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The Effect of *Psidium Guajava* Leaf Extract on Cognitive Function in Scopolamine-Induced Alzheimer's Disease Wistar Rats

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ABSTRACT

This study evaluated the impact of *P. guajava* leaf extract on cognitive function of scopolamine-induced Alzheimer's disease model of Wistar rats. The plant leaf was extracted by maceration using methanol as a solvent. Phytochemical screening and quantification of the bioconstituents of the leaf extract were done The study was done in two phases, with phase one lasting for two weeks and phase two lasting for twelve weeks. A total of 80 male Wistar rats weighing 100 – 230 g were divided into 5 groups each for the two phases (phase 1, n = 8; phase 2, n = 8). The rats in groups 2 to 5 were induced with Alzheimer's disease (AD) using 1 mg/kg of scopolamine (i.p.) for 7 days before commencement of treatment. Group 1 (normal control) received 2 mL/kg of distilled water only, group 2 (AD control rats) also received 2 mL/kg of distilled water while groups 3 – 5 were AD rats treated with 100 mg/kg, 300 mg/kg of *P. guajava* leaf extract and 5 mg/kg of donepezil respectively. Neurobehavioural tests for cognitive function were done in weeks one and two, then every two weeks using navigational maze test, radial maze test and Barnes maze test. At the end of the14th and 84th days of the experiment, the animals were euthanised with 100 mg/kg of ketamine then decapitated to remove the brain. The brain tissues for the ELISA were rinsed in normal saline, homogenised in phosphate buffer using a ceramic mortar and pestle placed on ice, transferred into sample bottles then taken to the laboratory in ice pack for the BDNF assay. The neurobehavioural results for cognitive function indicated that *P. guajava* leaf extract significantly ($p < 0.05$) improved learning and memory in a dose and time dependent manner. The extract was able to improve significantly the levels of BDNF in the brain homogenate. It was concluded from the results that *P. guajava* leaf may be used in the treatment of AD and it is recommended that the treatment should commence with the low dose (100 mg/kg b.wt.) then progress to the medium dose (300 mg/kg b.wt.).

KEYWORDS: scopolamine, *P. guajava*, BDNF, rats

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INTRODUCTION

Dementia is the loss of mental abilities that affects daily life. It is usually promoted by other neurodegenerative diseases such as AD (60 – 80 % of all cases). AD is characterised by unavoidable loss of neurons, formation of neurofibrillary tangles, tau protein aggregation, amyloid β-protein (Aβ) deposition and low levels of acetylcholine (Ach) (Rawat et al., 2022; Sun et al., 2022). Neurodegenerative disease can be described as a heterogeneous group of disorders caused by progressive, long-lasting degradation and loss of neuronal

cells in specific areas of the central nervous system (Pohl & Lim, 2018). Neurodegenerative diseases are known for their wide range of harmful conditions leading to progressive cell damage, nervous system connections and neuronal death. These pathologies culminate to loss of important motor and cognitive functions, such as mobility, learning and sensation, loss of memory, decision making, balance, talking, breathing and heart function (Silva & Pogacnik, 2020). Neurodegeneration affects millions of people worldwide, yet no integral cure has been developed (Rojas-Garcia et al., 2023).

The World Health Organisation (WHO, 2023) stated that Alzheimer's disease (AD) is one of the major diseases plaguing the elderly worldwide. They estimated that 6.7 million Americans aged 65 and above were living with Alzheimer's disease in 2023, 73 % of this number was aged 75 or older and about 1 in 9 people aged 65 and above (10.7 %) had Alzheimer's disease. By 2050, the number of people aged 65 and above with Alzheimer's disease may grow to a projected 12.7 million. 1 in 3 seniors die with Alzheimer's disease or other dementia. Between 2000 and 2022, deaths from Alzheimer's disease have more than doubled, while those from heart disease – the leading cause of death – have decreased (WHO, 2023).

According to WHO, (2023), more than 55 million people currently have dementia worldwide, over 60 % of whom live in low – and middle – income countries (WHO, 2023). Every year, there are nearly 10 million new cases. AD is the most common cause of dementia and may contribute to 60 – 70 % of cases. Dementia is currently the seventh leading cause of death and one of the major causes of disability and dependency among older people globally (WHO, 2023).

AD affects many regions of the brain such as hippocampus, cerebellum, thalamus, amygdala, frontal lobe etc. (Martin, 2015). The hippocampus is the first area of the brain to be affected by AD resulting in loss of short-term memory. A study showed that the volume and ratio of this region was reduced by 25 % in AD, 21 % in mixed dementia, 11 % in vascular dementia, and 5 % in normal pressure hydrocephalus (Martin, 2015). The hippocampus is a complex brain structure embedded deep into the temporal lobe. It can be distinguished externally as a layer of densely packed neurons, which curls into S-shaped structure on the edge of temporal lobe. In adult humans, the hippocampus on each side of the brain is about 3 – 3.5 cm^3 as compared to 320 – 420 cm^3 for the volume of the neocortex. The hippocampus plays a major role in learning, memory, and spatial navigation and emerging studies have also indicated it to be part of 'moral brain' (Fumagalli & Priori, 2012).

