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Spatial memory-enhancing effects and antioxidant activities of leaf and stem bark methanol extracts of *Prunus africana* in scopolamine-treated mice

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ABSTRACT

Introduction: Conventional medicines for Alzheimer's disease (AD) have little efficacy and are linked to several severe effects, necessitating alternative therapy. The current study investigated the memory-enhancing effects and antioxidant activities of stem bark and leaf MeOH extracts of *Prunus africana* in scopolamine-induced amnesic mice. Several inclusions build up in brain tissue during AD progression and the brain clears them oxidatively. This makes the antioxidant activity a vital requirement for plant extracts that are used with great success to manage AD.

Methods: In this study, for each plant extract, thirty Swiss albino mice were randomly assigned to six groups; extract-treated, reference drug control, normal control, and negative control groups. The mice were then subjected to the Morris water maze task for four consecutive days, euthanized and their whole brains were assessed for antioxidant activities.

Results: The studied extracts significantly ($P<0.01$) reduced escape latencies of experimental mice in a dose-related manner, depicting their considerable memory-enhancing effects. The extracts also displayed significant ($P<0.01$) enzymatic and non-enzymatic antioxidant activities.

Conclusion: The leaf and stem bark MeOH extracts of *P. africana* possess phytocompounds with spatial memory-enhancing effects and antioxidant activities.

Implication for health policy/practice/research/medical education:
The present study revealed new knowledge about the efficacy of the two *P. africana* extracts in the enhancement of spatial memory. In addition, the study also established the presence of phytocompounds with valuable medicinal properties, including enzymatic and non-enzymatic antioxidant activities which are necessary for alleviating oxidative stress linked to Alzheimer's disease (AD) pathology. Therefore, the extracts could be useful in managing AD.

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Introduction

Cognition is a term used to describe mental processes associated with acquiring, storing, manipulating, and retrieving information (1). Memory is one of the cognitive processes that enable animals to store and remember learned information. There are three types of memory; sensory, short-term, and long-term memories. Spatial memory and visual memory are the forms of memory and are represented within short-term and long-term memories. Spatial memory stores information on movements and locations whereas visual memory retains visual shapes and colours (2).

One of the most common tasks used to examine an animal's spatial memory is the Morris water maze (MWM), which involves the acquisition, storage, manipulation, and retrieval of information about the location of a hidden escape platform relative to colour cues displayed on the walls of the pool. Other methods used to measure spatial memory in animal models include dynamic mazes, pathway span tasks, radial arm mazes, psychomotor

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speed, and sustained attention tasks (3). Alzheimer's disorder and topographical disorientation are examples of cognitive diseases characterized by an inability to access one's cognitive map, misrepresentation of the surrounding environment, or inability to judge an object's position in relation to another (4).

The current conventional drugs for Alzheimer's disease (AD) are mostly cholinergic agonists, particularly acetylcholinesterase (AChE) inhibitors. Unfortunately, their efficacy is low, and they have short half-lives hence requiring higher and more frequent dose administration. They also have serious side effects. Many plants have been utilized for memory improvement and management of dementia-associated ailments. The mechanism actions of phytochemicals in anti-AD plants include possession of antioxidant and other neuroprotective properties, inhibition of cholinesterase, ability to restore mitochondrial energy, and clearance of precipitated proteins (5,6).

In Kenya, *Prunus africana* is used in the management of many diseases including mental disorders around the Mount Kenya region. Nevertheless, these claims are not supported by any scientific literature. Therefore, the current study investigated the spatial memory-enhancing effects of leaf and stem bark MeOH extracts of *P. africana* using the MWM task as well as enzymatic and nonenzymatic antioxidant activities.

Materials and Methods

Plant samples collection and preparation

Leaves and stem bark samples of *P. africana* were randomly collected from Kereita forest situated in Kiambu County, Kenya (Global position system coordinates; 1°0'28.1"S, 36°37'57.7"E). The collected plant materials were shade-dried at room temperature and then pulverized into a fine powder using an electric mill. The plant sample powders were packaged individually in khaki bags that were clean, dry, and well-sealed, and then stored at room temperature until extraction.

Extraction

One litre of MeOH and 500 g of each plant sample powder were mixed separately. The mixture was continuously swirled for 24 hours at room temperature. The resulting broth was filtered using grade 1 Whatman filter paper and the filtrate was concentrated using a rotary evaporator at 40°C under vacuum pressure. The formula described by Leal et al. (7) was used to calculate the extracts' percentage yields:

$$\text{Percentage yield} = \frac{\text{Mass of extract obtained(g)}}{\text{Mass of powder extracted(g)}} \times 100$$

Quantitative phytochemical (LC-MS) analysis

The extracts' phytochemical composition was quantified using the ACQUITY ultra-performance liquid chromatography (UPLC) I-class system (Waters Corporation, Milford, Massachusetts). Each extract's sample weighing 1.0 g was put in 1 mL of MeOH and ddH_2O in the ratio of 90:10, vortexed for 10 seconds, sonicated for 30 minutes, and then centrifuged for 10 minutes at 14 000 revolutions per minute (rpm). The supernatant was aspirated and analysed using an ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) (0.1 µL).

Spatial memory-enhancing effects of MeOH extracts of *Prunus africana*

Experimental design

The current study adopted a completely controlled randomized study design, from which the experimental design was derived. Briefly, for each extract, 30 Swiss albino mice were divided into six groups of five animals each. The groups were treated as summarized in Table 1. The plant extracts, reference drug and vehicle (normal saline) were orally administered followed by intra-peritoneal injection of scopolamine after 30 minutes daily for four consecutive days. The normal control group mice never received scopolamine. The anticholinesterase drug, donepezil (DNP), was used as the reference or standard drug. Scopolamine injection and behavioural testing were separated by a 30-minute break.

Protocol for Morris water maze task

Spatial memory test was conducted following a protocol described by Kim et al (8) with minor modifications. MWM is made up of a circular pool (2 feet in height and 5 feet in diameter) initially with a featureless inner surface. The experiment involved training, pre-trials, and testing sessions.

Table 1. Experimental design (n = 5)

Group	Category	Therapy
I	Normal control	Normal saline
II	Negative control	Normal saline + Scop (5 mg/kg bw)
III	Positive control	DNP (0.5 mg/kg bw) + Scop (5 mg/kg bw)
IV	Experimental group a	25 mg/kg bw extract + Scop (5 mg/kg bw)
V	Experimental group b	50 mg/kg bw extract + Scop (5 mg/kg bw)
VI	Experimental group c	75 mg/kg bw extract + Scop (5 mg/kg bw)

DNP, Donepezil; Scop, Scopolamine.

a. Training

Training of mice was undertaken on 2 consecutive days after acclimatization. Training was done to ensure that all the mice could swim comfortably for at least sixty seconds without drowning.

