Nig. J. Pure & Appl. Sci. Vol. 39 (Issue 1, 2026)
e-ISSN 2756-4045
(C) 2026 Faculty of Physical Sciences and Faculty of Life Sciences, Univ. of Ilorin, Nigeria
www.njpas.com.ng

Amelioration of Aluminum Chloride-Induced Alzheimer's Disease via Biochemical Pathways by Methanolic *Cucurbita Pepo* (Pumpkin) Fruit Extract in Wistar Rats

Muhammad, B.Y., *Lawal, A.H., Yahaya, A., Ahmed, S.T.

Department of Biochemistry Nasarawa State University Keffi, Nigeria

Date Received: 15-10-2025
Date Accepted: 13-04-2026
DOI: https://doi.org/10.48198/NJPAS/25.A36

ABSTRACT

This study evaluated the neuroprotective potential of *Cucurbita pepo* fruit methanol extract in an aluminium chloride-induced rat model of Alzheimer's disease. Phytochemical analysis revealed high concentrations of steroids (19.55±0.83 mg/g), phenols (14.70±0.05 mg/g), flavonoids (10.33±0.06 mg/g) and saponins (7.62±0.36 mg/g), indicating the presence of bioactive compounds. In behavioural assessments using the Elevated Plus Maze Test (EPMT), the disease control group exhibited a significant reduction ($p < 0.001$) in time spent in open arms, indicative of increased anxiety and cognitive impairment. Treatment with *C. pepo* extract significantly increased open arm time, comparable to the standard drug. Biochemical analyses revealed a substantial elevation of amyloid-beta (Aβ) and tau protein levels in the disease control group, while *C. pepo* extract administration at both low and high doses significantly reduced these pathological markers. Antioxidant assays showed decreased SOD and GPx activities and increased MDA levels in the disease control group, which were ameliorated following *C. pepo* extract treatment. Neurotransmitter analysis further indicated elevated AChE, MAO-A, and MAO-B levels, alongside reduced acetylcholine (ACh) in the disease group. These alterations were significantly reversed by *C. pepo* extract, especially at the high dose, mirroring the effects of vitamin E. Overall, *Cucurbita pepo* fruit extract ameliorated markers of oxidative stress, cholinergic dysfunction and reduced the level of amyloid and tau protein.

Keywords: *Cucurbita pepo*, Alzheimer's disease, antioxidants, neurotransmitters, neuroprotection

Introduction

Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative condition characterized by cognitive and functional decline, including episodic memory loss, language skills impairment, neuropsychiatric symptoms, and premature death (Zhao *et al.,* 2024). It involves the degeneration of cholinergic neurons in the brain, leading to reduced acetylcholine synthesis and secretion, amyloid-beta (Aβ) deposition, and plaque formation causing dementia (Zhao *et al.,* 2024). 60-80% of dementia cases are attributed to AD, affecting around 25 million people globally, with projections of 115.4 million cases by 2050 (Li *et al.,* 2024).

Oxidative stress occurs when there is imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive intermediates or repair the resulting damage (Ashikaa *et al.,* 2023). Key contributors to oxidative stress in AD include amyloid-beta (Aβ) plaques, which are hallmarks of the disease (Dhapola, *et al.,*2024). This oxidative damage

Corresponding Author: **Lawal, Abdulkadir Hassan**
Department of Biochemistry Nasarawa State University Keffi, Nigeria
Email: lawalahassan@nsuk.edu.ng Phone: +2348166789313

©2026 Creative Common License Attribution 4.0 International

affects various cellular components, including lipids, proteins, and nucleic acids (Majid and Garg, 2024). Neuroinflammation is another critical factor for AD as Aβ activates microglia and astrocytes to release cytokines like IL-6, TNF-α, and IL-1β (Li *et al.,* 2024).

Heavy metals, notably aluminium (Al), have been linked to neurodegenerative diseases like AD (Aschner *et al.,* 2024). Al exposure, commonly from diet, drinking water, cosmetics, and medications, leads to clinical features of AD, including memory deficits and neuronal loss (Aschner *et al.,* 2024). Herbal medicine has shown efficacy in treating neurodegenerative diseases, with phytochemicals improving cognitive functions (Sani, *et al.*, 2023).