Tau neurotoxicity provokes alterations in brain-derived neurotrophic factors (BDNF)/ tropomyosin receptor kinase B (TrkB/cAMP – response-element binding protein (CREB) signalling to contribute to neurodegeneration. Compounds activating TrkB may therefore provide beneficial effects in tauopathies (Te-Hsien et al., 2022). Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor that promotes neuronal survival and growth (Pisani et al., 2023). In the brain, BDNF is mainly synthesized in cell bodies of neurons and glial cells and then transported to presynaptic terminals and postsynaptic dendrites. The localisation of BDNF and its receptors, tropomyosin receptor kinase B (TrKB), to glutamate synapses regulate neurotransmitter release, ion channel activity, axonal pathfinding and neuronal excitability (Schinder & Poo, 2000). BDNF binds to tropomyosin-receptor kinase B (TrkB) to induce dimerisation and phosphorylation of TrkB, and subsequent activation of downstream extracellular-signal regulated kinase (ERK) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signalling pathway (Huang & Reichardt, 2003). ERK phosphorylates cAMP responsive element binding protein 1(CREB 1) to promote transcription of genes, such as BDNF and B-cell lymphoma 2 (BCL 2) anti-apoptosis regulator for neuronal survival, neurite outgrowth and neuroplasticity (Walton & Dragunow, 2000). In addition, PI3K – AKT pathway stimulates target gene expression via CREB phosphorylation to promote cell survival. Enhancement of BDNF expression rescues neuronal death and improves learning and memory in rodent and primate models of AD (Hsiao et al., 2014).

Under pathological condition such as AD, Huntington and Parkinson's disease, and normal aging conditions, abnormal levels of BDNF have been reported. The Profound BDNF deficits are reported in the hippocampus, parietal, entorhinal and frontal cortex for AD and in the striatum and motor cortex for Huntington disease (Hock et al., 2000). Neurotrophic factors are crucial for the aetiology of AD, in particular BDNF. BDNF protein and mRNA levels as well as proBDNF are reduced in the post-mortem brain of AD patients compared with age-matched controls; it has no changes in TrkB levels. The reduction is also found in mild cognitive impairment (MCI), a potentially prodromal stage of AD (Shimada et al., 2014). BDNF levels are correlated to the severity of the disease and with episodic memory performance in patients, suggesting that these diseases could be related to the pathogenesis of the disease. Down regulation of BDNF and proBDNF are thought to be underlying mechanism related to early AD (Peng et al., 2005).

Psidium guagava commonly known as guava in English is called goba in Hausa, guaba or gilofa in Yoruba, ugwoba in Igbo (Okoye, 2021) and ugwomba in Abua language. *P. guajava* is an evergreen shrub native to the Caribbean, Central America and South America and Mexico. It is widely cultivated in tropical and subtropical regions around the world. *P. guajava* belongs to the phylum magnoliophyta, class magnoliopsida and myrtaceae family (Dakappa et al., 2013; Okoye, 2021). The plant has a wide spreading network of branches. Its leaves have opposite arrangement with small petioles of about 3 to 16 cm. The leaves have clear green

colour with prominent veins (Rouseff et al., 2008). The plant produces white flowers with incurved petals having a nice fragrant. The flowers have four to six petals and yellow coloured anthers and pollination is by means of insects especially bees. *P. guajava* have been used in traditional medicine in many cultures throughout central America, the Caribbean, Africa and Asia for the treatment of inflammation, diabetes, hypertension, caries, wounds, pain relief, fever, diarrhoea, rheumatism, lung diseases and ulcers (Gutierrez et al., 2008). Several studies have demonstrated the potential of *P. guajava* in the management of AD in area of inhibiting acetylcholinesterase (Bhattacharya et al., 2024; Lorena et al., 2022; Silva et al., 2022) and inhibiting neurotoxicity and cognitive dysfunction (Usen & Enogieru, 2023).

MATERIALS AND METHODS

Plant Preparation

The fresh leaves of *P. guajava* were obtained from Agada 1 in Abua/Odual Local Government Area of River State, Nigeria and identified in the Department of Botany, Faculty of Science, Rivers State University, with a voucher number RSUPSH 0167. The fresh leaves were washed in running tap water and air dried in a shady place for two weeks. Thereafter, the leaves were chopped into pieces with the aid of a knife then pulverised using laboratory mortar and pestle into coarse powder form. 1500 g of the pulverized leaf were extracted by maceration using methanol as a solvent. The extract was heat-dried at 60 ℃ in a water bath. The dry yield was 150.5 g, which was stored at 4℃ in a refrigerator until used.

Induction of Alzheimer's Disease

Apart from the animals in the control group (group one), all other animals from group 2 to group 5 were induced Alzheimer's disease using 1 mg/kg b.wt. of scopolamine (i.p.) for 7 days. Scopolamine hydrobromide obtained from Sigma – Aldrich (USA), was dissolved in normal saline (0.9 % NaCl) (El-Marasy et al., 2018). The *P. guajava* leaf extract was administered after the 7 days of Alzheimer's disease induction.

Phytochemical Screening

The phytochemical screening of the *P. guajava* leaf extract were done using methods of Sofowora (1993), Trease and Evans (1989). The various phytoconstituents of the extract were presented in a table.

Alkaloidal Test

0.5 g of the plant extract was dissolved in 5 mL in 5 % hydrochloric acid on a steam bath and then filtered. 1 mL of the filtrate was treated with a few drops of Mayer's reagent and a second 1 mL portion was treated similarly in Dragendorff's reagent. A third part was treated with a few drops of picric acid (Sofowora, 1993).