On each training day, the circular tank was freshly filled with water (up to a height of 30 cm) and each mouse was allowed to swim twice, for 60 seconds, with a one-hour break between the two swimming sessions. Water temperature was maintained at 26 ± 1°C throughout the experiment.

b. Pre-trials

On the third day, mice in various groups were subjected to a session of pre-trial tests to explore their entry behaviour and hence their fitness for inclusion in the study. Four primary directions were marked on the inner wall of the pool as E (East), W (West), S (South) and N (North) and a white square platform (6 cm wide) was positioned in the NE quadrant to act as an escape platform. The platform was submerged 1 centimetre underneath the water surface such that it remained visible from the water surface. A red and a blue sticker were also fixed on N and E marks respectively to serve as colour cues.

Two specific locations of the pool were used as starting points: East (E) and South (S), for pre-trial one and pre-trial two, respectively. A 60-minute break was allowed between the two pre-trials. When a mouse moved to the platform, it was let to rest for ten seconds and then withdrawn from the pool. In case the mouse failed to locate the escape platform within 1 minute, it was carefully directed to the platform using a stick and allowed to relax for ten seconds before it was removed, dried, and returned to the home cage.

c. Testing

The experimental animals were tested for spatial memory development and retention based on differences in scores on each trial. On test days, the escape platform and the colour cues remained in the same positions but 500 g of white powdered milk was added to water to make the platform invisible from the surface. Testing was done on four consecutive days, two trials per day. The starting points were alternated daily and were applied in the order: 1st day, East (E); 2nd day, South (S); 3rd day, West (W), and 4th day, North (N). An inter-trial break of 60 minutes was allowed each day and observations were recorded using a video camera.

Investigation of antioxidant activities of *P. africana* extracts

Following the end of MWM testing, the animals were euthanized under diethyl-ether anaesthesia, and whole brains were harvested. A volume of 0.5 mL of ice-cold sodium phosphate solution with a pH of 7.4 was added to each brain tissue before it was homogenized. Subsequently, 0.1 mL of ice-cold phosphate buffer with a pH of 8.0 was added and centrifuged for ten minutes at 3000 rpm. The supernatants were used to carry out antioxidant assays.

Glutathione peroxidase activity

A protocol described by Sharma et al (9) was used to evaluate for glutathione peroxidase (GPx) activity. The assay mixture was constituted by mixing 0.1 mL of EDTA (1 mM), 1.49 mL of 0.1 M sodium phosphate solution (pH 7.4), 0.1 mL of 1.0 mM Sodium azide, 0.1 mL of 0.02 mM NADPH, 0.01 mL of 1.0 mM H_2O_2, and 0.1 mL brain supernatant to make a total capacity of 2 mL. Blank control was simultaneously prepared, but with no tissue homogenate. NADPH oxidation was determined using a spectrophotometer at 340 nm after every 30 seconds for 3 minutes. GPx activity was computed as millimoles of NADPH oxidized/minute/millilitre of protein, using an extinction coefficient of $0.00373M^{-1}$ (9).

$$\text{Glutathione peroxidase (µmol/min/ml)} = \frac{\Delta A340/\text{min}}{0.00373M^{-1}} \times \text{Sample dilution}$$

Where ΔA340/min is the reaction rate and $0.00373M^{-1}$ is the NADPH extinction coefficient

Glutathione reductase activity

Glutathione reductase (GR) activity was evaluated following a procedure described by Dringen and Gutterer (10) with minor changes. The assay mixture was constituted by mixing 0.1 mL of 1.0 mM EDTA, 0.1 mL of 1.0 mM oxidized glutathione, 0.01 mL of 1.0 mM H_2O_2, 1.6 mL of 0.1 M sodium phosphate solution (pH 7.4), and 0.1 mL brain supernatant to make a final volume of 2 mL. Glutathione reductase activity was recorded using a spectrophotometer at 340 nm every 30 seconds for up to 3 minutes. Enzyme activity was expressed as µmoles of NADPH oxidized/minute/millilitre of protein using an extinction coefficient of $6.22 \times 10^{-3}\,\mu M^{-1}\,cm^{-1}$ (10).

$$\text{Glutathione reductase } \left(\frac{\text{mU}}{\text{mL}}\right) = \frac{\Delta\ A340\ /\ \text{min}}{(6.22 \times 10^{-3}\mu M^{-1}cm^{-1}) \times V} \times DF$$

Where Δ A340/min is the reaction rate and 6.22×10^{-3}; µM-1cm-1 is extinction coefficient; DF= Sample dilution factor; and V = Volume of the sample in millilitres.

Superoxide dismutase activity

The activity of superoxide dismutase (SOD) was determined following a protocol designed by Sun et al (11) with slight modifications. Briefly, the reaction mixture comprised 0.5 mL brain supernatant, 1 mL sodium carbonate (50mM), 0.4 mL of 25 µM NBT, and 0.2 mL of 0.1mM EDTA. The reaction was activated by adding 0.4 mL of 1.0 mM hydroxylamine hydrochloride. Absorbance change was noted at 560 nm every 30 seconds for 3 minutes. Blank control was concurrently run with no

tissue supernatant. IC_{50} was determined and the activity of SOD was computed using the formula described by Sun et al (11);

$$\text{SOD activity}\left(\frac{\text{U}}{\text{ml}}\right) = \frac{A_0 - A_1}{A_0} \div 50\% \times \frac{\text{System volume}}{\text{Sample volume}} \times \text{Dilution factor}$$

Where A_0 = Absorbance without supernatant; A_1 = Absorbance in presence of supernatant; 50%= Enzymatic activity which causes 50% inhibition in the system.

Catalase activity

Catalase (CAT) activity was evaluated according to a procedure described by Hadwan et al (12) with minor changes. For each treatment, each reaction mixture comprised either 100 µL aliquot of the sample or distilled water (blank), 250 µL of 0.1M phosphate solution (pH 7.4), and 200 mL of 1 mM H_2O_2 were placed in three test tubes labelled 1, 2 and 3. Reactions were stopped after 0, 30 and 60 seconds for test tubes 1, 2, and 3, respectively, by the addition of 1 mL of 2mM dichromate-acetic acid mixture (100 mL of 2mM potassium dichromate dissolved in 300 mL of 2mM acetic acid). Subsequently, the content was put in a water bath at 100°C for 10 minutes, cooled and absorbance read at 620 nm. Blank control was simultaneously carried out with no tissue homogenate. Optical density values obtained at each treatment level were plotted against time, and CAT activity was computed using the formula described by Hadwan et al (12):

$$\text{Catalase activity (mmoles/min/ml)} = \frac{\Delta\mu\text{moles}(H_2O_2) \times d \times 100}{V \times t}$$