Pharmacological options provide only symptomatic relief and carry adverse effects (Cummings, 2021), highlighting the urgent need for safer alternatives. This research aims to study the ameliorative potential of methanol *Cucurbita pepo* (pumpkin) fruit extracts in aluminum chloride-induced neurotoxicity in wister rats.

2.0 Materials and Method

Chemicals and Reagents

Chemicals and reagents used were of analytical grade and they include: Concentrated Hydrochloric Acid, Acetic Anhydride, Wagner's Reagent, n-butanol, Diethyl ether, Sodium Azide Solution, Hydrogen Peroxide.

Preparation and Extraction of Pumpkin Extract

The pumpkin fruit was washed to remove impurities and then cut and blended into a smooth paste to facilitate the extraction process. Approximately 500 g of the fruit paste was macerated using methanol (volume of the methanol used) for 72 hours at room temperature. The mixture was filtered, concentrated at 45°C using a rotary evaporator, and the extract was stored at 4°C in airtight containers.

Experimental Animals

Thirty adult Wistar albino rats from Nasarawa State University, Keffi, were used. With ethical approval, they were housed under standard conditions.

Experimental Design

A randomized block design with six groups of male Wistar rats (n=5 each) was used. All animals, except controls, received daily intraperitoneal $AlCl_3$ (100 mg/kg) for 28 days.

The experimental groups:
Group I: Normal Control (Feed and water)
Group II: Disease Control (Feed, water, and 100 mg/kg of AlCl3)
Group III: low dose (Feed, water, $AlCl_3$, and 100 mg/kg of pumpkin extract)
Group IV: High dose (Feed, water, $AlCl_3$, and 200 mg/kg of pumpkin extract)
Group V: Standard (Feed, water, $AlCl_3$, and Vitamin E)
Treatments were given 2 hours post-$AlCl_3$. Behavioural assessments were performed during the study, and on day 28, rats were euthanized for neuroinflammatory, neurochemical, biochemical, and histopathological analyses.

Quantitative Analysis of the Plant Extract

Phytochemical components of the *Cucurbita pepo* fruits methanol extract were quantified using established spectrophotometric assays. Total phenolic and tannin contents were determined with the Folin–Ciocalteu method (Muhammad *et al.,* 2019) while flavonoids were estimated by the aluminum chloride colorimetric assay with rutin as standard. Alkaloids and terpenoids were analysed following the previous method standardized (Tiwari & Chanda, 2016) and sterols were measured using the Liebermann–Burchard reaction (Araújo *et al.,* 2013). Cardiac glycosides and saponins were analyzed according to Sofowora (Sofowora & Medicine, 1996), Quantification was performed against calibration standards, and all analyses were conducted in triplicate.

Behavioural Parameter Assessment: Elevated Plus Maze Test (EPMT)

The elevated plus maze test (EPMT) was employed to evaluate anxiety-like behaviour and long-term spatial memory following the methodology described by Pentkowski *et al.* (2018). The apparatus was elevated 40 cm above the ground. Each rat was placed in one of the open arms, facing away from the central platform. Over a 3-minute period, the number of entries

into the closed arms and on days 1, 7, 14, 21, and 28 were recorded.

Preparation of Tissue Homogenate for biochemical analyses

After 4 weeks of treatment, rat brains were collected for biochemical analysis. For monoamine oxidase A and B assays, the supernatant underwent further centrifugation, pellet washing, and resuspension with protein concentration adjusted to 1 mg/ml.

Estimation of Lipid Peroxidation

Lipid peroxidation was determined by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances method as described by Ohkawa *et al.* (1979).

Estimation of Glutathione Peroxidase (GPx)

GPx activity was assayed following the method of Nyman (1959). The reaction was initiated with hydrogen peroxide, and the decrease in NADPH absorbance at 340 nm was monitored over 5 minutes.

Estimation of Superoxide Dismutase (SOD)

SOD activity was measured using the method of Misra and Fridovich (1972). Tissue supernatant was mixed with carbonate buffer and epinephrine, and the change in absorbance at 480 nm was recorded over 2 minutes.

Quantification of Acetylcholinesterase (AChE)

AChE activity was quantified using Ellman's method. AChE activity was calculated and expressed as micromoles per minute per gram of tissue (µmol/min/g), as described by Shetab-Boushehri (2018) and Shunan *et al.* (2021).

