

## Evaluation of Methanolic Extract of *Cucurbita pepo* (Pumpkin) on Locomotion and Cognitive Function in A $\beta$ <sub>42</sub> Transgenic *Drosophila* Model of Alzheimer's Disease

Abubakar Yahaya<sup>1</sup>; Muhammad Bawa Yusuf<sup>1</sup>; Mohammed Auwal Ibrahim<sup>2</sup>; Moses Zira Zaruwa<sup>1</sup>; Chibuzo Carole Nweze<sup>1</sup>; Mohammed Lukeman Senusi<sup>1</sup>; Modibbo Muhammad Rabiu<sup>1</sup>; Abdulqadir Lawal Hassan<sup>1</sup>; and Muhammad Khadijat Musa<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi, Nigeria. <sup>2</sup>Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria.

Corresponding author: [yahayaabubakar@nsuk.edu.ng](mailto:yahayaabubakar@nsuk.edu.ng)

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### Abstract

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by cognitive and motor dysfunction, largely associated with the accumulation of amyloid-beta peptides. This study evaluated the neuroprotective effects of *Cucurbita pepo* (pumpkin) methanolic fruit extract on locomotor and cognitive deficits in A $\beta$ <sub>42</sub> transgenic *Drosophila melanogaster*, a well-established model of AD. The objectives were to analyze the phytochemical constituents of the extract and assess its impact on locomotion, learning, and memory. Qualitative and quantitative phytochemical analysis revealed a rich presence of bioactive compounds, notably flavonoids (28.60 $\pm$ 0.73 g/mg) and steroids, followed by phenols and tannins. AD-induced flies showed significantly impaired climbing ability, learning, and memory compared to controls. Treatment with Donepezil and graded doses (94.9 mg, 142.4 mg, and 189.8 mg) of *Cucurbita pepo* extract significantly improved all behavioral parameters, with the 142.4 mg dose producing effects comparable to Donepezil. These findings suggest that the extract possesses neuroprotective properties, likely due to its antioxidant and anti-inflammatory phytochemicals. It is concluded that *Cucurbita pepo* methanol extract may serve as a promising natural therapeutic agent for managing AD-related cognitive decline. Further studies are recommended to isolate active compounds, investigate molecular mechanisms, assess safety in mammalian models, and develop nutraceutical formulations.

**Keywords:** Alzheimer's Disease, *Cucurbita pepo*, *Drosophila melanogaster*, Cognitive Function, Phytochemicals.

### Introduction

#### Background of the Study

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder that primarily affects the elderly population. It is the most common cause of dementia globally, accounting for approximately 60–70% of all cases (Rajan *et al.*, 2021). With an aging global

population, the prevalence of AD is expected to rise sharply, with projections suggesting that a new case will be diagnosed every 33 seconds by the year 2050, leading to nearly one million new cases annually (Ferri *et al.*, 2005). Clinically, AD is characterized by progressive memory loss, cognitive decline, behavioral disturbances, and functional impairment. The neuropathological hallmarks of AD include extracellular accumulation of amyloid-beta ( $A\beta$ ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau proteins. Among the various forms of  $A\beta$  peptides,  $A\beta_{42}$  is the most neurotoxic and aggregation-prone, playing a central role in disease onset and progression (Hashemi *et al.*, 2023).

*Cucurbita pepo*, commonly known as pumpkin, is a plant species in the Cucurbitaceae family widely cultivated and consumed for its nutritional and medicinal properties. It has a long history of use in traditional medicine across various cultures and has been reported to possess anti-diabetic, antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial effects (Kainat *et al.*, 2022). The phytochemical composition of *Cucurbita pepo* includes flavonoids, phenolic acids, carotenoids, tannins, and polysaccharides—bioactive compounds that are increasingly recognized for their neuroprotective and antioxidant potential (Saavedra *et al.*, 2015). Despite these properties, scientific evidence supporting the role of *Cucurbita pepo* in managing neurodegenerative diseases like Alzheimer's remains limited and largely unexplored (Muhammad *et al.*, 2023).

*Drosophila melanogaster* has emerged as a valuable and cost-effective model organism for studying the genetic and molecular basis of human neurodegenerative diseases, including AD. The fruit fly shares highly conserved genetic pathways with humans and possesses orthologs for nearly 70% of human disease-related genes (Fortini *et al.*, 2000). Using the UAS-GAL4 binary expression system, transgenic *Drosophila* models expressing human  $A\beta_{42}$  in neuronal tissues have been developed, resulting in observable phenotypes such as impaired locomotion, reduced learning and memory, and shortened lifespan—mimicking key features of AD pathology (Kasanin *et al.*, 2022; Reiter *et al.*, 2001). These phenotypes provide reliable and quantifiable endpoints for evaluating the efficacy of therapeutic interventions. Moreover, *Drosophila* possesses an APP ortholog (dAPPI),  $\gamma$ -secretase components, and a  $\beta$ -secretase-like enzyme, thereby making it a genetically relevant model for AD research (Periz and Fortini, 2009; Yagi *et al.*, 2000).

This study aims to investigate the phytochemical constituents of *Cucurbita pepo* fruit methanol extract and evaluate its potential neuroprotective effects using the  $A\beta_{42}$  transgenic *Drosophila melanogaster* model of Alzheimer's disease. Behavioral assays such as negative geotaxis (for locomotion) and olfactory associative learning tests (for cognitive function) are employed to assess therapeutic efficacy. By integrating phytochemical analysis with functional behavioral endpoints, this research seeks to contribute to the growing interest in plant-based therapeutics for neurodegenerative diseases and provide preliminary evidence for the use of *Cucurbita pepo* as a candidate for AD management.

### Statement of the Research Problem

Alzheimer's disease (AD) remains a significant global health challenge with no definitive cure or preventive therapy. The increasing prevalence of AD, especially among the aging population, has imposed a heavy social and economic burden worldwide. Current therapeutic strategies focus mainly on symptomatic management and are associated with limited efficacy and undesirable side effects. Furthermore, the progressive nature of the disease and the complex mechanisms underlying its pathology, particularly the toxic accumulation of amyloid-beta ( $A\beta_{42}$ ) peptides, highlight the need for new, safer, and more effective therapeutic agents.

Natural products, especially plant-derived compounds with antioxidant and neuroprotective properties, are gaining increasing attention as potential therapeutic alternatives. *Cucurbita pepo* (pumpkin) is a widely consumed plant with documented medicinal benefits; however, its potential neuroprotective effects in Alzheimer's disease have not been thoroughly investigated, especially in transgenic models that closely mimic human AD pathology. Therefore, it is essential to evaluate the phytochemical constituents and possible therapeutic effects of *Cucurbita pepo* extract on neurodegenerative parameters such as locomotion and cognitive function using a reliable in vivo model like *Drosophila melanogaster* expressing human  $A\beta_{42}$ .

### Aim

To evaluate the phytochemical composition and neuroprotective effects of *Cucurbita pepo* methanol fruit extract on locomotor and cognitive functions in  $A\beta_{42}$  transgenic *Drosophila melanogaster*, a model of Alzheimer's disease.

### Objectives

1. To determine the phytochemical constituents of *Cucurbita pepo* methanol fruit extract.
2. To evaluate the effect of *Cucurbita pepo* extract on locomotor activity in  $A\beta_{42}$ -expressing *Drosophila melanogaster*.
3. To assess the effect of *Cucurbita pepo* extract on learning and memory function in  $A\beta_{42}$ -expressing *Drosophila melanogaster*.

### Materials and Methods

#### Materials

#### Chemicals and Equipment

All reagents used for this research were of analytical grade. These reagents include; Methanol, Donepezil, Hotplate, Soxhlet Apparatus, Rotary Evaporator (RE300) and Microplate reader (NeoMedica NW-67) Microscope. Stereo scope (Zeiss), Small paint brushes for pushing flies, 28.5 x 95 mm Polystyrene Vial (Capitol Vial, Inc.), Mini-Alarm Timer/Stopwatch (VWR International), Sharpie pens, Adhesive Tape, Quinine Hydrochloride (Sigma Aldrich; CAS Number: 6119-47-7), Distilled Water, Digital measuring

scale for precise dilution by weight, Quick Disconnect Connectors, High-Density Polyethylene (NALGENE VWR Catalog No. 62868-021), 15 mL Centrifuge Tube (VWR International) cut at the 2 mL mark from closed end to fit with the Quick Disconnect Connector adaptor for light source, Single-channel 200  $\mu$ L pipetter to load quinine solution onto filter paper lining the chamber, T-Maze (Simple Behavioral Systems;). Aluminum Foil, Light Source (Zeiss), A lamp with red light and Filter Paper etc.