Saponins Test

(a) Frothing Test: about 0.5 g of the extract was vigorously shaken with distilled water in a test tube. Frothing that persisted on warming was taken as preliminary evidence for the presence of saponins (Sofowora, 1993).

(b) Sodium Bicarbonate Test: 0.5 g of the extract was added with 5 % sodium bicarbonate and Fehling's solution A and B and boiled. Presence of brown precipitate was taken as a positive test (Trease & Evans, 1989).

Tannins Test

(a) Ferric Chloride Test: 0.5 g of the extract was stirred with 10 ml of distilled water and filtered. 5 % of ferric chloride reagent was added to the filtrate. The presence of a blue-black, green or blue green precipitate was considered as evidence for the presence of tannins (Trease & Evans, 1989).

(b) Bromine Water Test: 5 drops of the extract solution was mixed with 10 mL of distilled water and bromine water. Decolourisation of the bromine water was taken as an indication of the presence of tannins (Trease & Evans, 1989).

Flavonoids Test

Shinoda Reduction Test: Few pieces of magnesium metal were added to each plant extract solution. The solution was obtained by using concentrated hydrochlorice acid to dissolve the extract. The formation of orange (flavonones), red crimson (flavonols) or magenta colouration was an evidence for the presence of flavonoids (Trease & Evans, 1989).

Cardiac Glycosides

(a) Salkowskis Test (terpenoids): 0.5 % of the extracts was dissolved in 2 mL of chloroform. Concentrated sulphuric acid was carefully added by running it down the side of the test tube. A reddish brown coloure at the interphase indicated the of aglycone portion of cardiac glycosides (Sofowora, 1993).

(b) Keller-Kiliani Test: 0.5 % of each extract was dissolved in 2 mL of glacial acetic acid containing one drop of ferric chloride. This was then underplayed with 1 mL concentrated sulphurice acid. A brown ring characteristic obtained at the interphase indicated the presence of deoxy-sugar characteristic of cardenolide (Sofowora, 1993)

(c) Liebermann Test (steroids): 0.5 g of each extract was added to 3 mL of chloroform and filtered. Ten drops of acetic anhydride was added to the filtrate along with 2 drops of concentrated sulphuric acid. A reddish brown or violet ring at the interphase is a positive test while a bluish colour could be suspected to be steroids (Trease & Evans, 1989).

(d) Animals

(e) Eighty (80) male Wistar rats weighing 100 – 230 g were used for the study. Forty (40) rats were used for each phase (1 and 2) of the study. The phases 1 and 2 of the study represent experimental duration of 14 days and 84 days, respectively. After two weeks of

acclimatisation, the animals were divided into five groups of 8 rats each in plastic wire meshed cages. Group 1 served as normal control and received 2 mL/kg of distilled water only, group 2 received 1 mg/kg of scopolamine plus 2 mL/kg of distilled water, group 3 received 1 mg/kg of scopolamine plus 100 mg/kg of *P. guajava* leaf extract, group 4 received 1 mg/kg of scopolamine plus 300 mg/kg of *P. guajava* leaf extract, and group 5 received 1 mg/kg of scopolamine plus 5 mg/kg of donepezil. Throughout the experiment, the animals were allowed free access to water and feed (vital grower®) *ad libitum*. Animals were maintained in twelve hours of light and dark cycles at room temperature (25 ℃).

Experimental Design

The animals were divided into five groups (n = 16) and the treatment regimen was as indicated in Table 1. The experiment was done in two phases. Phase one lasted for 14 days following a 7 days of Alzheimer's disease induction while phase two took 84 days. The doses used for the study fell within a range that had been adjoined to repeatedly show efficacy in rat studies reporting the pharmacological effects of *P. guajava* leaf extract (Vijayakumar et al., 2018).

Table 1 Treatment Regimen of the Drug and Extract.

Group	Treatment	Dosage	Duration	
			Phase 1	Phase 2
1	distilled water	2 mL/kg only	2 weeks	12 weeks
2	scopolamine + distilled water	1 mg/kg + 2 mL/kg	2 weeks	12 weeks
3	scopolamine + *P. guajava*	1 mg/kg + 100 mg/kg	2 weeks	12 weeks
4	scopolamine + *P. guajava*	1 mg/kg + 300 mg/kg	2 weeks	12 weeks
5	scopolamine + donepezil	1 mg/kg + 5 mg/kg	2 weeks	12 weeks

n = 16

Neurobehavioural Studies

Neurobehavioural studies of motor and cognitive function which were done once every week for phase one then every two weeks for phase two, was carried out in the neurophysiology laboratory of physiology department, University of Port Harcourt, using navigational maze test, beam walk test, Barnes maze test,

Navigational Maze Test: This was used to assess spatial learning and memory. The apparatus consists of a box containing intricate paths formed by squares inside the box. It has an entrance from one face of the box and an exit at opposite side. Food is placed at the exit point to serve as aversive stimulus. The rat was placed at the entrance of the box, then the entrance was shut for the rat to fine the food at the exit. The rat was allowed to look for the exit within 5 minutes. The time taken by the rat to fine the exit was recorded. Rats with impaired memory took longer time to locate the exit.