Estimation of Monoamine Oxidase (MAO) A and B Activities

The MAO-A and B activity was measured following Charles *et al.* (1977). Products were extracted with butyl acetate and quantified spectrophotometrically at 280 nm.

Quantification of Total Beta-Amyloid Protein and Tau Protein, via ELISA

The quantification of total beta-amyloid protein and tau protein, levels in rat brain tissue extracts was conducted using enzyme-linked immunosorbent assay (ELISA) purchased from Solarbio Life Sciences (Beijing, China) according to the manufacturer's protocol. Assays were carried out as per the manufacturer's guidelines, and optical density (OD) values were recorded using micro plate reader.

Statistical Analysis

Data were expressed as Mean ± Standard deviation (SD). Statistical analysis was performed using SPSS package. Statistical differences among groups were analyzed using one-way analysis of variance (ANOVA) and Ducan's Multiple Range Test (DMRT) was used as a post-hoc test to compare group means. Differences were considered statistically significant at $p < 0.05$.

Ethical approval

Ethical approval was obtained from the Ethical Committee of the Research and Publication, Nasarawa State University, Keffi, Nigeria in accordance with institutional regulation. The approval number is RP/ETH/2025/10.

Results

Quantitative Phytochemical Analysis of Cucurbita pepo Fruit Methanol Extracts

The results in Table 1 indicate that steroids, phenols, flavonoids and saponins were the predominant phytochemicals in Cucurbita pepo fruit methanol extract.

Table 1: Quantitative Phytochemical Analysis of the *C. pepo* Fruit Extract

Phytochemical	Concentration (mg/g)
Phenols	14.70 ± 0.05^{b}
Tannins	1.00 ± 0.05^{a}
Saponins	7.62 ± 0.36^{b}
Alkaloids	-
Steroids	19.55 ± 0.83^{b}
Cardiac glycosides	3.64 ± 0.20^{a}
Glycosides	3.25 ± 0.18^{a}
Flavonoids	$10.33\pm.0.06^{b}$

Result presented as Mean ± Standard deviation, Different alphabets at superscript of the means indicates significant differences at P<0.05.

Effects of C. pepo Fruit Methanol Extracts on Aluminium Chloride-Induced Alzheimer Rats on Time Spent in Open Arms (s) at Different Days of Treatment

In table 2 the disease treatment with *Cucurbita pepo* fruit extract and the standard drug significantly increased ($p < 0.05$) open arm duration relative to the disease control group.

Antioxidant Effect of C. pepo Fruit Methanol Extracts

The results in Table 3 show that the disease control group had significantly reduced SOD and GPx activities with elevated MDA levels compared to the normal control. Treatment with *Cucurbita pepo* extract or vitamin E significantly improved SOD and GPx activities while reducing MDA levels.

Antioxidant Effect of C. pepo Fruit Methanol Extracts

The results in Table 3 show that the disease control group had significantly reduced SOD and GPx activities with elevated MDA levels compared to the normal control. Treatment with *Cucurbita pepo* extract or vitamin E significantly improved SOD and GPx activities while reducing MDA levels.

Table 2: Effects of *C. pepo* Fruit Methanol Extracts on Aluminium Chloride-Induced Alzheimer Rats on Time Spent in Open Arms (s) at Different Days of Treatment

Day	Normal Control (s)	Disease Control (s)	Low dose (s)	High dose (s)	Standard (s)
1	77 ± 4.18	33 ± 3.65	55 ±4.90[a]	58 ± 4.70 [a]	62±34.16 [a]
7	76 ± 4.50	30 ± 3.58	62 ±3.52 [a]	67 ± 5.60 [a]	72 ± 6.52 [a]
14	79 ± 4.40	28 ± 3.48	61 ±3.24 [a]	77 ± 4.90 [a]	79 ± 5.13 [a]
21	74± 4.40	27 ± 4.10	62 ±4.24 [a]	73 ± 4.80 [a]	74 ± 4.13 [a]
28	76 ± 5.90	30 ± 3.99	60 ±3.17 [a]	71 ± 5.61 [a]	81 ± 4.15 [a]

Values are expressed as means ± SD. Superscript [a] indicate values that are significantly different compared with the disease control group at P<0.05

Table 3: Effects of *C. pepo* Fruit Methanol Extracts on Antioxidants Biomarkers

Group	SOD (IU/mg)	GPx (IU/mg)	MDA (mg/dm)
Group I: Normal Control	18.56±0.26[b]	42.26±5.14[b]	0.64±0.02[b]
Group II: Disease Control	16. 72±0.05[a]	30.43±1.04[a]	2.53±0.18[a]
Group III: low dose	19.19±0.24[b]	49.52±4.71[b]	1.62±0.30[b]
Group IV: High dose	18.65±0.33[b]	51.33±5.36[b]	0.56±0.14[b]
Group V: Standard	18.84±0.56[b]	42.32±3.02[b]	2.05±0.37[a]

Values are expressed as means ± SD. Values with different superscript letters (a, b) within the same column are significantly different at P<0.05.