Where Δ µmoles (H_2O_2) = Difference in H_20_2 amount added to the colorimetric reaction between a sample and blank; d = CAT reaction original sample dilution; t = Time of CAT reaction in minutes; V= sample volume (mL) in CAT reaction; 100 = Aliquot dilution from CAT reaction in colorimetric reaction (15.5 mL from 1 mL)

Total glutathione content

A method described by Rahman et al (13) with some modifications was used to assess total glutathione content. Briefly, an aliquot of 1.0 mL of brain supernatant was added to 1.0 mL of sulphosalicylic acid (4%w/v) then centrifuged for 5 minutes at 1200 rpm, and the supernatant was filtered. Aliquots of 0.1 mL of the supernatant, 0.2 mL of DTNB (40 mg/10 mL of phosphate buffer, 0.1M, pH 7.4), and 2.7 mL of 0.1M phosphate solution (pH 7.4) were mixed to a total volume of 3.0 mL. Blank control was simultaneously run with no tissue homogenate. Optical densities of the resulting solutions were determined at 412 nm at exactly 30 seconds after the addition of DTNB. A standard calibration curve was constructed and glutathione content was computed using the formula described by Rahman et al (13):

$$\text{Glutathione content (µM)} = \frac{\text{Absorbance at 412 nm} - \text{y intercept}}{\text{Slope}} \times 2 \times \text{Sample dilution}$$

Statistical data analysis

Descriptive statistics on escape latencies were carried out using any-maze tracking software, version 6.33, UK, based on the videos captured. The data from antioxidant assays were transferred to a spreadsheet for descriptive statistics. Kurtosis (values between -1.96 to +2.96), skewness (values between -1.96 to +1.96), and Shapiro–Wilk ($P > 0.01$) were used to test for data normality before inferential statistics were carried out. Statistical differences among treatment groups were analyzed using one-way analysis of variance (ANOVA). In case of statistical differences, Tukey's multiple comparisons were computed for pair-wise comparison. Paired t-test was utilized to compare the scores of the first and second trials on each day. The efficacies of the two extracts at different doses were compared using an independent t-test.

Results

Quantitative phytochemical screening of leaf and stem bark MeOH extracts of *Prunus africana*

The stem bark and leaf MeOH extracts of *P. africana* recorded yields of 4.71% and 8.23%, respectively. The liquid chromatography-mass spectrometry (LC-MS) analysis of the two extracts detected various phytochemicals with different retention times and belonging to various classes of phytochemicals. The most abundant phytochemical identified by the LC-MS analysis of the stem bark extract was cyanidin-O-galactoside (20.34%). Chlorogenic acid, quercetin, and quercetin 3,3'-dimethyl ether-4'-glucoside, among others, were also detected with abundances of 18.14%, 12.32%, and 10.26%, respectively (Table 2).

The most abundant phytochemical in the leaf extract according to the LC-MS analysis was quercetin 3,3'-dimethyl ether-4'-glucoside (17.35%). Oleic acid, ursolic acid, quercetin, catechin, and chlorogenic acid, among others, were also identified with abundances of 8.91%, 8.27%, 8.04%, 8.00%, and 7.74%, respectively (Table 3). The retention time, molecular formula, chemical class and concentration of the identified phytochemicals are presented in Table 2 and Table 3.

Effect of leaf and stem bark MeOH extracts of *Prunus africana* on escape latencies in Morris water maze task

Generally, the stem bark extract demonstrated a dose-dependent reduction in escape latencies following the administration of scopolamine in mice (Table 4). Notably, the stem bark extract dose restored escape latencies to normal on the second trial of the 3rd and 4th days (Table

Table 2. LC-MS analysis of stem bark MeOH extract of *Prunus africana*

Rt	Compound name	Molecular formula	Chemical class	Concentration (ng/g)	Abundance (%)
1.44	Catechin	$C_{15}H_{14}O_6$	Flavonoid	47.66	6.23
1.53	Prunasin	$C_{14}H_{17}O_6$	Cyanogenic glucoside	18.91	2.47
1.58	Chlorogenic acid	$C_{16}H_{18}O_9$	Polyphenol	138.74	18.14
1.69	Oleic acid	$C_{18}H_{34}O_2$	Fatty acid	52.87	6.91
3.10	p-Coumaric	$C_9H_8O_3$	Polyphenol	0.57	0.07
4.85	Rutin	$C_{27}H_{30}O_{16}$	Flavonoid	4.30	0.07
5.65	Zeatin	$C_{10}H_{13}N_{5O}$	Cytokinin	5.31	0.69
8.39	Quercetin 3,3'-dimethyl ether-4'-glucoside	$C_{22}H_{22}O_{12}$	Flavonoid	78.51	10.26
8.57	Apigenin 6-C-glucoside	$C_{26}H_{28}O_{14}$	Polyphenol	0.07	0.01
8.79	Cyanidin-O-galactoside	$C_{21}H_{21}ClO_{11}$	Flavonoid	155.63	20.35
8.98	Kaempferol	$C_{15}H_{10}O_6$	Flavonoid	3.36	0.44
9.05	Luteolin	$C_{15}H_{10}O_6$	Flavonoid	5.56	0.73
9.21	Apigenin	$C_{15}H_{10}O_5$	Flavonoid	32.93	4.30
9.60	Quercetin	$C_{15}H_{10}O_7$	Flavonoid	94.23	12.32
10.74	Campesterol	$C_{28}H_{48}O$	Phytosterol	11.84	1.55
10.86	beta-Sitostenone	$C_{29}H_{48}O$	Phytosterol	52.16	6.82
10.95	beta-Sitosterol	$C_{29}H_{50}O$	Phytosterol	19.06	2.49
11.02	Prunetrin	$C_{22}H_{22}O_{10}$	Flavonoid	19.57	2.56
11.09	Ursolic acid	$C_{30}H_{48}O_3$	Triterpenoid	11.93	1.56
11.22	Oleanolic acid	$C_{30}H_{48}O_3$	Triterpenoid	11.67	1.53

RT: Retention time (minutes).