## Experimental Animals

*Drosophila melanogaster* strain of Alzheimer's Disease UAS-A $\beta$ <sub>42</sub> and elav-Gal-4 were utilized in this study. To obtain flies expressing A $\beta$ <sub>42</sub> pan-neuronally (experimental group: elav-Gal<sub>4</sub>; UAS-A $\beta$ <sub>42</sub>) Gal<sub>4</sub>-UAS transgene expression system was used with the pan-neuronal elav-Gal<sub>4</sub> driver (Bloomington stock #458). Because the elav<sub>155</sub>-Gal<sub>4</sub> transgene is located on the X chromosome, we collect virgin females from elav-Gal<sub>4</sub> flies and cross them with male UAS-A $\beta$ <sub>42</sub> flies.

## Plant Material

The pumpkin (*Cucurbita pepo*) was bought from Keffi market, washed, pilled and air dried. The fruit was grinded into powder for extraction.



Figure 1: Pumpkin (*Cucurbita pepo*) Fruits

## Methods

### Extraction of Plant Sample

The dried *Cucurbita pepo* fruit sample was ground to a fine powder using a laboratory grinder and passed through a 0.5 mm mesh sieve to increase surface area for efficient extraction, following the method described by James *et al.* (2014). Approximately 14 g of the powdered sample was placed into a porous cellulose thimble and subjected to Soxhlet

extraction using 98% methanol as the solvent. The extraction was carried out at a temperature of 75°C for 12 hours. After extraction, the methanol solution was filtered using Whatman No. 1 filter paper to remove particulate matter. The filtrate was then concentrated to dryness under reduced pressure at 45°C using a rotary evaporator. The resulting crude methanol extract was weighed and stored in an airtight container at 4°C until further analysis.

### **Phytochemical Analysis**

#### **Qualitative Phytochemical Screening**

The qualitative phytochemical screening was carried out according to the procedure stated by Vishnu *et al.* (2019).

#### **Test for Phenol**

To 5 ml of extract, 3 ml of 10% acetate solution was added and mixed gently. The formation of bulky white precipitate indicates positive for phenol (Vishnu *et al.*, 2019).

#### **Test for Tannins**

To 5 ml of extract, few drops of 5% ferric chloride solution were added. The production of dark green color indicates the presence of tannins (Vishnu *et al.*, 2019).

#### **Test for Flavonoids**

A measure of 1 ml of the extract was taken and 10% of lead acetate was added. The formation of yellow precipitate indicates positive inference for flavonoids (Vishnu *et al.*, 2019).

#### **Test for Alkaloids**

To 5 ml of extract, 2 ml of HCl was added and 1 ml of Dragendroff's reagent was added. An orange or red precipitate indicates positive results for alkaloids (Vishnu *et al.*, 2019).

#### **Test for Glycosides (Borntrager's test)**

To 2 ml of the extract, 3 ml of chloroform was added and shaken. The chloroform layer was separated and 10% ammonia solution was added. The pink color formation indicates the presence of glycosides (Vishnu *et al.*, 2019).

#### **Test for Cardiac Glycosides (Keller-Killani test)**

A 5ml of the extract was mixed with 2ml of glacial acetic acid and a drop of ferric chloride solution was added followed by the addition of 1ml of conc. H<sub>2</sub>SO<sub>4</sub>. A brown ring in the interface indicates the presence of deoxy sugars of cardenoloides (Vishnu *et al.*, 2019).

**Test for Saponins**

A 0.5mg of extract was vigorously shaken with few of distilled water. The formation of frothing indicates positive for saponins (Vishnu *et al.*, 2019).

**Test for Steroids**

To 2ml of extract, 2ml of chloroform and 2ml of concentrated  $H_2SO_4$  was added. The appearance of red color and yellowish-green fluorescence indicates the presence of steroids (Vishnu *et al.*, 2019).

**Quantitative Analysis of the Plant Extract****Total Phenolic Content**

The total phenolic content was determined according to the method describe by Mauryan and Singh (2010). This is basically a color reaction that produces a blue color which is measured spectrophotometrically. Filin Ciocalteu Reagent was used to evaluate the amount of total phenolic content and garlic acid was used as standard express as mg/g garlic acid equivalent (GAE). The concentration of 0.01, 0.02, 0.03, 0.04, and 0.05 mg/ml was prepared in methanol. The concentration of 1 mg/ml of plant extract was prepared in methanol and 0.5 ml of the sample was placed on a test tube and it was mixed with 2.5ml of 10-fold diluted Filin Ciocalteu Reagent. The mixture was added to 2ml of 7.5% of sodium bicarbonate. The tubes were allowed to stand for 30mins covered with para-film at room temperature before taking the absorbance at 760nm. Reading was taken in triplicates.

**Estimation of Total Tannins**

Determination of total tannins content was evaluated for total phenolic content by using Folin-Ciocalteu's reagent (Rajeev *et al.*, 2012). Plant extract (10mg) was dissolved in 10 ml of distilled water to make final concentration of 1 mg/ 1ml. Different aliquots of garlic acid (0.5, 0.4, 0.3, 0.2 and 0.1 mg) was dissolved in 1 ml of distilled water to make the final concentrations 100, 80, 60, 40 and 20 ugh/ml. After preparing 3.5% v/v sodium carbonate solution, 0.1ml garlic acid solution of every concentration (100-20 mg/l) was taken into different volumetric flask. Then 7.5 ml of distilled water and 0.5 ml FC reagent was added to it. After 5mins, 1 ml 3.5% sodium carbonate solution and 10 ml distilled water was added to that flask. After 30mins, UV absorbance was determined at 725nm. Total tannins content of the extract was measured using garlic acid standard calibration curve and stated as mg garlic acid equivalent, GAE/100g of dried plant extract.

**Total Flavonoids Content**

The total flavonoids content was determined according to the method describe by Zengnin *et al.* (2011). A standard solution (10-50  $\mu$ gh/ml) of rutin was added to 75  $\mu$ l of sodium nitrite ( $NaNO_2$ , 5%) solution and mixed properly. After 5-6 minutes, 0.5 ml of alluminium chloride ( $AlCl_3$ , 100g/l) was added. 0.5 ml of sodium hydroxide ( $NaOH$ , 4%) was added after 5 minutes. The final volume was kept at 2.5ml with distilled water and thoroughly mixed. The

absorbance of the mixture was determined at 510nm against the same mixture without the sample as blank. Total flavonoids content was expressed as mg rutin/g dry weight (mg/g DW) through the calibration curve of rutin. All samples were analyzed in triplicates.

#### **Total Alkaloids Content**

The total alkaloids content was determined according to the method describe by Raheleh *et al.* (2013). 100ppm solution of atropine (1mg in 10 ml of distilled water) was used as standard. From this stock solution, exactly 0.5, 1.0, 1.5, 2.0 and 2.5 ml of atropine solutions was transferred to five different separating funnels. To each separating funnel, 5 ml of phosphate buffer (pH 4.7) and 5 ml Bromocresol green (BCG) solution was added and mix vigorously. The formed complex mixture was extracted with chloroform (5ml). The chloroform fraction was collected in a 10 ml volumetric flask and make up the volume with chloroform. Absorption at a wavelength of 470nm of each flask was measured and a calibration graph was drawn. For the presentation of sample, the plant extract (1mg/ml) was dissolved in 2N HCl and then filtered. The pH of the extract was adjusted to neutral with 0.1N NaOH. 1ml of this solution was transferred to separating funnel and to the mixture of Bromocresol green solution along with 5ml of phosphate buffer was added and mixed properly. The form mixture was extracted further with 5ml of phosphate chloroform and transferred to 10ml volumetric flask and make the volume with chloroform. The absorbance of the complex in chloroform was measured at 470nm.