Table 4: Effect *C. pepo* Fruit Methanol Extract on Brain Neurotransmitters

Group	AChE(µ moles/min/mg protein)	ACh (ng/mg protein)	MAO-A (nmoles/mg protein).	MAO-B (nmoles/mg protein).
Group I: Normal Control	0.20±0.07[b]	10.00±0.56[b]	0.47±0.04[b]	0.20±.05[b]
Group II: Disease Control	0.78±0.16[a]	5.27±0.93[a]	0.62±0.04[a]	0.43±0.11[a]
Group III: low dose	0.24±0.10[b]	6.77±0.65[a]	0.53±0.04[a]	0.20±0.01[b]
Group IV: High dose	0.48±0.10[b]	8.73±0.68[b]	0.51±0.04[a]	0.28±0.05[b]
Group V: Standard	0.16±0.03[b]	7.26±1.68[a]	0.19±0.09[b]	0.17±0.01[b]

Values are expressed as means ± SD. Values with different superscript letters (a, b) within the same column are significantly different at P<0.05.

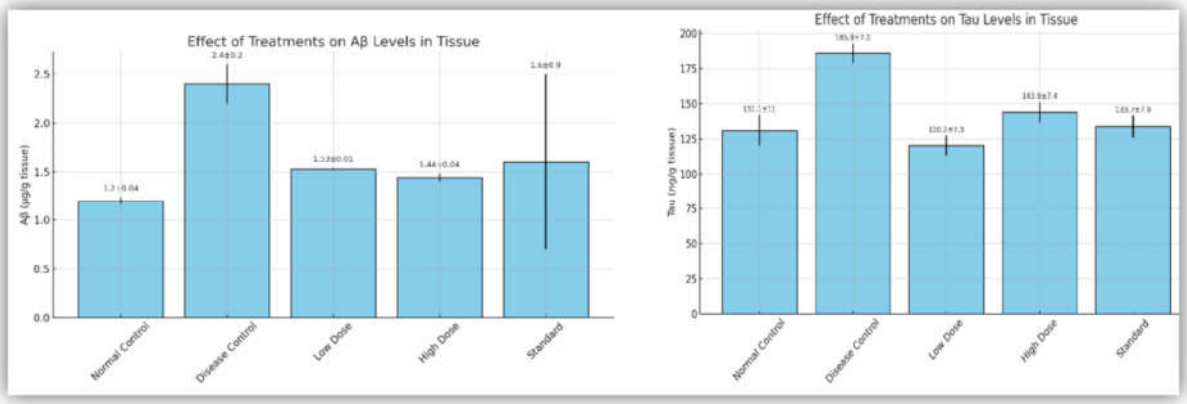

Figure 1: Amyloid-β (Aβ) and Tau Protein Levels in Different Experimental Groups

The bar chart shows that the Disease Control group had markedly higher Aβ and Tau protein levels than the Normal Control. Treatment with *Cucurbita pepo* extract (low and high doses) or the standard drug lowered both Aβ and Tau levels, suggesting neuroprotective effects. Error bars indicate SEM, and superscripts denote significant group differences.

Discussion

The presence of bioactive phytochemicals in *Cucurbita pepo* fruit extract suggests its potential neuroprotective role in Alzheimer's disease (AD). The quantitative analysis revealed that steroids and phenols are the most abundant constituents. Steroids, the dominant phytochemicals, have been reported to modulate neuro-inflammation driven by overactive microglia and astrocytes, with phytosterols shown to suppress inflammatory pathways and protect cognition (Jie *et al.,* 2022). Phenols have been reported to modulate amyloid precursor protein (APP) processing, reducing the formation of toxic Aβ aggregates (Rojas-García, *et al.,* 2023).