Table 3. LC-MS analysis of leaf MeOH extract of *Prunus africana*

Rt	Compound name	Molecular formula	Chemical class	Conc(ng/g)	% Abundance
1.46	Catechin	C15H14O6	Tannins	70.39	8.00
1.53	Prunasin	C14H17O6	Cyanogenic glucoside	11.51	1.31
1.58	Chlorogenic acid	C16H18O9	Phenolic acid	68.09	7.74
1.61	Oleic acid	C18H34O2	Fatty acid	78.43	8.91
1.70	Octadecanoic acid	CH3(CH2)16COOH	Fatty acid	60.39	6.86
2.79	p-Coumaric	C9H8O3	Polyphenol	2.11	0.24
4.86	Rutin	C27H30O16	Flavonoid	7.02	0.80
5.65	Zeatin	C10H13N5O	Cytokinin	2.74	0.31
8.38	Quercetin 3,3'-dimethyl ether-4'-glucoside	C22H22O12	Flavonoid	152.74	17.35
8.61	Apigenin 6-C-glucoside	C26H28O14	Polyphenol	9.43	1.07
8.80	Cyanidin-O-galactoside	C21H21ClO11	Flavonoid	21.63	2.46
8.97	kaempferol	C15H10O6	Flavonoid	18.01	2.05
9.04	Luteolin	C15H10O6	Flavonoid	47.94	5.45
9.23	Apigenin	C15H10O5	Flavonoid	67.39	7.66
9.61	Quercetin	C15H10O7	Flavonoid	70.79	8.04
10.76	Campesterol	C28H48O	Phytosterol	10.35	1.18
10.93	beta-Sitosterol	C29H50O	Phytosterol	46.44	5.28
11.03	Prunetrin	C22H22O10	Flavonoid	61.96	7.04
11.08	Ursolic acid	C30H48O3	Triterpenoid	72.79	8.27

RT: Retention time (minutes).

Table 4. Effect of stem bark MeOH extract of *Prunus africana* on escape latencies in mice treated with scopolamine in the first and second trials using Morris water maze

Treatment	Escape latency (s)							
	Day 1		Day 2		Day 3		Day 4	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Normal saline	5.54 ± 0.18^{d}	4.16 ± 0.09^{d}	4.80 ± 0.07^{e}	3.80 ± 0.03^{e}	3.90 ± 0.09^{e}	3.14 ± 0.07^{e}	3.10 ± 0.09^{d}	2.44 ± 0.03^{d}
SA + Normal saline	27.64 ± 0.82^{a}	26.84 ± 0.63^{a}	26.86 ± 0.61^{a}	27.34 ± 0.48^{a}	25.86 ± 0.58^{a}	26.00 ± 0.70^{a}	26.28 ± 0.73^{a}	25.02 ± 0.63^{a}
SA + DNP	9.38 ± 0.25^{c}	6.34 ± 0.17^{c}	7.80 ± 0.16^{d}	6.50 ± 0.15^{d}	5.70 ± 0.16^{d}	4.90 ± 0.10^{cd}	4.98 ± 0.14^{c}	4.10 ± 0.11^{d}
SA + 25 mg/kg E	14.04 ± 0.20^{b}	10.22 ± 0.20^{b}	13.54 ± 0.12^{b}	10.22 ± 0.20^{b}	11.38 ± 0.25^{b}	8.78 ± 0.15^{b}	8.24 ± 0.12^{b}	6.60 ± 0.15^{b}
SA + 50 mg/kg E	12.34 ± 0.25^{b}	9.04 ± 0.15^{b}	11.06 ± 0.23^{c}	8.58 ± 0.18^{c}	9.14 ± 0.15^{c}	6.52 ± 0.013^{c}	5.80 ± 0.09^{c}	4.58 ± 0.12^{c}
SA + 75 mg/kg E	9.84 ± 0.33^{c}	6.88 ± 0.20^{c}	7.72 ± 0.16^{d}	6.12 ± 0.12^{d}	5.80 ± 0.10^{d}	4.48 ± 0.07^{de}	4.82 ± 0.10^{c}	3.82 ± 0.09^{cd}

Values are presented as mean ± standard error of the mean (n=5). Values with distinct superscript letters column-wise differ significantly using one-way ANOVA and Tukey's multiple comparisons ($P < 0.01$). DNP, Donepezil; SA, Scopolamine; E, Extract.

4). On the 1st day (both trials), stem bark extract doses of 25 and 50 mg/kg were equally ($P > 0.01$) effective (Table 4). On all the other days (both trials), the effect of a stem-bark extract dose of 50 mg/kg was considerably higher compared with the effect of the extract dose of 25 mg/kg ($P < 0.01$, Table 4). On trial 2 of the third day, 50 and 75 mg/kg bw doses were equally effective as the reference drug but significantly more effective than the lowest extract dose (Table 4). However, the 50 and 75 mg/kg bw extract doses had similar efficacies on both trials of the 4th day.

For both trials on the 1st and 2nd days, the stem bark extract doses of 25 and 50 mg/kg bw were statistically less efficacious ($P < 0.01$) than the extract dose of 75 mg/kg bw. Nonetheless, the effect of the extract dosage of 75 mg/kg bw statistically matched ($P > 0.01$) that of donepezil (Table 4). Also, this was the case on trial 1 on the 3rd day.

Throughout the four days of the current research, the stem bark extract and the reference drug reduced escape latencies significantly more in the 2nd trials than in the corresponding 1st trials (Supplementary file 1, Figures S1a-d). However, escape latencies in negative control mice were not different significantly between the two trials.

The leaf extract of *P. africana* also demonstrated a dose-dependent reduction in escape latencies in experimental mice (Table 5). Notably, unlike the stem bark extract, none of the leaf extract doses restored escape latencies to normal (Table 5). On the 1st day (trial 1), 2nd day (trial 2), and 3rd day (trial 1), the leaf extract doses of 25 and 50 mg/kg bw were equally effective ($P > 0.01$; Table 5). In all the other trials, the effect of the leaf extract dose of 50 mg/kg bw was significantly higher than the effect of 25 mg/kg bw (Table 5). In both trials, on the 4th day, the effect of a leaf extract dose of 75 mg/kg bw was statistically non-significant compared with the effect of donepezil. Moreover, the 50 and 75 mg/kg bw extract doses had similar efficacies ($P > 0.01$) in both trials on the 1st and 4th days (Table 5).

For both trials on the 2nd and 3rd days, the leaf extract dosages of 25 and 50 mg/kg bw were considerably less efficacious ($P < 0.01$) than the extract dose of 75 mg/kg bw. On both trials, on the 4th day, the efficacy of the highest extract dosage of 75 mg/kg bw was equal to ($P > 0.01$) the effect of donepezil (Table 5).