#### **Estimation of Total Steroids**

Estimation of total sterols in the extract was done according to the modified methods method of Raheleh *et al.* (2013). The Liebermann-Burchad (LB) reagent was employed for the quantitative estimation of sterol. It was prepared by adding 0.5ml of concentrated sulphuric acid in 10ml of acetic anhydride. To 1ml of each of the extract (1mg/ml), chloroform was added to make the volume to 5ml in a test tube. A volume of 2 ml of LB reagent was be added and mixed well. These tubes were then covered with black paper and kept in the dark for 15mins to avoid any exposure to light. The reaction mixture was turns green which was measured spectrophotometrically by using UV-Vis Spectrophotometer at 640nm against the blank. Beta-sitosterol was used as the standard to prepare a calibration curve. All the test was carried out in triplicates and the results was expressed as Beta-sitosterol equivalent ( $\mu\text{g}$  of Beta-sitosterol/mg of extract).

#### **Determination of Cardiac Glycosides**

The procedure described by Sofowora (1995) was used. 10ml of the extract was pipetted into a 250ml conical flask. 50ml chloroform was added and shaken on vortex mixer for 1 hour. The mixture was filtered into 100ml conical flask. 10ml of pyridine and 2ml of 29% of sodium nitroprusside were added and shaken thoroughly for 10mins. 3ml of 20% NaOH was added to form a brownish and yellow color. Glycosides standard (Digitoxin) was used at

concentration range from 0-50mg/ml from stock solution and the absorbance was read at 510nm.

#### **Determination of Total Saponnins**

The total saponins content was determined spectrophotometrically using the modified method of Uematsu *et al.* (2000). To 2ml of each extract, 1ml of reagent A (p-anisaldehyde 0.5 in ethyl acetate 99.5) and 1ml of reagent B (H<sub>2</sub>SO<sub>4</sub> 50% in ethyl acetate 50%) was mixed and homogenized in a vortex. The methanol Extract of *Cucurbita pepo* and standard (Digitoxin) was dissolved in 2ml ethyl acetate. Then, 1ml of reagent A and 1ml of reagent B was added. The mixture was stirred and incubated at 60°C for 10mins in a water bath. The solution was cooled at room temperature for 10mins and the absorbance of the color developed solution was recorded at 430nm. Ethyl acetate was used as a control for the measurements of absorbance. Solution containing 75-175 µg standard (digitoxin) in 2ml ethyl acetate was used to obtain a calibration curve. The total saponins was determined from the calibration curve and expressed as milligrams of digitoxin equivalent per grams of extract (mgDE/g).

#### **Determination of Total Glycosides Content (TGC)**

Balet's reagent colorimetric method was used for the determination of the total glycosides content of the Extract as previously reported by Tonight *et al.* (2016) with modification. The extract (1mg/ml) was dissolved with 6ml of distilled water and 1ml of 12.5% (w/v) lead acetate solution was added. The mixture was made up to 10ml with distilled water and filtered. The filtrate (5ml) will be transferred to a volumetric flask, 1ml of 4.77% (w/v) Na<sub>2</sub>HPO<sub>4</sub> solution was added and made up to 10ml with distilled water and filtered. Baljet's reagent (10ml) was added to 1ml of the clear filtrate and the mixture was allowed to stand for 1hour and diluted with 20ml of distilled water. The absorbance of the mixture was read against the blank at 495nm with a UV-Vis Spectrophotometer. The total glycosides content was determined from the calibration curve and expressed as milligrams of digitoxin equivalent per gram of Extract (mgDE/g).

#### **Drosophila Diet Preparation (850ml)**

850ml of distilled water was divided into 500ml and 350ml portions. The 500ml portion was added to a cooking pot and boiled. A small quantity of the boiling water was used to dissolve 10g of yeast. Then, 8g of agar-agar was added to the remaining boiling water and for 10 minutes with periodic stirring. Out of 350ml, 200ml portion of water was used to dissolve 50g of cornmeal. The dissolved cornmeal was added to the boiling agar-agar mixture and heated for 10 minutes with periodic stirring. The dissolved yeast was added to the seething mixture and stirred periodically while allowing it to seethe for 15 minutes. The food was then cooled to 65°C, and 1g of Nipagin previously dissolved in 10ml of ethanol was added and stirred. After cooling to about 35°C (semi solid state), aliquots of the media were supplemented with the appropriate concentration of extract/drug and dispensed into vials

(10-30cm) or as required. The remaining food was refrigerated until needed. Flies could be added as soon as the media solidified and cooled (Sani *et al.*, 2023).

### Collection of Virgin Female Flies

To obtain flies expressing A $\beta$ <sub>42</sub> pan-neuronally (experimental group: elav-GAL4; UAS-A $\beta$ <sub>42</sub>) or control flies (elav-GAL4), we use the GAL4-UAS transgene expression system with the pan-neuronal elav-GAL4 driver (Bloomington stock #458). Because the elav-GAL4 transgene is located on the X chromosome, we collect virgin females from elav-GAL4 transgenic flies and cross them with male UAS-A $\beta$ <sub>42</sub> transgenic flies.

The modified method of Sekiya and Iijima (2021) was adopted for crossing the flies. Briefly, vials of elav-GAL4 flies for collection of virgin females and vials of UAS-A $\beta$ <sub>42</sub> for male were prepared under standard laboratory condition of 24°C–25°C, 40%–50% humidity with 12 h light/dark cycle. After the adult flies were eclosed around day 10–11, 30 virgin females of elav-GAL4 and 15 males of UAS-A $\beta$ <sub>2</sub> were placed into the culture vials. After 4–5 days, the parental (Fo) flies were transferred to new vials to increase the number of vials. The F1 males from control (elav-GAL4) and the experimental group (elav-GAL4; UAS-A $\beta$ <sub>42</sub>) were collected ten days after setting up the cross. The flies were anaesthetized and 50 flies were placed per plastic vials (25 mm diameter) containing fly food. These flies are 0 day old. The vials were changed every 3–4 days to obtain age-matched flies from the experimental and control groups.

### Experimental Design

**Group 1:** Gal-4 (Negative control, wild strain)

**Group 2:** A $\beta$ -42 (Positive control, mutant strain induced with AD and + untreated)

**Group 3:** 10 $\mu$ l of Donepezil (Standard control, mutant strain induced with AD + Drug)

**Group 4:** 94.9mg of *Cucurbita pepo* (mutant strain induced with AD + Extract)

**Group 5:** 142.4mg of *Cucurbita pepo* (mutant strain induced with AD + Extract)

**Group 6:** 189.8mg of *Cucurbita pepo* (mutant strain induced with AD + Extract)

### Homogenization and Biochemical Assays

The modified protocol for homogenization of *Drosophila* according to Experiments with *Drosophila* for Biology Courses, 2021 (Lakhotia and Ranganath, 2021) was adopted. Briefly, at the end of the experiments, 30 *Drosophila* flies were collected in a microcentrifuge tube, weighed then homogenized in 0.1M Phosphate buffered saline, pH 7.4 (1:10) flies/volume and with the help of a hand homogenizer. The homogenates were centrifuged at 10,000 rpm for 10 min; the supernatants were separated from the pellet into Eppendorf tubes kept at -20°C freezer and used subsequently for biochemical assays. The Assay was carried out for each of the 3 replicates of control and treated flies

### Negative Geotaxis Assay

The Negative Geotaxis Assay was done following the modified method of Mershin *et al.* (2004), flies from the experimental group (elav-GAL4; UAS-A $\beta$ 42) and control group (elav-GAL4) were collected and aged. A total of 150 flies were used per group, with 30 flies placed into each plastic vial (25 mm diameter) containing fly food. This resulted in 5 vials per group. Flies were tapped to the bottom of the vials and transferred to fresh fly food vials every 3 days. The negative geotaxis assay was performed once, starting when the flies were 2 weeks old. On Day 14, flies were tapped to the bottom of the food vials and transferred to new, empty plastic vials (25 mm diameter) marked with genotype and a scale representing height. The vials were laid horizontally for 5 minutes to acclimate the flies to their new environment.