Flavonoids and saponins were present in *C. pepo* in moderate amounts. Studies have shown that flavonoids can inhibit β-secretase (BACE1), an enzyme responsible for cleaving APP into Aβ peptides (Meshginfar, *et al.,* 2021). By reducing BACE1 activity, flavonoids help decrease Aβ production, thereby mitigating amyloid plaque deposition, a hallmark of AD pathology. Saponins, have been shown to enhance Aβ clearance via neprilysin activation and maintain acetylcholine (ACh) levels through cholinergic activity (Zhang *et al.,* 2023).

Oxidative stress, central to AD, was evident in the disease control group with reduced SOD and GPx and increased MDA, indicating lipid peroxidation (Muhammad, *et al.,* 2021). Treatment with *C. pepo* (low and high doses) and vitamin E improved antioxidant status by raising SOD and GPx and lowering MDA, with low dose showing slightly stronger effects, suggesting a biphasic response. These effects were comparable to vitamin E.

Cholinergic dysfunction in the disease control group was marked by elevated AChE activity and reduced ACh. *C. pepo* extract reversed this by lowering AChE and raising ACh similar to cholinesterase inhibitors, while also reducing MAO-A and B activities, thereby mitigating oxidative stress and neuroinflammation. Furthermore, hallmark AD pathologies-Aβ plaques and hyperphosphorylated tau were increased in the disease control group (Muhammad *et al.,* 2021) but significantly reduced with *C. pepo* treatment, indicating interference with Aβ accumulation and tau phosphorylation (Dhapola *et al.,* 2024).

Notably, the neuroprotective effects of *C. pepo* were comparable to those of vitamin E, a well-established antioxidant known for its protective

role against neurodegeneration. This similarity suggests that *C. pepo* may exert its effects through antioxidant and anti-inflammatory mechanisms, which are crucial in slowing AD progression.

However, inducing aluminum chloride to cause neurotoxicity in the rats may not fully represent human Alzheimer's disease due to the complexity differences. The solvent used (methanol) may not reveal all the constituents and concentrations compare to the commonly consumed *Cucurbita pepo* preparations. The doses used in both aluminum and the *Cucurbita pepo* extract may not reflect realistic level of exposure in human. Overall, *C. pepo* exerts multi-targeted neuroprotective actions comparable to vitamin E through antioxidant, anti-amyloidogenic, anti-tau, cholinergic-enhancing, and anti-inflammatory mechanisms.

Conclusion

This study highlights the neuroprotective potential of *Cucurbita pepo* fruit extract in combating Alzheimer's disease (AD)-like symptoms. Rich in phytochemicals such as steroids, phenols, flavonoids, and saponins, the extract demonstrated strong antioxidant, anti-inflammatory, and anti-amyloid effects. It significantly reduced oxidative stress markers, inhibited key enzymes like AChE and MAOs, and lowered Aβ and tau protein levels. These effects show that *C. pepo* does not merely attenuate isolated pathological conditions but target multiple pathways implicated in AD-related diseases. These findings suggest that *C. pepo* may serve as a natural and effective therapeutic agent for managing AD-related neurodegeneration.

Acknowledgement

The work was sponsored by TETFUND through institutional based research grant (IBR) 2024

Conflict of Interest

The authors declared no conflict of interest.

References

Araújo, L. B., Silva, S. L., Galvão, M. A., Ferreira, M. R., Araújo, E. L., Randau, K. P., and Soares, L. A. J. R. B. d. F. (2013). Total phytosterol content in drug materials and extracts from roots of Acanthospermum hispidum by UV-VIS spectrophotometry. *Revista Brasileira de Farmacognosia, 23*(5), 736-742.

Aschner, M., Skalny, A. V., Santamaria, A., Rocha, J. B., Mansouri, B., Tizabi, Y., and Tinkov, A. A. (2024). Epigenetic Mechanisms of Aluminum-Induced Neurotoxicity and Alzheimer's Disease: A Focus on Non-Coding RNAs. *Journal of Neurotoxicity Research, 49(11), 2988-3005*.

Ashikaa, B. A., Zaruwa, M. Z., Muhammad, B. Y., Bamidele, T. O., Daikwo, M. A. J. J. o. C., and Research, A. M. (2023). Neuroprotective Effect of Stachytarpheta angustifolia Methanol Extract Against Aluminium Chloride ($AlCl_3$) Induced Alzheimer's Disease in Albino Rats. *Journal of Complementary and Alternative Medical Research,* 21(2), 22-31.