Throughout the four days of this study, the effects of the reference drug and leaf extract on escape latencies in the 2nd trial were substantially higher than in the 1st trial. However, escape latencies in negative control mice did not change significantly between the two trials

Table 5. Effects of leaf MeOH extract of *Prunus africana* on escape latencies in mice treated with scopolamine in the first and second trials using Morris water maze

Treatment	Time (s)							
	Day 1		Day 2		Day 3		Day 4	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Normal saline	5.54 ± 0.18^{e}	4.16 ± 0.09^{e}	4.80 ± 0.07^{f}	3.80 ± 0.03^{e}	3.90 ± 0.09^{e}	3.14 ± 0.07^{f}	3.10 ± 0.09^{e}	2.44 ± 0.03^{d}
SA + Normal saline	27.64 ± 0.82^{a}	26.84 ± 0.63^{a}	26.86 ± 0.61^{a}	27.34 ± 0.48^{a}	25.86 ± 0.58^{a}	26.00 ± 0.70^{a}	26.28 ± 0.73^{a}	25.02 ± 0.63^{a}
SA + DNP	9.38 ± 0.25^{d}	6.34 ± 0.17^{d}	7.80 ± 0.16^{e}	6.50 ± 0.15^{d}	5.70 ± 0.16^{d}	4.90 ± 0.10^{e}	4.98 ± 0.14^{d}	4.10 ± 0.11^{c}
SA + 25 mg/kg E	16.20 ± 0.15^{b}	12.98 ± 0.21^{b}	15.92 ± 0.24^{b}	11.64 ± 0.13^{b}	12.46 ± 0.12^{b}	10.78 ± 0.10^{b}	9.50 ± 0.18^{b}	7.96 ± 0.11^{b}
SA + 50 mg/kg E	14.54 ± 0.21^{bc}	11.10 ± 0.12^{c}	12.72 ± 0.16^{c}	10.40 ± 0.18^{b}	11.48 ± 0.14^{b}	9.08 ± 0.16^{c}	7.60 ± 0.13^{c}	5.40 ± 0.13^{c}
SA + 75 mg/kg E	13.04 ± 0.15^{c}	10.22 ± 0.11^{c}	10.30 ± 0.20^{d}	8.40 ± 0.17^{c}	9.28 ± 0.20^{c}	6.90 ± 0.12^{d}	6.70 ± 0.10^{cd}	4.74 ± 0.12^{c}

Values are presented as mean ± standard error of the mean (n=5). Values with distinct superscript letters column-wise differ significantly using one-way ANOVA and Tukey's multiple comparisons ($P < 0.01$). DNP, Donepezil; SA, Scopolamine; E, Extract.

(Supplementary file 1, Figures S2a-d).

In all the conducted trials, the latency to locate the escape platform by mice administered with stem bark extract was considerably lower ($P<0.01$) relative to those of mice treated with leaf extract at all dosages tested (Figure 1a-c).

Antioxidant activity of methanolic *Prunus africana* extracts

This study established that the three studied dosages of stem bark extract of *P. africana* increased GPx activity in a dose-dependent trend. Mice that received the reference drug and the stem bark extract at various doses showed significantly higher GPx activities compared with negative control mice. The stem bark extract dosage of 50 mg/kg bw and the reference drug restored enzyme activity to normal levels. The stem bark extract dosage of 75 mg/kg bw was the most potent and its effect was non-significant ($P>0.01$) compared to the effect of donepezil (Table 6).

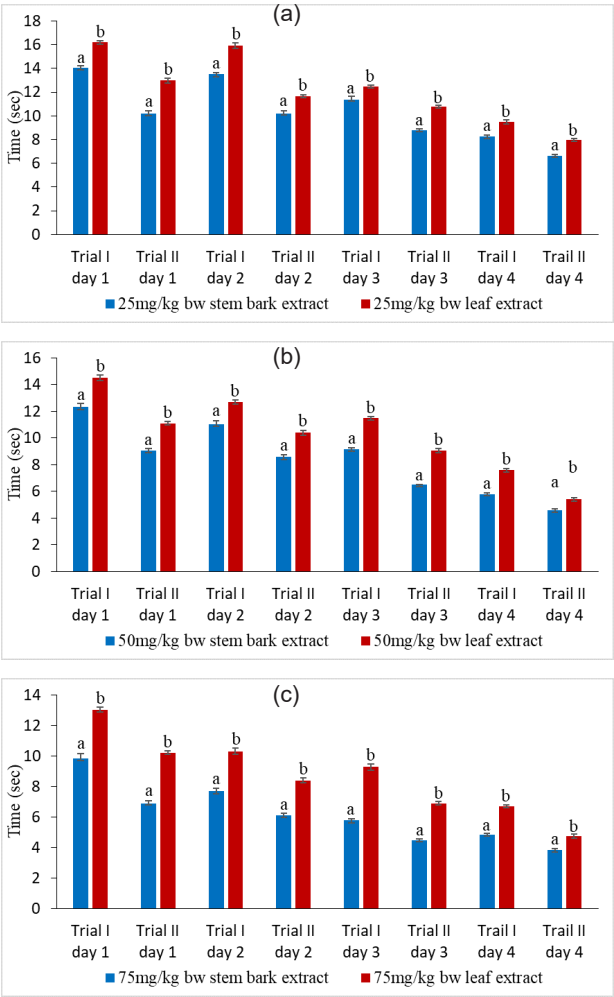

Figure 1. Comparison of the effects of leaf and stem bark MeOH extracts of *Prunus africana* on escape latencies at (a) 25 mg/kg bw, (b) 50 mg/kg bw and (c) 75 mg/kg bw doses. Bars with different lowercase letters within the same trial and day differ significantly using an independent *t* test ($P<0.01$).

Also, leaf extract increased GPx activity in a dose-dependent pattern. The enzyme activities in mice that were given the leaf extract or the reference drug were significantly greater relative to those of mice from the negative control group. The 75 mg/kg bw leaf extract dose restored GPx activity to normal. The effect of leaf extract at 50 mg/kg bw was statistically similar to those extract dosages of 75 and 25 mg/kg bw ($P>0.01$; Table 7).

P. africana stem bark extract significantly increased ($P<0.01$) GPx activity better than leaf extract dosages of 50 and 75 mg/kg bw. However, their antioxidant effects statistically matched ($P>0.01$) at the dosage of 25 mg/kg bw (Figure 2). Moreover, the three studied doses of stem bark extract increased glutathione reductase activity in a dose-dependent trend. Mice that received the reference drug and stem bark at various doses were found to have significantly higher glutathione reductase activity relative to the negative control mice. The effect of stem bark extract at the concentration of 25 mg/kg bw was statistically similar to that of 50 mg/kg bw dose and donepezil. Further, the effect of stem bark extract at 50 mg/kg bw was statistically alike ($P>0.01$) relative to the effect at 75 mg/kg bw extract dosage and the standard drug (Table 6).

The study also established that the three *P. africana* leaf extract doses increased glutathione reductase activity dose-dependently. Glutathione reductase activities in mice that received leaf extract at various doses or the reference drug were significantly greater relative to the negative control mice. The effect of leaf extract at the concentration of 25 mg/kg bw was statistically similar to that of 50 mg/kg bw dosage and the standard drug. Furthermore, the effect of leaf extract at 50 mg/kg bw was comparable to the effect of the highest extract dose, 75 mg/kg bw, and the standard drug. The two lower leaf extract concentrations (25 and 50 mg/kg bw) and reference drug restored enzyme activity to normal ($P>0.01$; Table 7).