**First Trial:** The vials were tapped against a foam pad or layered Kimtowels to knock the flies to the bottom. The vials were then stood upright in front of a white board. After 10 seconds, a photograph was taken to capture the distribution of flies within the vial. Healthy flies exhibited negative geotaxis by climbing up the vial walls. The recording time of 10 seconds was chosen as most control flies reached the top of the vial within this period. The recording time was adjusted based on experimental conditions. The vials were laid horizontally for 3–5 minutes before proceeding to subsequent trials.

**Second to Fifth Trials:** Steps from the first trial were repeated for a total of five trials. After the assay, flies were transferred back to their original plastic vials containing fly food and maintained. Flies were categorized based on their position within the vials (top, middle, bottom) using the captured photographs. The percentage of flies in each section of the vial was calculated, averaging the results from five trials per vial. The average percentages and standard errors were computed for 5–6 vials per treatment.

### Aversive Phototaxis Suppression Assay (APS Assay)

APS assay method was Adapted from Le Bourg and Buecher (2002) with modifications, this assay exploits the positive phototactic behavior in flies to train them to associate light with aversive stimuli (in this case, bitter taste of quinine). After a training/conditioning phase, wild type flies will be able to associate the lighted area with aversive taste and avoid it. Flies with compromised learning capacities will fail to make this association. Furthermore, the APS assay can also be used to measure short term memory function of flies (Seugnet *et al.*, 2009), by subjecting already trained flies to the same test 6 hours post conditioning to test their ability in remembering the learned task.

**Preparing the Quinine Solution:** Quinine hydrochloride was dissolved in distilled water to make a 0.1M stock solution (1.98g in 50mL distilled water). The stock solution can be kept at -20°C for up to a year in small aliquots. The working solution was prepared of 1  $\mu$ M by diluting the stock solution in distilled water.

**Preparing the T-Maze:** T-maze consists of the center column with the trap door and two independent chambers, a "dark" chamber and a "lighted" chamber.

Chamber preparation: Two 15mL plastic centrifuge tubes were cut at 2 mL mark from the bottom closed end with a manual saw. The connector adapters were fit at each end, and the connection was seal with parafilm. The adapter at the end of the tube serves as the input slot for the gooseneck light source. One tube was connected with the light source and this tube will be the "lighted" chamber. The other tube was wrapped with aluminum foil to serve as the "dark" chamber. 180uL of quinine solution was added to the filter paper and place in the light chamber. The T-maze was assembled by screwing the lighted and dark chambers on each side of the center column, with the trap door in the middle closed. Then the light source was inserted into the lighted chamber.

#### **Training flies to suppress phototaxis with aversive stimuli**

The dark chamber was unscrewed from the T-maze and a single fly was transfer into this chamber then the chamber was immediately screwed back to the maze. The lights in the room was turn off and the red lamp turn on. The fly was allowed to acclimatize in the dark chamber for 30 seconds and the light was slowly switched on illuminating the lighted chamber. Slowly open the trap door that separates the two chambers. If the fly walks to the lighted chamber within 10 seconds, it is considered positively phototactic and is ready to be trained for the assay. Failure in phototaxis indicates problems in the visual system and flies with negative phototaxis need to be excluded from the assay. For Aversive Phototaxis Suppression (APS) training, the fly that demonstrated positive phototaxis was tap back to the dark chamber, close the trap door and turn off the light. Allow 30 seconds of acclimatizing. During this time the filter paper with quinine solution was inserted into the lighted chamber. Slowly open the trap door and turn on the light. The fly was allowed to walk into the quinine coated lighted chamber. After one minute, tap the fly back to the dark chamber and repeat this 9 more times. (Typically, wild type flies avoid the lighted chamber after 3 to 5 training trials). Immediately after training, five test trials was conducted. In each test trial, allow 10 seconds after light is turned on for the trained fly to walk to the lighted chamber. Failure to walk to the lighted vial was recorded as "Pass", which is equivalent to "task learned through reinforcement". The Pass rate over five consecutive trials was recorded as PCo (0 hr post conditioning).

**Assessing Short-Term Memory Function in Flies:** After the flies were trained and initial PCo was recorded, each fly was placed back into its original food vial and kept for six hours. Six hours post training, each fly was subjected to 5 trials again in the same way as before, and the number of times the fly avoids (pass) or goes into (fail) the lighted vial was recorded. This pass rate was recorded as PC6 (6 hrs post conditioning), which is an indicator of short term memory.

### Statistical Analysis

All data obtained were analyzed using SPSS statistical software version 21. For comparison of multiple groups, the one-way analysis of variance (ANOVA) with a Duncan's post hoc test for homogenous subset was used to define statistically significant differences among the groups. Probability values ( $P < 0.05$ ) were considered as statistically significant. All the results obtained were converted to Bar chart using GraphPad Prism version 10.

### Results

#### Qualitative Phytochemical Analysis

The results for qualitative phytochemical analysis (Table 4.1) show the presence of Cardiac glycosides, Glycosides, Flavonoids, Tannins, Steroids, and Phenols, while Terpenoids were absent. From the results, Flavonoids and Steroids were present in abundance, followed by Phenols and Tannins, then Cardiac Glycosides, Glycosides, and Saponins.

The phytochemicals of *Cucurbita pepo* fruits methanol Extract were quantitatively analyzed and presented in Figure 4.1 below. The result shows that Flavonoids ( $28.60 \pm 0.73$  g/mg) had the highest concentrations among the phytochemicals. However, the concentrations decrease in the following order; Steroids ( $18.03 \pm 0.49$  g/mg), Phenols ( $15.15 \pm 0.46$  g/mg), Tannins ( $11.71 \pm 0.75$  g/mg), Saponins, ( $8.41 \pm 0.81$  g/mg), Cardiac glycosides ( $3.95 \pm 0.27$  g/mg) and Glycosides ( $3.55 \pm 0.27$  g/mg).

**Table 1: Qualitative Phytochemical Analysis of *Cucurbita pepo* Fruit Methanol Extract**

| Phytochemicals     | Inference |
|--------------------|-----------|
| Cardiac Glycosides | +         |
| Glycosides         | +         |
| Flavonoids         | +++       |
| Terpenoids         | -         |
| Tannins            | ++        |
| Steroids           | +++       |
| Saponnins          | +         |
| Phenols            | ++        |