Cummings, J. (2021). New approaches to symptomatic treatments for Alzheimer's disease. *Journal of Molecular Neurodegeneration, 16*(1), 2. doi:10.1186/s13024-021-00424-9

Dhapola, R., Beura, S. K., Sharma, P., Singh, S. K., and HariKrishnaReddy, D.. (2024). Oxidative stress in Alzheimer's disease: current knowledge of signaling pathways and therapeutics. *Journal of Molecular Biology Reports, 51*(1), 48.

Jie, F., Yang, X., Wu, L., Wang, M., Lu, B. , and nutrition. (2022). Linking phytosterols and oxyphytosterols from food to brain health: origins, effects, and underlying mechanisms. *Journal of Critical Reviews in Food Science and Nutrition, 62*(13), 3613-3630.

Li, K., Ye, H., Dong, Z., Zhao, M., Xu, Q., and Xu, J. (2024). The Health and Economic Burden of Ozone Pollution on Alzheimer's Disease and Mild Cognitive Impairment in China. *Journal of Environmental Research,* 119506.

Majid, A., and Garg, S. J. (2024). Reactive oxygen species may cause amyloid beta aggregation by free radical polymerization. *Journal of Applied Polymer Science, 141*(14), e55190.

Muhammad, B. Y., Shaban, N. Z., Elrashidy, F. H., Ghareeb, D. A. (2019). Antioxidant, Anti-inflammatory, Antiproliferative and Antimicrobial Activities of Combretum glutinosum and Gardenia aqualla Extracts in vitro. *Journal of Free Radicals and Antioxidants, 9*(2), 66-72.

Muhammad, B. Y., Zaruwa, M. Z., Muhammad, R. A., and Ahmed, M. M. (2021). Survey of Snuff Use and Preliminary Study of Effect of Two Brands on, Brain Antioxidants and Acetylcholinestrase Enzyme of Wister Albino Rats. *Al-Azhar International Medical Journal, 2*(3), 60-66.

Rojas-García, A., Fernández-Ochoa, Á., Cádiz-Gurrea, M. d. l. L., Arráez-Román, D., and Segura-Carretero, A. (2023). Neuroprotective effects of agri-food by-products rich in phenolic compounds. *Journal of Nutrients, 15*(2), 449.

Sani, H., Muhammad, B. Y., Moses, A. Z. Z., and Bamidele, T. (2023). Phytochemistry and Ameliorative Effects of Peanut Skin Ethanol Extract against Lead Toxicity in Drosophila Melanogaster. *Journal of Academia Arena 15*(4), 26-33.

Sani, H., Muhammad, B. Y., Moses, Z. Z., Bamidele, T. (2023). Phytochemical and ameliorative effects of Peanut skin ethanol extract against Lead toxicity in Drosophila Melanogaster. *Equity Journal of Science and Technology, 10*(1), 23-29.

Sethuraman, S. P., Ramachandran, K. P. (2024). Phytochemical Profiling, In-vitro Antioxidant and Cytotoxic Effects of Luisia tenuifolia Extracts Against Human Skin Squamous Carcinoma. *Journal of Applied Biochemistry and Biotechnology, 196*(1), 400-416.

Sofowora, A. J. T. (1996). Research on medicinal plants and traditional medicine in Africa. *Journal of Alternative and Complementary medicine, 2*(3), 365-372.

Tiwari, R. K., and Chanda, S. (2016). Quantitative analysis of secondary metabolites in aqueous extract of Clerodendrum serratum. *International Journal of Pharmaceutical Sciences and Research,* 7(1), 354-359.

Zhang, R., Zeng, M., Zhang, X., Zheng, Y., Lv, N., Wang, L., . . . Yang, L. (2023). Therapeutic candidates for Alzheimer's disease: saponins. *International Journal of Molecular Sciences, 24*(13), 10505.

Zhao, M., Ma, G., Yan, X., Li, X., Wang, E., Xu, X.-X., . . . Zeng, J. J. M. P. (2024). Microbial infection promotes amyloid pathology in a mouse model of Alzheimer's disease via modulating γ-secretase. *Journal of Molecular Psychiatry,* 29(5), 1491-1500.