On comparing the two extracts, *P. africana* stem bark extract was considerably better ($P<0.01$) than the leaf extract in raising the activity of glutathione reductase at the higher concentrations of 50 mg/kg bw and 75 mg/kg bw. Nevertheless, the antioxidant effects of the two extracts were not significantly different at the lowest dosage ($P>0.01$) of 25 mg/kg bw (Figure 3).

Results from the current study noted a substantial rise in SOD activity in animals that were given various doses of stem bark extract and the increase revealed a dose-dependent pattern. Mice from the negative control group showed significantly lower SOD activities compared with those that were given DNP or the different stem bark extracts doses. The effect of stem bark extract at the dosage of 50 mg/kg bw was comparable to that of donepezil. In addition, the stem bark extract dosage of 75 mg/kg bw and the reference drug restored enzyme activity to normal

Glutathione peroxidase activity

Table 6. Effect of stem bark extract of *Prunus africana* on antioxidant activities in mice treated with scopolamine

Treatment	Antioxidant activities				
	GPx (mmol/min/m)	GR (mU/ml)	SOD (U/ml)	CAT (mmoles/min/mL)	GHS (µM)
Normal saline	30.89 ± 0.36^{b}	12.65 ± 0.62^{c}	45.17 ± 0.23^{ab}	2.13 ± 0.01^{ab}	73.93 ± 0.77^{b}
SA + Normal saline	6.76 ± 0.44^{d}	4.91 ± 0.35^{d}	22.58 ± 0.34^{e}	1.30 ± 0.05^{d}	43.46 ± 0.76^{e}
SA + DNP	35.89 ± 1.07^{a}	14.24 ± 0.35^{bc}	44.57 ± 0.34^{bc}	1.95 ± 0.02^{bc}	78.75 ± 0.76^{a}
SA + 25mg/kg E	25.85 ± 0.52^{c}	14.73 ± 0.37^{b}	41.35 ± 0.20^{d}	1.89 ± 0.03^{c}	63.06 ± 0.68^{d}
SA + 50mg/kg E	31.53 ± 0.46^{b}	15.97 ± 0.37^{ab}	43.63 ± 0.11^{c}	2.02 ± 0.04^{abc}	69.34 ± 0.57^{c}
SA + 75mg/kg E	35.82 ± 0.36^{a}	17.49 ± 0.37^{a}	45.91 ± 0.13^{a}	2.15 ± 0.04^{a}	81.44 ± 0.72^{a}

Values are presented as mean ± standard error of the mean (n = 5). Values with distinct superscript letters column-wise differ significantly using one-way ANOVA and Tukey's multiple comparisons ($P < 0.01$). DNP, Donepezil; SA, Scopolamine; E, Extract; GPx, Glutathione peroxidase; GR, Glutathione reductase; GHS, Glutathione content; SOD, Superoxide dismutase; CAT, Catalase.

Table 7. Effect of leaf extract of *Prunus africana* on antioxidant activities in mice treated with scopolamine

Treatment	Antioxidant activities				
	GPx (mmol/min/m)	GR (mU/mL)	SOD (U/mL)	CAT (mmoles/min/mL)	GHS (µM)
Normal saline	30.89 ± 0.36^{b}	12.65 ± 0.62^{b}	45.17 ± 0.23^{a}	2.13 ± 0.01^{a}	73.93 ± 0.77^{b}
SA + Normal saline	6.76 ± 0.44^{d}	4.91 ± 0.35^{c}	22.58 ± 0.34^{d}	1.30 ± 0.05^{d}	43.46 ± 0.76^{e}
SA + DNP	35.89 ± 1.07^{a}	14.24 ± 0.35^{ab}	44.57 ± 0.34^{a}	1.95 ± 0.02^{bc}	78.75 ± 0.76^{a}
SA + 25mg/kg E	24.77 ± 0.31^{c}	12.65 ± 0.62^{b}	40.86 ± 0.13^{c}	1.85 ± 0.02^{c}	57.80 ± 0.65^{d}
SA + 50mg/kg E	27.88 ± 0.56^{bc}	13.48 ± 0.33^{ab}	43.11 ± 0.16^{b}	1.91 ± 0.02^{bc}	66.43 ± 0.52^{c}
SA + 75mg/kg E	30.67 ± 0.46^{b}	15.49 ± 0.34^{a}	45.43 ± 0.19^{a}	2.03 ± 0.03^{ab}	79.31 ± 0.29^{a}

Values are presented as mean ± standard error of the mean (n = 5). Values with distinct superscript letters column-wise differ significantly using one-way ANOVA and Tukey's multiple comparisons ($P < 0.01$). DNP, Donepezil; SA, Scopolamine; E, Extract; GPx, Glutathione peroxidase; GR, Glutathione reductase; GHS, Glutathione content; SOD, Superoxide dismutase; CAT, Catalase.

($P > 0.01$; Table 6).

Similarly, the leaf MeOH extract at the three studied doses increased the activity of SOD dose-dependently. Notably, mice treated with leaf extract at the tested dosages and the reference drug showed significantly higher SOD activities relative to those of mice from the negative control group. The mice that received the leaf extract dosage of 75 mg/kg bw and the reference drug had their SOD activity restored to normal ($P > 0.01$; Table 7). Notably, the two extracts at the three levels tested noted comparable SOD activity ($P > 0.01$) (Figure 4).

The stem bark extract at the three doses raised CAT activity in a dose-dependent trend. The mice administered with various doses of stem bark extract and DNP showed significantly higher CAT activities in contrast to the negative control mice. Furthermore, the stem bark extract dosage of 25 mg/kg bw caused statistically similar CAT activity relative to that of 50 mg/kg bw and donepezil. Conspicuously, at 75 mg/kg bw, the stem bark extract and the reference drug restored enzyme activity to normal

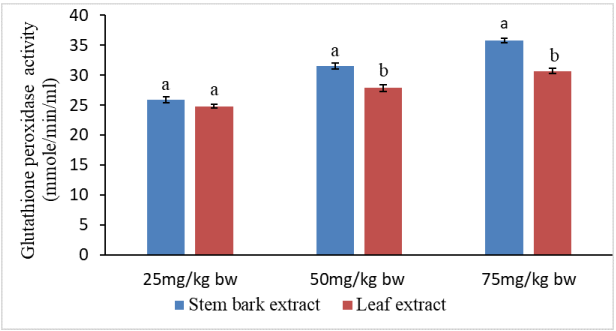

Figure 2. Comparison of the effects of leaf and stem bark MeOH extracts of *Prunus africana* on glutathione peroxidase activity. Bars with different lowercase letters at the same dosage differ significantly using an independent t-test ($P < 0.01$).