**+ = Present; - = Absent**

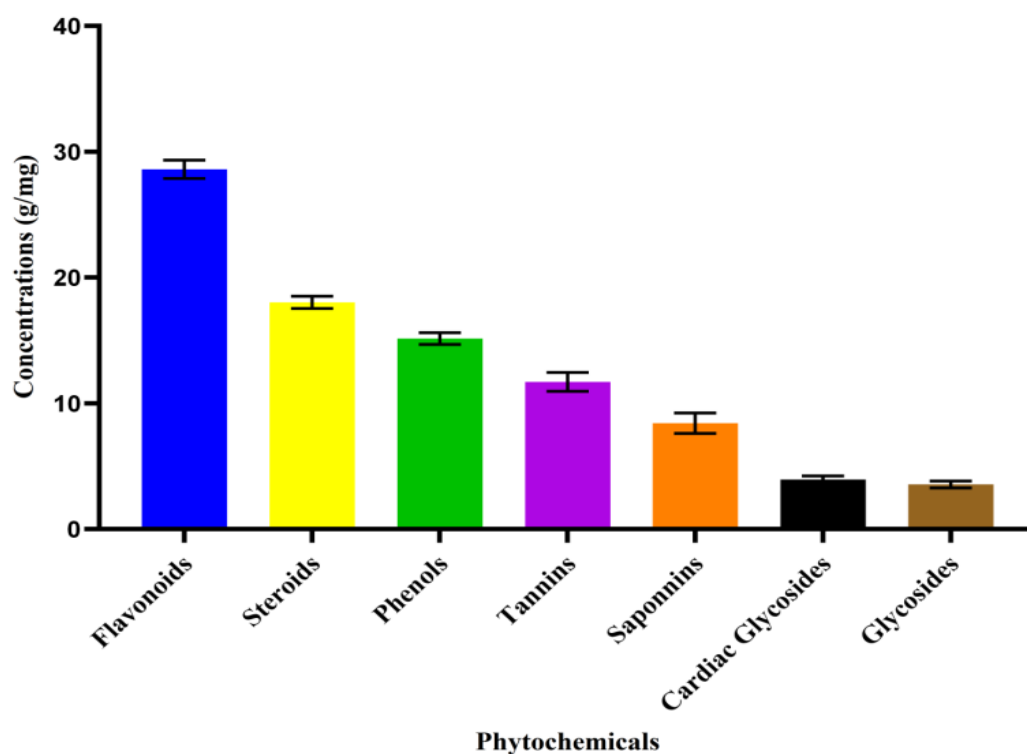


Figure 2: Quantitative Phytochemicals

#### Effects of *Cucurbita pepo* Extract on Locomotor Function of *Drosophila melanogaster*

In Figure 3 below, the result for the percentage Locomotive ability on *Drosophila melanogaster* shows a significant decrease ( $P < 0.05$ ) in AB42 (48%) group when compared with the wild strain Gal-4 (80%). Treatment with the standard drug, Donepezil (86%) shows a significant increase ( $P < 0.05$ ) in the locomotive function. Similarly, treatment with different dosages of the *Cucurbita pepo* Fruit extract, 94.9mg (70%), 142.4mg (78%) and 189.8mg (72%) shows a significant increase ( $P < 0.05$ ) in the percentage of the locomotive function when compared with the mutant AB42 group. Furthermore, Donepezil (86%) and 142.4mg *Cucurbita pepo* (78%) were more effective, almost similar to that of the wild strain Gal4 group (84%).

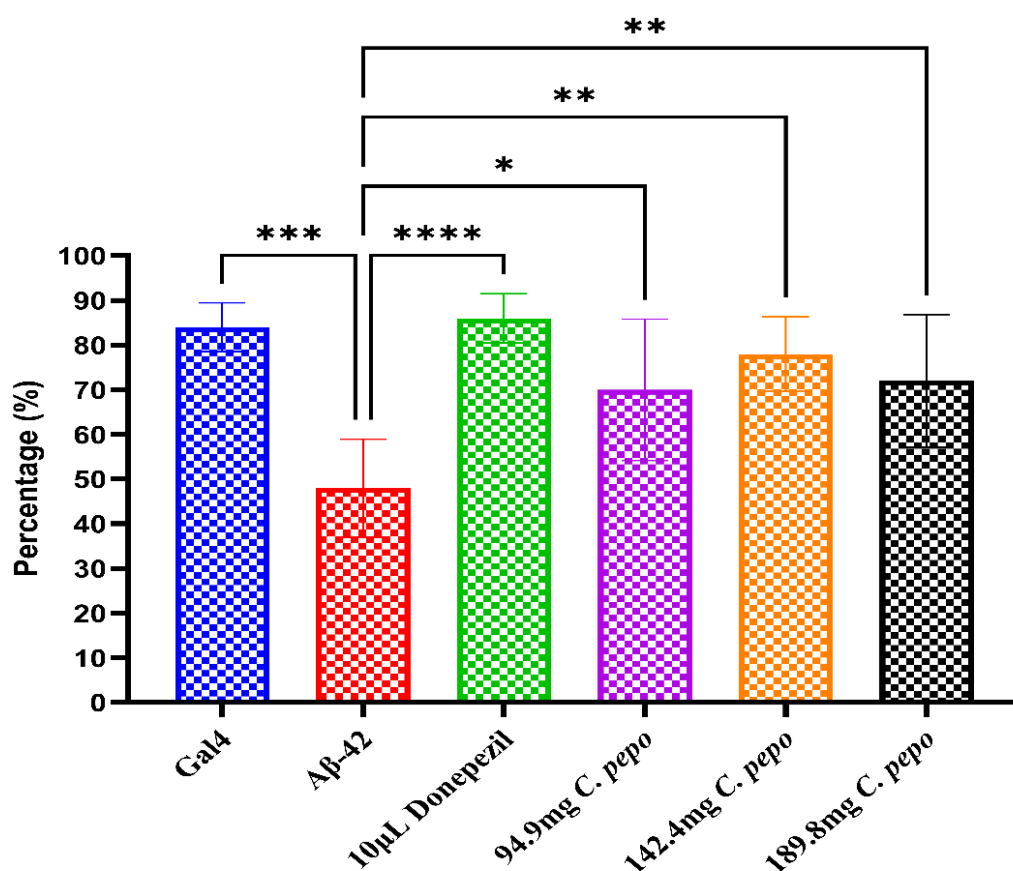


Figure 3: Effects of *Cucurbita pepo* Extract on Locomotive Ability of *Drosophila melanogaster*

**Group 1:** Gal-4 (Negative control, wild strain) **Group 2:** Aβ-42 (Positive control, mutant strain induced with AD and + untreated) **Group 3:** 10μl of Donepezil (Standard control, mutant strain induced with AD + Drug) **Group 4:** 94.9mg of *Cucurbita pepo* (mutant strain induced with AD + Extract) **Group 5:** 142.4mg of *Cucurbita pepo* (mutant strain induced with AD + Extract) **Group 6:** 189.8mg of *Cucurbita pepo* (mutant strain induced with AD + Extract).

#### Effects of *Cucurbita pepo* Extract on Learning Function of *Drosophila melanogaster*

The results for the effects of *Cucurbita pepo* extract on learning function of *Drosophila melanogaster* were presented in Figure 4 below. The results show a significant decrease ( $P < 0.05$ ) in learning ability of the *Drosophila melanogaster* in AB<sub>42</sub> group (40%) when compared with the wild strain Gal<sub>4</sub> group (80%). There is significant increase in percentage of the learning function of the *Drosophila melanogaster* upon treatment with Donepezil (88%) as compared with the mutant group AB<sub>42</sub> (40%). Similarly, a significant increase ( $P < 0.05$ ) was observed in group treated with 142.4mg of *Cucurbita pepo* (80%) as compared with the mutant group AB<sub>42</sub> (40%). However, no significant differences ( $P > 0.05$ ) were

observed in groups treated with 94.9mg and 189.8mg of *Cucurbita pepo* when compared with the mutants AB<sub>42</sub> group (40%). Among the treatment groups, Donepezil and 142.4mg *Cucurbita pepo* were found to be more effective in reversing the learning ability of mutant *Drosophila*.

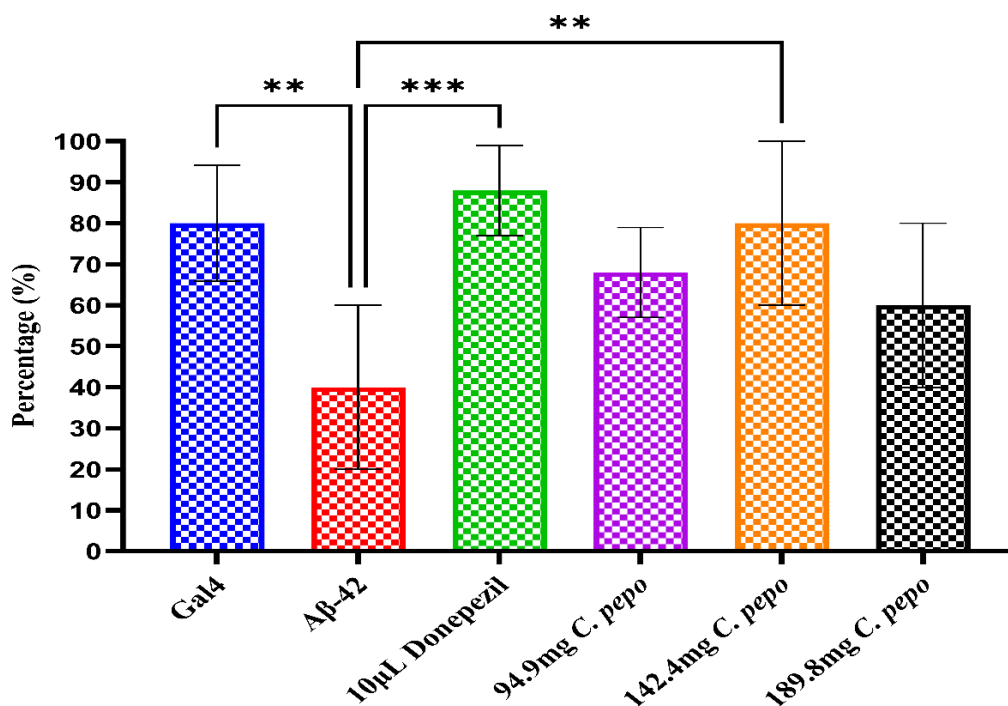


Figure 4: Effects of *Cucurbita pepo* Extract on Learning ability of *Drosophila melanogaster*

**Group 1:** Gal-4 (Negative control, wild strain) **Group 2:** Aβ-42 (Positive control, mutant strain induced with AD and + untreated) **Group 3:** 10μl of Donepezil (Standard control, mutant strain induced with AD + Drug) **Group 4:** 94.9mg of *Cucurbita pepo* (mutant strain induced with AD + Extract) **Group 5:** 142.4mg of *Cucurbita pepo* (mutant strain induced with AD + Extract) **Group 6:** 189.8mg of *Cucurbita pepo* (mutant strain induced with AD + Extract).