Radial Maze Test: This maze measured spatial learning and memory in rats. The apparatus consists of equidistantly spaced arms (8), each about 50 cm long, 10 cm wide, and 20 cm high from the floor, radiating from a small circular central platform. At the end of each arm there was a food site, the contents of which were not visible from the central platform. The rats were trained for three (3) days by placing food in all the arms on the first day, then reduced it to half of the arms. This was to ensure the rats understood the maze. During the test, the rats were placed in the centre of the maze and allowed to explore. The rats must remember which arm they have visited to avoid re-entry and collect all rewards. The design ensures that, after checking for food at the end of each arm, the rat is always forced to return to the central platform before making another choice. Reference memory was assessed when the rats only visit the arms of the maze which contained the reward (food). Failure to do so implied memory deficit. Working memory was assessed when the rats enter each arm a single time (once). Re-entry into the arms implicated a working memory deficit. The number of arms explored in 5 minutes were recorded (Tarragon et al., 2012).

Barnes Maze Test: Barnes maze test was used to assess spatial learning and memory in the rats. It consists of a circular maze platform of 100 cm in diameter with 20 holes of 5 cm in diameter each. A small, black escape box was placed under one of the holes. The procedure was done in a well illuminated room to serve as aversive stimuli to motivate the rats to search for shelter in the escape box. A video camera was placed above the centre circular arena to record all movements. Habituation was carried out by placing the rat in the escape box within the Barnes maze for 3 minutes and in the arena for 1 minute. Immediately after the habituation, the rats were trained to locate the escape box. This was done by placing the rat at the centre of the platform covered by a starting box for 20 seconds, then released to find the escape box. Rats that fail to fine the escape box within 5 minutes were guided to it and kept there for 1 minute before returning to the home cage. The training was done for 3 days with 3 trails lasting for 5 minutes each. Trials were done following the training to assess the effect of the drugs. The time taken

to find the escape box (latency) was measured in seconds. Rats with impaired spatial learning and memory took longer time to find the escape box and made more errors (Peris et al., 2024).

Biochemical Assay

A 100 µL of dilution of standard solution was added to blank and sample wells each. The plates were covered with the sealer, incubated for 90 min at 37℃. The liquid from each well was decanted without washing. 100 µL of biotinylated detection Ab working solution was immediately added to each well and covered with a new sealer then incubated for 1 hour at 37℃. The solution from each well was decanted and 350 µL of wash buffer was added to each well, soaked for 1min and aspirated the solution from each well then patted dry against clean absorbent paper. This step was repeated 3 times. 100 µL of HRP conjugate working solution was added to each well, covered with a new sealer and incubated for 30 min at 37℃. The solution was decanted from each well and the process repeated for 5 times. 90 µL of substrate reagent was added to each well, covered with a new sealer and incubated for 30 min. at 37℃. The plate was protected from light. The microplate reader was preheated for about 15 min before optical density measurement. 50 µL of stop solution was added to each well in the same order as the substrate solution. The optical density value of each well was determined at once with a micro-plate reader set at 450 nM. This was done in the Uuniversity of Port Harcourt.

Ethical Permit

Ethical permit was sought for and obtained from the Ethical Permit Committee for Animal Use of the Faculty of Basic Medical Sciences, Rivers State University, and the experiment was done in accordance with the guidelines of the permit.

3.14 Statistical Analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) software package (version 28). A one-way ANOVA was employed to determine the significance of the mean of the data obtained from the study of the neurobehavioural and biochemical analysis and a multiple comparisons (LSD) *post-hoc* test was used to compare individual groups. Results were presented as mean ± SEM (standard error of the mean) and difference were considered significant at the level of $p < 0.05$.

RESULTS

Phytochemical Study

Table 4.1 provides a preliminary screening of various phytochemicals present in *Psidium guajava* leaf using three different solvent extracts; N-hexane, methanol, and hydromethanol. The table indicates that methanol had a better extraction potential compared to N-hexane and hydro-methanol. Alkaloids, flavornoids, carbohydrate, glycoside, saponnin, tannin and phlobatannin are moderately present in the methanol extract while, anthraquinones is slightly present. N-hexane and hydro-methanol extracts showed slight presence of the phytochemicals analysed with anthraquinones absent in the N-hexane extract.

Table 2: Preliminary Screening of the Phytochemicals Present in *Psidium guajava* Leaf Extract using Different Solvents.

Phytochemicals	Tests	N-Hexane	Methanol	Hydro-Methanol
Alkaloids:	Meyers test	+ ve	+ ve	+ ve
	Drangendorffs test	+ ve	+ + ve	+ ve
	Hagers test	+ ve	+ + ve	+ ve
Flavornoids:	Shinoda test	+ ve	+ + ve	+ ve
	ACl_3	+ ve	+ + ve	+ ve
	NaOH	+ ve	+ + ve	+ ve
Carbohydrate:	Fehling solution	+ ve	+ + ve	+ ve
	Molisch test	+ ve	+ + ve	+ ve
Anthraquinones:	Free anthraquinone test	- ve	+ ve	+ ve
	Combined anthraquinone	– ve	+ ve	+ ve
Glycoside:	Keller Killiani	+ ve	+ + ve	+ ve
	Liebermann-Burchard	+ ve	+ + ve	+ ve
	Salkwoski test	+ ve	+ + ve	+ ve
	Keddle test	+ ve	+ + ve	+ ve
Saponin:	Frosthing test	+ ve	+ + ve	+ ve
	Emulsion test	+ ve	+ + ve	+ ve

Tannin:	5 % Ferric chloride	+ ve	+ + ve	+ ve
Phlobatannin:	1 % Hydrochloric acid	+ ve	+ + ve	+ ve

Key: + indicates slightly present, ++ indicates moderately present while – indicates absence of the phytochemicals tested.