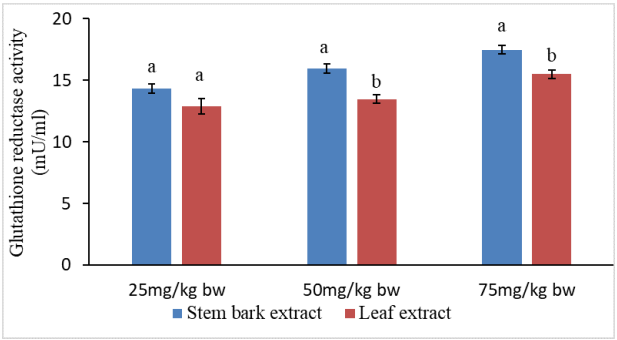

Figure 3. Comparison of the effects of leaf and stem bark MeOH extracts of *Prunus africana* on glutathione reductase activity. Bars with different lowercase letters at the same dosage differ significantly using an independent t-test ($P < 0.01$).

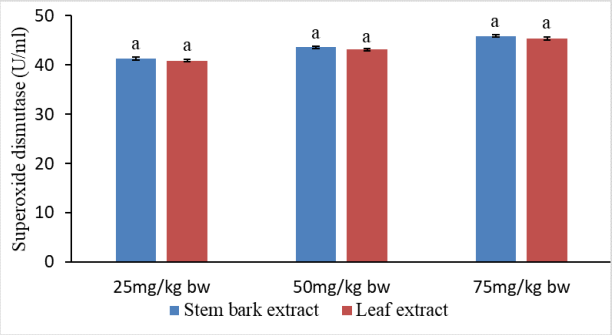

Figure 4. Comparison of the effects of leaf and stem bark MeOH extracts of *Prunus africana* on superoxide dismutase activity. Bars with different lowercase letters at the same dosage differ significantly using an independent t-test ($P < 0.01$).

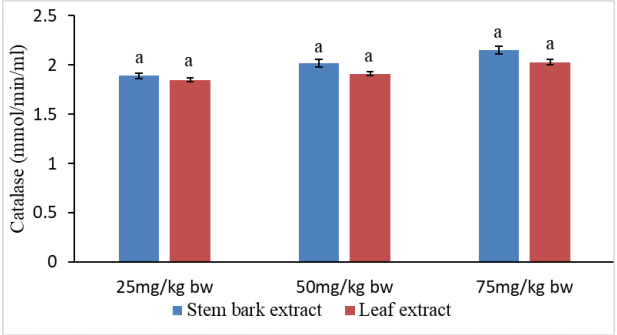

Figure 5. Comparison of the effects of leaf and stem bark MeOH extracts of *Prunus africana* on catalase activity. Bars with different lowercase letters at the same dosage differ significantly using an independent t-test ($P < 0.01$).

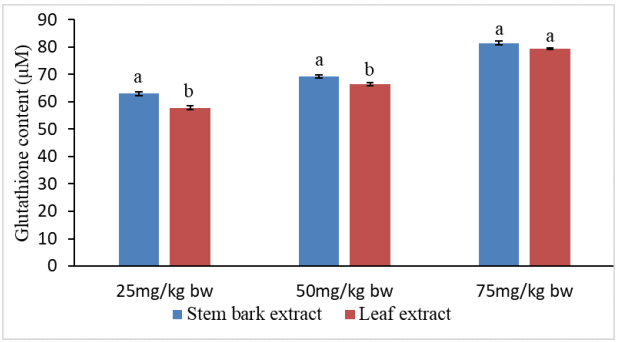

Figure 6. Comparison of the effects of leaf and stem bark MeOH extracts of *Prunus africana* on total glutathione content. Bars with different lowercase letters at the same dosage differ significantly using an independent t-test ($P < 0.01$).

($P > 0.01$; Table 6). Similarly, the leaf extract at the three doses increased CAT activity in a dose-dependent pattern. The three leaf extract concentrations were as effective as the reference drug statistically. The findings of the study indicated that the mice administered with leaf extract at various doses and the reference drug had significantly higher CAT activity compared with the negative control mice. Furthermore, the effects of 25 and 50 mg/kg bw doses of leaf extract were statistically similar and those of 50 and 75 mg/kg bw were also similar. Only the leaf extract dose of 75 mg/kg bw restored CAT activity to normal ranges ($P > 0.01$; Table 7). Importantly, the comparison of efficacies of stem bark and leaf MeOH extracts noted no significant variation ($P > 0.01$) between the two at the three levels (Figure 5).

Findings from this study established that glutathione content significantly increased in animals given various concentrations of stem bark extract than those of mice in the negative control group. The increase was in a dose-dependent pattern. Furthermore, the effect of the stem bark extract dose of 75 mg/kg bw on glutathione content was statistically similar to that of the standard drug ($P > 0.01$; Table 6). Also, leaf extract at the three tested doses increased glutathione content in a dose-dependent trend. The leaf extract at various doses and donepezil caused a substantial increase in glutathione content relative to the negative control group. Markedly, the activity of leaf extract dose of 75 mg/kg bw on glutathione content statistically matched that of donepezil ($P > 0.01$; Table 7).

In comparison, at the two doses of 25 and 50 mg/kg bw, the effect of the stem bark MeOH extract on glutathione content was significantly greater than that of leaf extract. However, at the dosage of 75 mg/kg bw, the two extracts had statistically similar ($P > 0.01$) effects on glutathione content (Figure 6).

Discussion

Several previous studies have indicated that oxidative stress influences pathogenesis in neurodegenerative disorders like AD in a major way. Thus, one of the strategies for managing the progression of neurodegenerative disorders is by use of free radical scavengers such as herbal antioxidant agents (14).

LC-MS results in the current study indicated the presence of several flavonoids, including quercetin, cyanidin 3-O-beta-D-galactoside, apigenin, catechins, luteolin, prunetrin, quercetin-3,3'-dimethylether-4-glucoside, rutin, and kaempferol. Flavonoids and their secondary metabolites play an important role in the alleviation of oxidative stress in age-related cognitive deficits including AD. Antioxidant activity therefore might be responsible for the enhanced cognitive function displayed by mice that received the extracts in MWM.

Flavonoids act through several other mechanisms, including the activation of signalling pathways necessary for regulating synaptic plasticity, minimizing neuro-inflammation, and stimulating vascular effects capable of initiating the growth of new nerve cells or enhanced recovery of damaged neurons in the central nervous system (15). These properties of flavonoids are crucial in the recovery process of neurons after scopolamine treatment.

According to Chan et al (16), quercetin, a flavonoid, increases the antitumor effects of trichostatin A by

upregulating the expression of p53 protein *in vitro* and *in vivo*. Also, it is reported to influence many essential pathways involved in memory acquisition, cognition, and long-term potentiation, which are compromised during neurodegenerative diseases or other mental disorders (16).