#### Effects of *Cucurbita pepo* Extract on Memory Function of *Drosophila melanogaster*

The results for the effects of *Cucurbita pepo* extract on memory function of *Drosophila melanogaster* were presented in figure 5 below. From the result, the percentage of the memory function in the mutant AB<sub>42</sub> group (16%) significant decrease when compared with the wild strain Gal<sub>4</sub> (72%). Treatment with Donepezil (76%) significantly increased the percentage of the memory function when compared with the untreated AB<sub>42</sub> group (16%). Similarly, the groups treated with the 94.9mg *Cucurbita pepo* (70%), 142.4mg *Cucurbita pepo* (80%) and 189.8mg *Cucurbita pepo* (68%) reverse the decline in memory function with

a significant increase ( $P < 0.05$ ) in percentage of the memory function when compared with the Alzheimer's diseases mutant AB<sub>42</sub> group (16%).

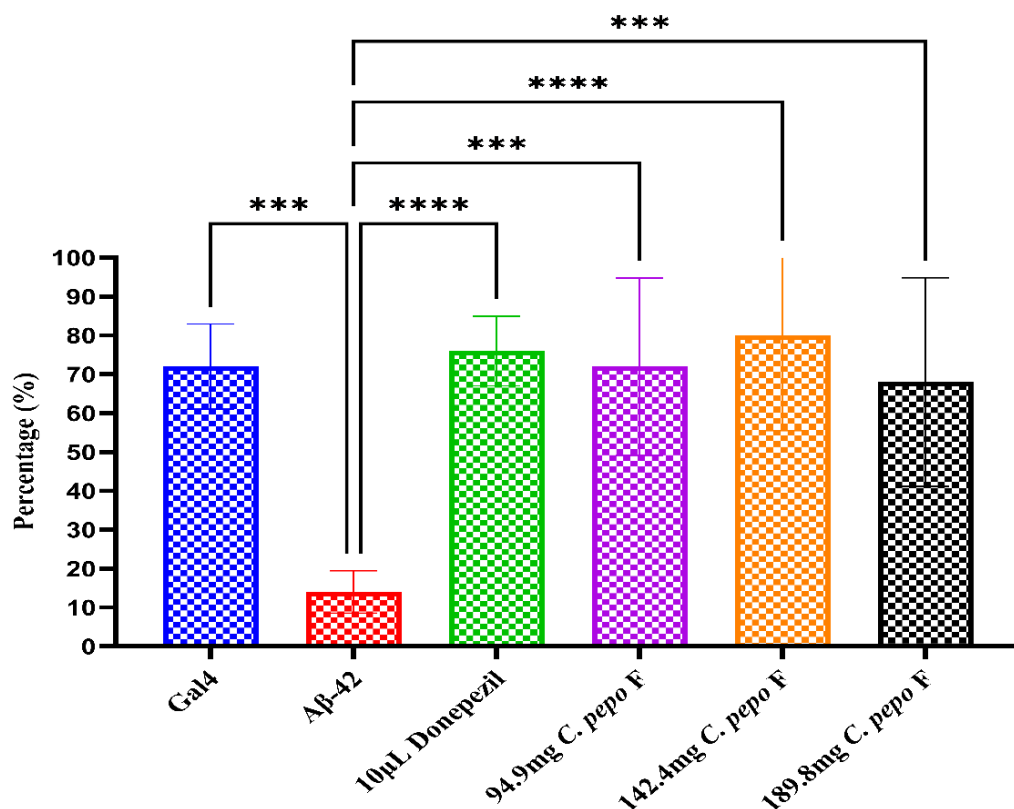


Figure 5: Effects of *Cucurbita pepo* Extract Memory Function of *Drosophila melanogaster*.

**Group 1:** Gal-4 (Negative control, wild strain) **Group 2:** Aβ-42 (Positive control, mutant strain induced with AD and + untreated) **Group 3:** 10μl of Donepezil (Standard control, mutant strain induced with AD + Drug) **Group 4:** 94.9mg of *Cucurbita pepo* (mutant strain induced with AD + Extract) **Group 5:** 142.4mg of *Cucurbita pepo* (mutant strain induced with AD + Extract) **Group 6:** 189.8mg of *Cucurbita pepo* (mutant strain induced with AD + Extract).

## Discussion

Pumpkin (*Cucurbita pepo*) is not only a widely consumed food but also a source of bioactive compounds with demonstrated therapeutic properties, attributable to its rich phytochemical and nutritional profile (Anyanwu *et al.*, 2024). Phytochemicals such as flavonoids, alkaloids, phenols, tannins, and saponins play a pivotal role in mitigating neurodegenerative conditions through antioxidative, anti-inflammatory, and neuroprotective mechanisms (Polyzos *et al.*, 2024). Flavonoids and phenolic acids, for example, can scavenge free radicals, chelate transition metals, and upregulate endogenous

antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT), thereby protecting neurons from oxidative damage, a major contributor to Alzheimer's disease (AD) pathology. Meanwhile, saponins and tannins have been implicated in stabilizing cell membranes and suppressing neuroinflammation through the inhibition of pro-inflammatory mediators such as NF- $\kappa$ B, IL-6, and TNF- $\alpha$ .

The Negative Geotaxis Assay is a standard behavioral test used to evaluate locomotive performance and neuromuscular integrity in *Drosophila melanogaster* (Minocha *et al.*, 2022). It assesses the fly's innate tendency to climb against gravity after mechanical stimulation, which serves as a readout for motor function. In AD models, such as flies expressing human A $\beta$ -42 peptide, impaired motor ability is often observed due to neuronal degeneration and synaptic dysfunction caused by oxidative stress and toxic protein aggregates (Nitta *et al.*, 2022). Consistent with this, our findings showed a significant decline in climbing ability in the A $\beta$ -42-expressing group compared to the Gal-4 control group, confirming the validity of the disease model.

Interestingly, treatment with Donepezil (a clinically approved acetylcholinesterase inhibitor) and *Cucurbita pepo* methanol fruit extract significantly improved climbing performance. This suggests that both treatments may counteract A $\beta$ -42-induced motor deficits via neuroprotective pathways. The phytochemicals in *Cucurbita pepo* likely contribute to this improvement by reducing oxidative stress, modulating cholinergic signaling, and suppressing neuroinflammatory responses. These mechanisms are consistent with previous studies indicating that flavonoid-rich extracts can restore neuromuscular function by preserving mitochondrial integrity and reducing lipid peroxidation in neural tissues (Sharma and Shanti, 2022; Eickelberg *et al.*, 2022).

The Aversive Phototaxis Suppression (APS) Assay is employed to measure cognitive behaviors such as learning and memory in *Drosophila* (Pokkunuri *et al.*, 2022). This paradigm leverages the fly's natural attraction to light and its ability to learn and avoid light when paired with an aversive stimulus like quinine. In our study, A $\beta$ -42-expressing flies displayed significant impairments in memory formation compared to controls, consistent with known A $\beta$ -42 pathology that interferes with key memory-related proteins such as cAMP response element-binding protein (CREB) and brain-derived neurotrophic factor (BDNF) (Zhao *et al.*, 2021; Ong *et al.*, 2022).