Quantitative Analysis of *P. guajava* Leaf Extract

Table 4.2 provides a quantitative screening of various phytochemicals present in *Psidium guajava* leaf in the three solvents extracts. The results indicate that methanol is the most effective solvent for extracting flavonoids, alkaloids, saponin, glycosides and anthraquinone yielding the highest concentration at 0.0020 Pg/ml, 0.0014 Pg/mL, 0.0014 Pg/mL, 0.0020 Pg/mL and 0.0010 Pg/mL respectively. While hydromethanol is the most effective solvent for extracting carbohydrate yielding the highest concentration of 0.0022 Pg/mL and tannin at 0.0014 Pg/mL. N-hexane did better than hydromethanol in extracting flavonoids at 0.0019 Pg/mL compared to 0.0015 Pg/mL, glycoside at a concentration of 0.0019 Pg/mL compared to 0.0018 Pg/mL; and phlobatannin at a concentration of 0.0015 Pg/mL compared to 0.0012 Pg/mL. Overall, these findings highlight the importance of solvent selection in maximizing the extraction of specific phytochemicals from *Psidium guajava* leaves, with methanol being particularly effective for flavonoids, glycoside, phlobatannin, alkaloids, saponins, and anthraquinone. Hydromethanol being optimal for carbohydrates and tannins. The table shows that *P. guajava* leaf extract contained higher quantities of carbohydrate, glycoside and flavonoids in that order while anthraquinone is the least.

Table 3: Result of the Quantitative Analysis of *P. guajava* Leaf Extract

Phytochemicals	N-Hexane (Pg/mL)	Methanol (Pg/mL)	Hydro-methanol (Pg/mL)
Alkaloids	0.0012	0.0014	0.0012
Flavornoids	0.0019	0.0020	0.0015
Carbohydrate	0.0017	0.0019	0.0022
Saponin	0.0012	0.0014	0.0012
Glycoside	0.0019	0.0020	0.0018
Tannin	0.0012	0.0012	0.0014
Anthraquinone	0.0000	0.0010	0.0009
Phlobatannin	0.0013	0.0015	0.0012

Neurobehavioural Studies

Navigational maze test: The results of the navigational maze study indicate distinct patterns of spatial learning and memory across different treatment groups over a period 12 weeks. The control group (Group 1) consistently outperformed all other groups, demonstrating the lowest maze completion times, which signifies effective spatial memory retention. On the other hand, the scopolamine group (Group 2) exhibited progressive significant (*$p \leq 0.05$) longer altered cognitive performance which increased from 154.00 ± 9.34 second in week 1 to 245.40 ± 17.01 seconds in week 12, with the longest time observed in week 10 (277.40 ± *22.60 s), reflecting impaired cognitive function. The *P. guajava* leaf extract groups (Groups 3 and 4) showed mixed results; Group 3 (100 mg/kg) demonstrated significant ($p < 0.05$) improvement with the shortest time observed in week 1 (72.80 ± 33.04 s), and longest time in week 10 (146.00 ± 0.00 s), while Group 4 (300 mg/kg) experienced an initial decline in performance which improved later with the longest time in week 2 (217.40 ± 8.75 s), and shortest time in week 12 (112.00 ± 2.00 s), suggesting a dose and time dependent effects. Group 5, receiving donepezil (5 mg/kg), showed significant (*$p \leq 0.05$) improvements compared to the scopolamine group and notable enhancements throughout, with the best performance seen in the earlier weeks of treatment (55.80 ± 5.19 s in week 2) and (200.60 ± 18.80 s in week 10). This is shown in Table 5.

Table 4: Effect of *P. guajava* leaf extract on the pattern of spatial learning and memory using the navigational maze test

Groups/ Treatment	Week 0 (Baseline) Time (s)	Week 1 Time (s)	Week 2 Time (s)	Week 4 Time (s)	Week 6 Time (s)	Week 8 Time (s)	Week 10 Time (s)
Group 1	91.10 ± 3.60	90.20 ± 31.40	99.00 ± 11.78	82.20 ± 23.65	96.60 ± 12.93	82.20 ± #23.65	60.60 ± #32.28
Group 2	100.00 ± 6.00	154.00 ± 9.34*	197.60 ± 5.02*	233.80 ± 5.79*	213.80 ± 5.79*	297.40 ± *2.60	277.40 ± *22.60
Group 3	98.56 ± 8.10	72.80 ± 33.04#	117.00 ± 7.28#	99.00 ± 52.16#	120.60 ± 5.72#	130.00 ± *0.00#	146.00 ± 0.00*#
Group 4	102.01 ± 1.20	146.80 ± 50.42	217.40 ± *#8.75	213.00 ± *57.00	192.20 ± 42.12*	135.00 ± *0.00#	133.00 ± *0.00#
Group 5	88.20 ± 5.40	124.80 ± 47.49	55.80 ± 5.19*#	112.00 ± 8.00#	158.20 ± 53.45#	165.40 ± *#50.07	200.60 ± *18.80

Values are presented in mean ± SEM, n= 5. * Means values are statistically significant ($p \leq 0.05$) when compared to the control group 1, # means values are statistically significant ($p \leq 0.05$) when compared to scopolamine only group.

