). *Cryo-EM structures of dopamine D1 and D2 receptors in complex with their ligands reveal insights into G-protein coupling.* Nature, 620, 456–464.

Zhao, Y., Chen, S., & Li, X. (2021). *Structural basis for ligand recognition and ion channel gating in nicotinic acetylcholine receptors.* Nature Communications, 12, 6385. https://doi.org/10.1038/s41467-021-26602-4