Remarkably, treatment with Donepezil and *Cucurbita pepo* extract (142.4 mg) significantly reversed these cognitive deficits. This therapeutic effect may be mediated by the extract's ability to enhance synaptic plasticity and neuronal survival through modulation of CREB-BDNF signaling, reduction of oxidative damage, and attenuation of inflammatory cytokine production. These findings are aligned with prior studies where antioxidant-rich plant extracts improved memory and learning in AD models, further supporting the neurotherapeutic potential of *Cucurbita pepo* (Baghel *et al.*, 2021; Singh *et al.*, 2020; Chaudhuri *et al.*, 2021).

While the results are promising, several limitations should be acknowledged. Firstly, although *Drosophila melanogaster* serves as a powerful and cost-effective model for

studying neurodegeneration, its neuroanatomical and physiological differences from humans limit the direct translatability of the findings. Key aspects such as blood-brain barrier complexity, immune responses, and pharmacokinetics differ significantly between flies and humans. Thus, further validation using mammalian models is essential before clinical relevance can be established. Secondly, the phytochemical composition of the methanol extract was not characterized in detail in this study. Without profiling the specific compounds and their concentrations, attributing the observed therapeutic effects to individual constituents remains speculative. In future work, LC-MS/MS or HPLC analysis could be employed to identify and quantify the bioactive molecules responsible for the effects. Moreover, the study did not include molecular-level assays (e.g., immunoblotting or gene expression analyses) to directly measure changes in oxidative stress markers, inflammatory cytokines, or synaptic proteins. Such data would provide stronger mechanistic insights into how *Cucurbita pepo* confers neuroprotection. Lastly, while Donepezil served as a positive control, a dose-response curve for *Cucurbita pepo* extract was not established, limiting our ability to determine the optimal therapeutic window or potential toxicity.

The current findings demonstrate that *Cucurbita pepo* methanol fruit extract significantly improves motor coordination and memory function in A $\beta$ -42-expressing *Drosophila melanogaster*, likely via antioxidant and anti-inflammatory mechanisms. Despite inherent model limitations, this study provides a strong rationale for further exploring *Cucurbita pepo* as a source of novel phytotherapeutics for Alzheimer's disease.

## Conclusion and Recommendations

### Conclusion

The present study provides compelling evidence for the therapeutic potential of *Cucurbita pepo* methanolic fruit extract in ameliorating Alzheimer's Disease (AD)-like symptoms using an A $\beta$ 42 transgenic *Drosophila melanogaster* model. The phytochemical analysis revealed the presence of abundant bioactive compounds, particularly flavonoids and steroids, known for their antioxidant and neuroprotective properties. These constituents likely contribute to the observed improvements in locomotor activity, learning, and memory functions in AD flies treated with the extract. Among the three tested doses, 142.4 mg of *Cucurbita pepo* extract showed the most promising neuroprotective effects, comparable to the standard drug Donepezil. The extract improved motor coordination, as assessed by the Negative Geotaxis Assay, and cognitive performance, as measured by the Aversive Phototaxis Suppression Assay. These beneficial effects are likely mediated through the antioxidative and anti-inflammatory properties of its phytochemical constituents, including flavonoids, phenols, and saponins, which may mitigate A $\beta$ -42-induced oxidative stress and neuronal damage. This suggests that the bioactive constituents of *Cucurbita pepo* can mitigate A $\beta$ 42-induced neurodegeneration, potentially through antioxidative and anti-inflammatory pathways. The study validates the folkloric

use of *Cucurbita pepo* in neurocognitive disorders and positions it as a potential source for developing safer, plant-based therapeutics for Alzheimer's disease.

However, several limitations must be acknowledged. First, the use of *Drosophila* as a model organism, while powerful, does not fully recapitulate the complexity of human neurobiology, thus limiting the generalizability of the findings to human disease. Second, the specific phytochemicals responsible for the observed effects were not identified or quantified, which limits mechanistic interpretation. Furthermore, molecular data (e.g., gene or protein expression levels of neurodegenerative or inflammatory markers) were not included, which could have provided more direct insight into the pathways involved. Lastly, a dose-response analysis of the extract was not conducted, and toxicity studies were not performed.

### Recommendations and Future Directions

1. **Phytochemical Profiling:** Future studies should include detailed phytochemical analysis of *Cucurbita pepo* extracts using techniques such as HPLC or LC-MS/MS to identify and quantify bioactive compounds responsible for its neuroprotective effects.
2. **Mechanistic Investigations:** Additional experiments involving molecular techniques (e.g., RT-qPCR, Western blotting, or ELISA) are recommended to assess changes in oxidative stress markers (e.g., SOD, CAT, MDA), inflammatory cytokines (e.g., TNF- $\alpha$ , IL-6), and key neuroplasticity proteins (e.g., BDNF, CREB).
3. **Toxicological Evaluation:** Conducting acute and chronic toxicity studies in both invertebrate and mammalian models would help assess the safety profile of the extract, especially at varying dosages.
4. **Mammalian Model Validation:** To enhance translational relevance, future research should replicate this study in higher-order animal models, such as mice or rats, which possess more comparable neuroanatomical structures and biochemical pathways to humans.
5. **Dose-Response Studies:** Determining the minimum effective and maximum safe doses of *Cucurbita pepo* extract is crucial for establishing its therapeutic potential and guiding clinical applications.

### References

- Anyanwu, C.F., Georgewill, O.A., & Georgewill, U.O. (2024). Effect of *Cucurbita pepo* (Pumpkin) seed Extract on the cyclicity and reproductive hormones of female Wistar rats. *J Herbmed Pharmacol*.13(4):620-629. <https://doi:10.34172/jhp.2024.52543>
- Baghel, M., Yadav, K. S., & Sharma, R. (2021). Natural compounds targeting neuroinflammation in Alzheimer's disease: A review. *Pharmacological Reports*, 73(6):1407–1420. <https://doi.org/10.1007/s43440-021-00285-6>
- Carmine-Simmen, K., Proctor, T., Tschape, J., Poeck, B., Triphan, T., Strauss, R., Kretzschmar, D. (2009). Neurotoxic effects induced by the *Drosophila* amyloid-beta peptide suggest a conserved toxic function. *Neurobiol Dis*, 33:274–281.