Radial maze test: The results from the radial maze study, measured by the number of arms explored, revealed significant differences in spatial learning and memory capabilities across treatment groups over a 12 week period of study. The control group (Group 1) consistently explored the highest number of arms, starting at 3.00 ± 0.71 arms in week 1 and increasing to 4.80 ± 1.11 arms in week 12, reflecting effective cognitive function. Conversely, the scopolamine group (Group 2) showed a significant (*$p \leq 0.05$) decline from 1.80 ± 0.68 arms in week 1 to 0.00 ± 0.00 arms by week 12, indicating profound impairment in cognitive function. Groups treated with *P. guajava* leaf extract (Groups 3 and 4) exhibited variable effects in the course of the weeks, with Group 3 (100 mg/kg) maintaining relatively stable exploration, having the best performance of 4.60 ± 1.21 arms in week 2 and steeping to 1.60 ± 0.81 arms in week 8. However, it picked up again in the last weeks but the earlier effect was better. Group 4 (300 mg/kg) showed insignificant improvement in the earlier weeks which worsen at week 4 with 0.60 ± 0.40 arms but subsequently improved significantly (*$p \leq 0.05$), reaching a peak of 3.60 ± 0.40 arms in week 12. This again suggest a time dependent beneficial effect (# $p \leq 0.05$ compared to the scopolamine group). The donepezil demonstrated a steady positive significant effect with the best result seen in week 4 (4.40 ± 0.68 arms) and the least in week 12 (2.20 ± 0.49 arms). Better results with donepezil treatment were observed in the earlier weeks of treatment (Table 5)

Table 5: Effect of *P. guajava* leaf extract on spatial learning and memory using radial maze test

Groups/Treatment	Week 0 (Baseline) No. of Arms/ 5 min	Week 1 No. of Arms/ 5 min	Week 2 No. of Arms/ 5 min	Week 4 No. of Arms/ 5 min	Week 6 No. of Arms/ 5 min	Week 8 No. of Arms/ 5 min	Week 10 No. of Arms/ 5 min	Week 12 No. of Arms/5 min
Group 1	4.10 ± 0.62	3.00 ± 0.63	4.00 ± 0.71	4.60 ± 0.40	4.20 ± 0.74	4.60 ± #1.12	4.00 ± 1.55	4.80 ± 1.11
Group 2	3.60 ± 1.40	1.80 ± 0.68*	2.40 ± 0.80*	2.80 ± 0.74*	1.20 ± 0.97*	1.20 ± *0.37	0.40 ± 0.25*	0.00 ± 0.00*
Group 3	4.83 ± 1.12	3.60 ± 0.98#	4.60 ± 1.21#	2.80 ± 1.24	3.60 ± 0.87#	1.60 ± 0.81	2.80 ± 0.58#	2.50 ± 0.97#
Group 4	4.52 ± 5.00	2.60 ± 1.08	3.00 ± 0.89	0.60 ± *#0.40	3.4 ± 1.08#	3.40 ± 1.40#	3.20 ± 0.	3.60 ± 0.40#
Group5	4.00 ± 3.70	3.20 ± 0.97#	4.20 ± 1.53#	4.40 ± *#0.68	3.80 ± 0.86#	2.60 ± *0.25	2.00 ± 0.63#	2.20 ± 0.49#

Values are presented in mean ± SEM, n= 5. * Means values are statistically significant ($p \leq 0.05$) when compared to the control group 1, # means values are statistically significant ($p \leq 0.05$) when compared to scopolamine only group.

Barnes maze test: The data from the Barnes maze test illustrates the effects of various treatments on spatial learning and memory across a 12 week period. The control group (Group 1) consistently demonstrated good memory performance with the longest latency of 26.80 ± 12.31 seconds at week 1 and shortest time of 17.60 ± 3.16 seconds by week 2. In contrast, the scopolamine group (Group 2) exhibited significant ($p < 0.05$) impairment in learning and memory all through the weeks compared with the normal control, with latency times increasing from 64.20 ± 1.32 seconds at week 1 to a notable 124.40 ± 16.35 seconds at week 10. This indicates severe deficits in spatial learning and memory throughout the study. The 100 mg/kg *P. guajava* group (Group 3) showed a significant ($p < 0.05$) improvement in memory compared to the scopolamine group, throughout the weeks with the shortest time seen in week 1 (20.60 ± 3.39 seconds) and the longest time in week 10 (54.80 ± 49.43 seconds). The 300 mg/kg *P. guajava* group (Group 4) had consistent performance, showing a latency time of 57.40 ± 66.56 seconds at week 4, improving with duration of treatment to 24.60 ± 5.78 seconds at week 12 ($p \leq 0.05$ compared to the scopolamine group). The donepezil group (Group 5) also performed well, with its performance being better in the earliest weeks of treatment having the shortest time of 17.20 ± 3.52 seconds in week 2 and longest time of 80.80 ± 58.12 seconds at week 8, demonstrating significant improvements compared to the scopolamine group across all weeks (Table 6).