- Chaudhuri, D., Brody, T., & VijayRaghavan, K. (2021). Neural basis of locomotion in *Drosophila*: A model to study neurodegenerative disorders. *Frontiers in Neuroscience*, 15:648239. <https://doi.org/10.3389/fnins.2021.648239>
- Eickelberg, V., Lüersen, K., Staats, S., & Rimbach, G. (2022). Phenotyping of *Drosophila melanogaster*-A Nutritional Perspective. *Biomolecules*. 12(2):221. <https://doi:10.3390/biom12020221>.
- Ferri, C.P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., Hall, K., Hasegawa, K., Hendrie, H., Huang, Y. (2005). Global prevalence of dementia: a Delphi consensus study. *Lancet*, 366:2112–2117.
- Fortini, M.E., Skupski, M.P., Boguski, M.S., Hariharan, I.K. (2000). A survey of human disease gene counterparts in the *Drosophila* genome. *J Cell Biol.*, 150:23–30.
- Hashemi, K.S., Aliabadi, M.K., Mehrara, A., Talebi, E., Hemmati, A.A., Rezaeiye, R.D., Ghanbary, M.J., Motealleh, M., Dayeri, B., & Alashti, S.K. (2023). A meta-analysis of microarray datasets to identify biological regulatory networks in Alzheimer's disease. *Front. Genet.* 14:1-13. <https://doi:10.3389/fgene.2023.1225196>.
- James, R., Malcolm, K., Dariel, B. & Joanna, V. (2014). Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties. *Journal of Microbiology and Biology Education*. 15(1):45-46.
- Kenchappa, P.G., Karthik, Y., Vijendra, P.D., Hallur, R.S., Khandagale, A.S., Pandurangan, A.K., Jayanna, S.G., Alshehri, M.A., Alasmari, A., Sayed, S., Shantaram, M., & Mushtaq, M. (2023). *In vitro* evaluation of the neuroprotective potential of *Olea dioica* against A $\beta$  peptide-induced toxicity in human neuroblastoma SH-SY5Y cells. *Frontiers in pharmacology*, 14, 1139606. <https://doi.org/10.3389/fphar.2023.1139606>
- Lakhotia, S.C., & Ranganath, H.A. (2021) Experiments with *Drosophila* for Biology Courses. 1st ed. 1-32. Indian; Academy of Sciences, Bengaluru. Pp.281.
- Le Bourg, E., & Buecher, C. (2002). Learned suppression of photopositive tendencies in *Drosophila melanogaster*. *Animal Learning & Behavior*, 30:330-341.
- Mauryan, S. & Singh, D. (2010). Quantitative Analysis of Total Phenolic Content in *Adhatoda vasica* Nees Extract. *International Journal of PharmTech Research*. 2(4):2403-2406.
- Mershin, A., Pavlopoulos, E., Fitch, O., Braden, B.C., Nanopoulos, D.V., & Skoulakis, E.M. (2004). Learning and memory deficits upon TAU accumulation in *Drosophila* mushroom body neurons. *Learn Mem.* 11:277-87.
- Minocha, T., Birla, H., Obaid, A.A., Rai, V., Sushma, P., Shivamallu, C., Moustafa, M., Al-Shehri, M., Al-Emam, A., Tikhonova, M.A., Yadav, S.K., Poeggeler, B., Singh, D., & Singh, S.K. (2022). Flavonoids as Promising Neuroprotectants and Their Therapeutic Potential against Alzheimer's Disease. *Oxid Med Cell Longev*. 28:6038996. <https://doi:10.1155/2022/6038996>.
- Muhammad, B.Y., Barau, S.A., Zaruwa, M.Z., Yahaya, A., & Salihu, M. (2023). Amelioration of lead-induced neurotoxicity by *Cucurbita pepo* (pumpkin) fruit extract in wister rats. *Pak. J. Nutr.*, 22:11-18. Doi: [10.3923/pjn.2023.11.18](https://doi.org/10.3923/pjn.2023.11.18)
- Nitta, Y., & Sugie, A. (2022). Studies of neurodegenerative diseases using *Drosophila* and the development of novel approaches for their analysis. *Fly (Austin)*. 16(1):275-298. <https://doi:10.1080/19336934.2022.2087484>.
- Ong, A.G., Villanueva, M.R., & Medina, P.M. (2020). *Bacopa monnieri* supplementation increases learning and short-term memory retention of sleep-deprived *Drosophila melanogaster*. *Journal of Applied Pharmaceutical Science*, 10(12):104-110. <https://doi.10.7324/JAPS.2020.101214>
- Periz, G., Fortini, M.E. (2004). Functional reconstitution of gamma-secretase through coordinated expression of presenilin, nicastrin, Aph-1, and Pen-2. *J Neurosci Res.*, 77:309–322.
- Pokkunuri, S., & Puwalowski Kimberly, P. (2022). A novel Alzheimer's Disease therapeutic model: Attenuating hyperphosphorylated Tau and Amyloid  $\beta$  (A $\beta$ ) aggregates by characterizing

- antioxidative, antiinflammatory, and neuroprotective properties of natural Extract. *Journal of Emerging Investigators*, 5(1):1-17.
- Polyzos, N., Fernandes, Â., Calhelha, R.C., Petrović, J., Soković, M., Ferreira, I.R., Barros, L., & Petropoulos, S.A. (2024). Biochemical Composition of Pumpkin Seeds and Seed By-Products. *Plants* 13(2395):1-14. <https://doi.org/10.3390/plants13172395>
- Prüßing, K., Voigt, A., & Schulz, J.B. (2013). *Drosophila melanogaster* as a model organism for Alzheimer's disease. *Molecular Neurodegeneration*, 8(35):1-11. <http://www.molecularneurodegeneration.com/content/8/1/35>.
- Raheleh, Z., Mona, F., Zeinab, M. & Golam, R. G. (2013). Extraction and comparison of alkaloids in different organs during different phonological periods of *Nitraria schoberi*. *Annals of Biological Research*. 4:130-135.
- Rajan, K.B., Weuve, J., Barnes, L.L., Mcaninch, E.A., Wilson, R.S. and Evans, D.A. (2021) Population Estimate of People with Clinical Alzheimer's Disease and Mild Cognitive Impairment in the United States (2020-2060). *Alzheimer's & Dementia*, 17, 1966-1975. <https://doi.org/10.1002/alz.12362>
- Rajeev, S., Pawan, K. V. & Gagandeep, S. (2012). Total phenolic, flavonoids and tannin contents in different Extract of *Artemisia absinthium*. *Journal of Intercultural Ethnopharmacology*. 1(2):101-104.
- Reiter, L.T., Potocki, L., Chien, S., Gribskov, M. and Bier, E. (2001) A Systematic Analysis of Human Disease-Associated Gene Sequences in *Drosophila melanogaster*. *Genome Research*, 11, 1114-1125. <https://doi.org/10.1101/gr.169101>
- Rubin, G.M., Yandell, M.D., Wortman, J.R., Gabor, G.L., Nelson, C.R., Hariharan, I.K., Fortini, M.E., Li, P.W., Apweiler, R., & Fleischmann, W. (2000). Comparative genomics of the eukaryotes. *Science*, 287:2204-2215.
- Sani, H., Muhammad, B.Y., Zaruwa, M.Z., & Bamidele, T.O. (2023). Peanut skin Ethanol Extract Ameliorates Lead-Induced Oxidative Stress in *Drosophila*. *Academ Arena*, 15(4):26-33. ISSN 1553-992X (print); ISSN 2158-771X (online). <http://www.sciencepub.net/academia.05>. <https://doi:10.7537/marsaaj150423.05>.
- Sekiya, M. & Iijima, K.C. (2021). Protocol Phenotypic analysis of a transgenic *Drosophila* model of Alzheimer's amyloid- $\beta$  toxicity. *STAR protocols*. 2:1-24. <https://doi.org/10.1016/j.xpro.2021.100501>.
- Seugnet, L., Suzuki, Y., Stidd, R., & Shaw, P.J. (2009). Aversive phototactic suppression: evaluation of a short-term memory assay in *Drosophila melanogaster*. *Genes Brain Behav* 8:377-89.
- Sharma, U., & Shanti, I.R. (2022). Effects of Crude Extract of *Areca catechu* L. on Locomotory Behaviour in *Drosophila melanogaster*. *Asian Journal of Biological and Life Sciences*, 11(3):745-750.
- Singh, T., Patel, D. K., & Roy, A. (2020). Neuroprotective potential of natural compounds in Alzheimer's disease models. *Current Neuropharmacology*, 18(8):679-693. <https://doi.org/10.2174/1570159X18666200227152356>
- Sofowora, A. (1993). *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.
- Sun, Y., Yolitz, J., Wang, C., Spangler, E., Zhan, M. and Zou, S. (2013) Aging Studies in *Drosophila melanogaster*. *Methods in Molecular Biology*, 1048, 77-93. [https://doi.org/10.1007/978-1-62703-556-9\\_7](https://doi.org/10.1007/978-1-62703-556-9_7)
- Tonight, T., Ghazi saeidi, N., Hadjiakhoondi, A. & Yassa, N. (2016). Determination of cardiac glycosides and total phenols in different generations. *Research Journal of Pharmacognosy*. 3(2):25-31.
- Vishnu, B., Sheerin, F. M. A. & Sreenithi, V. (2019). A Guide to Phytochemical Analysis. *International Journal of Advance Research and Innovative Ideas in Education*, 5(1):2395-4396.
- Zengnin, G., Aktumsek, A., Guler, G. O. & Cakmak, Y. S. (2011). Antioxidant Properties of Methanolic Extract and Fatty Acid Composition of *Centaurea urvillei* DC. SubspPhayekiana Wagenitz. *Records of Natural Products*. 52:123-132.

Zhao, J., Fu, Y., & Sun, Y. (2021). Alzheimer's disease and Drosophila: A model organism for AD research. *Frontiers in Genetics*, 12:638271. <https://doi.org/10.3389/fgene.2021.638271>