Table 6: Effect of *P. guajava* leaf extract on the pattern of spatial learning and memory using Barnes maze test

Groups/Treatment	Week 0 (Baseline) Time (s)	Week 1 Time (s)	Week 2 Time (s)	Week 4 Time (s)	Week 6 Time (s)	Week 8 Time (s)	Week 10 Time (s)	Week 12 Time(s)
Group 1	25.66 ± 13.00	26.80 ± 12.31	17.60 ± 3.46	19.60 ± 5.80	29.60 ± 11.41	18.60 ± 48.88	24.00 ± 52.34	24.00 ± 55.00
Group 2	25.28 ± 5.10	64.20 ± 1.32*	67.20 ± 15.09*	90.60 ± 13.63*	99.60 ± 13.42*	112.20 ± 49.39*	124.40 ± 16.35*	121.40 ± *62.89*
Group 3	23.62 ± 6.07	20.60 ± 3.39#	29.80 ± 1.16#	30.20 ± 54.98#	36.60 ± 9.26#	45.60 ± 10.45#	54.80 ± 49.43#	43.60 ± *16.28#
Group 4	24.25 ± 8.00	39.20 ± 20.41	35.00 ± 7.02	57.40 ± *66.56	32.40 ± 8.02#	33.00 ± 10.18#	35.00 ± 49.65#	24.60 ± 5.78#
Group 5	19.80 ± 5.32	29.80 ± 56.14	17.20 ± 3.51#	25.60 ± 8.60#	36.80 ± 55.03#	80.80 ± 58.12	45.80 ± 29.17#	35.60 ± *16.53#

Values are presented in mean ± SEM, n= 5. * Means values are statistically significant ($p \leq 0.05$) when compared to the control group 1, # means values are statistically significant ($p \leq 0.05$) when compared to scopolamine only group.

Brain-derived Neurotrophic Factor: Table 4.12 presents the levels of brain-derived neurotrophic factor (BDNF) (Pg/mL) measured in various treatment groups at both 14th day and 84th day of the study, providing insight into neurotrophic support in response to different interventions. The control group (Group 1) exhibited a stable BDNF response, starting at 202.96 ± 0.17 Pg/mL at the 14th day and slightly increasing to 206.00 ± 44.79 Pg/mL by the 84th day, indicating a consistent neurotrophic environment. In contrast, the scopolamine group (Group 2) showed significantly reduced BDNF levels, decreasing from 177.73 ± 0.73 Pg/mL at the 14th day to 149.67 ± 5.44 Pg/mL at the 84th day (both $p \leq 0.05$), suggesting that scopolamine impairs neurotrophic support and potentially contributes to neurodegeneration. The 100 mg/kg *P. guajava* group (Group 3) exhibited a favourable response, with initial BDNF levels at 195.49 ± 0.56 Pg/mL, increasing dramatically to 490.33 ± 17.02 Pg/mL by the 84th day ($p \leq 0.05$ compared to the control group), indicating a significant enhancement in neurotrophic factors through *P. guajava* treatment. Similarly, the 300 mg/kg treated group (Group 4) showed a higher starting point of 295 ± 1.64 Pg/mL and an impressive increase to 480.67 ± 18.96 Pg/mL by the end of the study, reflecting the potent neuroprotective effects associated with this treatment. The donepezil group (Group 5) also demonstrated a notable improvement in BDNF, with levels starting at 194.57 ± 0.27 Pg/mL at the 14th day and rising to 394.32 ± 16.29 Pg/mL at the 84th day, showing a significant increase compared to the scopolamine group.

Table 7 Effect of *P. guajava* Leaf Extract on Brain-Derived Neurotropic Factor (BDNF)

Groups/Treatment	BDNF (Pg/mL) 14th day	BDNF (Pg/mL) 84th day
Group1 (control group)	202.96 ± #0.17	206.00 ± #44.79
Group 2 (1 mg/kg Scopolamine)	177.73 ± *0.72	149.67 ± 5.44*
Group 3 (1 mg/kg scopolamine + 100 mg/kg *P. guajava*)	195.49 ± 0.56	490.33 ± 17.02#
Group 4 (1 mg/kg scopolamine + 300 mg/kg *P. guajava*)	295 ± *1.64	480.67 ± 18.96#
Group 5 (1 mg/kg scopolamine + 5 mg/kg Donepezil)	194.57 ± #0.27	394.32 ± 16.29#

Values are presented in mean ± SEM, n = 5. * Means values are statistically significant ($p \leq 0.05$) when compared to the control group 1, # means values are statistically significant ($p \leq 0.05$) when compared to scopolamine only group.

DISCUSSION

Impairment of learning and memory, and lack of motor coordination and balance are some of the clinical implications of Alzheimer's disease (Silva & Pogacnik, 2020). Pathologically, tau accumulation recognized as neurofibrillary tangles resulting from hyperphosphorylated tau proteins, accumulation of Aβ (senile plaques) and neuroinflammation are some of the hallmarks of Alzheimer's disease (Reddy et al., 2017; Tackenberg et al., 2020). In this study, many spatial memory and learning, and motor coordination tests were employed to investigate the effect of *P. guajava* leaf extract on cognitive function as well as motor coordination in AD rats. Phytochemical screening for possible bioactive and biochemical compounds of medicinal values in plants has been a traditional practice in trying to evaluate the efficacy of plant parts on certain diseases.

The phytochemical screening of *P. guajava* leaf extract using N-hexane, methanol and hydro-methanol solvents indicated the presence of alkaloids, flavonoids, carbohydrate, anthraquinnones, glycoside, saponin, tannin and phlobatannin with methanol having the highest yield. Quantification of these phytochemicals indicates that methanol extracted the highest quantity with flavonoids and glycoside being the highest and anthraquinnone the least. This implies that the choice of solvent is important for maximum yield of phytochemicals. This result agrees with those of Thenmozhi & Rajan (2015) and Usen & Enogieru (2023). Phytochemicals such as those present in *P. guajava* leaf have therapeutic effects on AD and dementia through different mechanisms (Brodowska, 2017; Liu et al., 2020; Shishta et al., 2020; Ullah et al., 2020). Hence, *P. guajava* is an important plant with the potentials to treat certain diseases.

The impact of *P. guajava* leaf extract on cognitive function in scopolamine-induced Alzheimer's disease Wistar rats was studied using navigational maze test, radial maze test and Barnes maze test. Treatment of 1 mg/kg b.wt. of scopolamine for a period of one week induced serious spatial memory impairment (Bhuvanendran et al., 2018; Das et al., 2019; More et al., 2016; Thongsopha et al., 2024). The navigational maze, Barnes maze and radial maze tests indicated that *P. guajava* leaf extract improved learning and memory in the AD treated groups. The navigational maze test showed a significant ($p < 0.05$) longer time of completion in the scopolamine group (group 2) implying cognitive impairment. Treatment with the 100 mg/kg b.wt dose indicated a significant ($p < 0.05$) improvement of learning and memory as judged by the reduction in time of completion. The Barnes maze and radial maze tests also showed significant ($p < 0.05$) learning and memory impairment in the scopolamine control group, while the extract and donepezil treated groups indicated significant improvement. The 300 mg/kg and the donepezil treated groups also had significant ($p < 0.05$) reduction in completion time. These results agree with the works of Silva et al. (2022), Usen & Enogieru (2023) and Bhattacharya et al. (2024). The 100 mg/kg b.wt. dose was found to show better efficacy at the early stage of treatments while the 300 mg/kg b.wt. dose did better at the later stage of treatment. Donepezil also ameliorated memory impairment in a manner similar to the 100 mg/kg b.wt. dose of *P. gaujava*. This result is very beneficial to health practitioners involved in the management of AD as it showed how best this treatment may be prescribed – starting with the low dose then to medium dose.

The brain-derived neurotrophic factor analysis at the 14th and 84th days of the study showed a significant ($p < 0.05$)

reduction of BDNF in the homogenised brain tissues of scopolamine-induced rats when compared with the normal control. Deficiency of BDNF in the brain is a well-known biochemical hallmark of AD (Shimada et al., 2014). Reduced levels of BDNF have been implicated as a key factor leading to the dementia due to AD (Hock et al., 2000; Laske et al., 2006; Peng et al., 2005; Xue et al., 2022). Treatment with the *P. guajava* leaf extract demonstrated a good and consistent elevation of the level of BDNF in the brain. The 100 mg/kg b.wt. dose significantly ($p < 0.05$) improved the BDNF level, and had a positive effect in both phases of the study. The 300 mg/kg b.wt. dose too showed a significant increase in the level of BDNF over the duration of treatment. The standard drug, donepezil was also effective in raising the BDNF levels when compared with the scopolamine group. These results have strongly supported the outcome of the neurobehavioural test for spatial learning and memory using navigational, radial and Barnes maze tests, which clearly demonstrated a significant amelioration of memory impairment by *P. guajava* leaf extract. The elevated level of BDNF may have activated the necessary memory pathways such as CREB, NMDA receptors, and TrkB (Delezie & Hanschin, 2018; Namekata et al., 2010) which led to the improvement in memory impairment observed in the treatment groups.

These effects may be attributed to the phytochemicals with antioxidant properties contained in the extract. Flavonoids, phytol, 2, 4-di-tert-butylphenol, 1, 4-benzendiol, alkanoids, saponins, and tannins are antioxidants with anti-inflammatory properties (Brodowska, 2017; Carvalho et al., 2020) known to possess neuroprotective ability owing to their ability to cross the blood-brain barrier, scavenge reactive oxygen species and control signalling pathways involved in cognition, neuroinflammation and neuronal survival (Carvalho et al., 2020; Tachakittirungrod et al., 2007; Ullah et al., 2020). Flavonoids also enhance the production of short-chain fatty acids hence, offering neuroprotection. Alkaloids have also demonstrated neuroprotective and anti-AD properties and butyrylcholinesterase anti-agonists. They also inhibit α-synuclein aggregation and function as agonists of dopaminergic and nicotinic cholinergic receptors, conferring their anti-neurodegenerative properties (Kim et al., 2024).

CONCLUSION

The *P. guajava* leaf extract was found to significantly mitigate cognitive impairment in scopolamine-induced Alzheimer's disease rats leading to impressive improvement in learning and memory. The extract also significantly stimulated the production of BDNF – a neurotrophic factor important in neuronal growth, plasticity and long term potentiation. These findings point to the possible benefit of *P. guajava* leaf extract in the management of dementia due to AD induced by scopolamine.

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