Another flavonoid, apigenin, has been demonstrated to possess several valuable neuroprotective effects through its antioxidant, chemoprevention, and anti-inflammatory properties (17). Apigenin is also known to reduce the expression of tumour necrosis factor-*alpha* (TNF-α) and interleukin-1 beta (IL-1β) in lipopolysaccharides (LPS)-treated human monocytes and mouse macrophages (18).

Also, apigenin mediates anti-inflammatory effects by inhibiting pro-oxidant enzymes, including inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in LPS-treated mouse macrophages (18). Soib et al (19) reported that apigenin suppresses neuro-inflammation by inactivating nuclear factor-kappa B (NF-κB) and attenuating cellular-induced inflammation by reducing lymphocyte and neutrophil adhesion to endothelial cells. Additionally, apigenin blocks allergen-stimulated airway inflammation. These findings attest that apigenin-initiated anti-inflammatory activities may be linked with many intracellular signalling pathways like extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase (MAPK), NF-κB, and c-jun N-terminal protein kinase (JNK) (20).

According to Muhammad et al (21), triterpenoids and flavonoids from fruits of *Alphitonia neocaledonica* possess anti-tyrosinase and antioxidant activities. Anti-tyrosinase activity is important since hydroxyl groups of tyrosine residues provide an ideal phosphorylation site for various kinases involved in APP metabolism and neurotransmission processes.

The current study revealed the presence of a triterpenoid, ursolic acid (UA). UA has been demonstrated to appreciably decrease free radical levels in cultured rat neuronal preparation. Minor to severe damage to the nervous system typically occurs due to oxidative harm and excitotoxicity (22). UA has been observed to block excitotoxicity and oxidative damage, demonstrating its neuroprotective potential. Additionally, UA reduces apoptotic signalling and mediates anti-inflammatory activities in the brain.

The LC-MS analysis further detected the presence of a phytosterol, campesterol, which was more abundant in stem bark extract compared with leaf extract. According to Gül et al (23), campesterol possesses strong antioxidant and anti-inflammatory effects, which hasten neuronal regeneration possibly by alleviating oxidative stress. Campesterol reduces the effects of AChE, TNF and corticosterone in living organisms and increases antioxidant activity, which helps in the protection of neurons (23). It has been identified as a potential candidate for developing therapeutic agents to treat AD-related oxidative damage. The drastic recovery from scopolamine incursion during the current study can be attributed to such phytocompounds.

In all the trials conducted, mice administered with leaf or stem bark extracts of *P. africana* exhibited a substantial reduction in time spent before finding the escape platform relative to negative control animals. The escape latencies initially increased by scopolamine treatment were significantly reduced by *P. africana* extracts in a dose-dependent trend during each of the four days of testing. This shows that the extracts contained some phytocompounds with spatial memory-enhancing properties.

Since the inter-trial duration was set at one hour, a significant reduction in escape latencies between trials indicates an improvement in short-term or working memory. These results suggest that *P. africana* extracts attenuate short-term memory impairment induced by scopolamine. This observation can be explained in terms of the presence of various antioxidants revealed in the extracts coupled to anticholinesterase activity among other mechanisms.

According to Jeong et al (24), mice treated with UA for 84 days improved physical endurance in a concentration-dependent pattern as revealed by increased exercise duration, perseverance, and speed of swimming. Some possible mechanism actions of UA include increased strength of skeletal muscles and reduced generation of fatigue-associated parameters like lactic acid, alanine and aspartate aminotransferases, alkaline phosphatase and creatinine (24).

Bang et al (25) demonstrated that ursolic acid could promote exercise endurance in men by elevating irisin levels, maximal muscle strength, and insulin-like growth factor 1 (IGF-1). According to Gabay et al (26), UA coupled to campesterol may act through physiological and structural cellular modifications that are indirectly or directly associated with psychomotor skills involved in swimming leading to reduced escape latencies.

Glutathione (GSH) is among the prime endogenous antioxidants in the brain. Other antioxidants, including GPx, GR, SOD and CAT mostly depend on GSH reserves for optimum activity. Glutathione acts by scavenging reactive oxygen radicals where it becomes reversibly oxidized to glutathione disulfate. Results from this study demonstrated a substantial increase in activities of GPx, GR, SOD, CAT and glutathione content in mice that were administered with the two extracts relative to those noted in negative control mice. The observed rise in the activity of antioxidant enzymes suggests that the ameliorating activities of *P. africana* extracts on memory deficits triggered by scopolamine may be associated with the alleviation of oxidative stress levels via enzymatic and nonenzymatic antioxidant activities. According to Lu et al (27), UA increased glutathione/oxidized glutathione ratio, and enzymatic activity of antioxidants like SOD and CAT

in subarachnoid hemorrhage rat models. According to Menard et al (28), such antioxidants are strong inhibitors of lipid peroxidation and have neuroprotective properties via the maintenance of proper neural membrane integrity and neutralization of the neurotoxic effects of β-amyloid.

The two extracts did not have equal efficacies at the various dose levels tested. Stem bark extract exhibited significantly higher efficacy relative to the leaf extract in terms of reduction of time duration at the three studied dosages in the entire experiment. Differences in efficacy can be attributed to differences in phytochemical profiles in terms of proportions and diversity among other factors. The enhanced potency of stem bark extract may have been caused by the phytocompounds, which were concentrated more in stem bark extract relative to the leaf extract. Such phytocompounds include prunasin, zeatin, quercetin, chlorogenic acid, campesterol, and cyanidin-O-galactoside. But it is also possible that lower concentrations of some phytochemicals can also result in higher efficacy since the effectiveness of a medicine is not always directly related to the quantities administered. In some cases, phytochemicals are required in very small quantities for optimum results to be realized as in the case of coenzymes, vitamins, hormones, and micronutrients. Moreover, only stem bark extract contained oleanolic acid and β-sitostenone. Beta-sitostenone is a phytosterol with antioxidant activity and is used for the treatment of androgen-dependent diseases including benign prostatic hyperplasia.

On the other hand, oleanolic acid, a naturally existing pentacyclic triterpenoid, is known to have anti-malarial, antiretroviral, and anti-inflammatory activities. Recently, oleanolic acid has been established to possess anticancer effects through topoisomerase inhibition. Notably, octadecanoic acid (stearic acid), which is a fatty acid, was only detected in the leaf extract.

The current study also revealed the presence of cyanidin-3-O-galactoside which is a flavonoid. It has been demonstrated to up-regulate the expression of ERK in the hippocampus in senescence-accelerated rodents leading to increased spatial memory in mice (15). It is a major component of stem bark extract and was relatively scantly distributed in the leaf extract. This could be one of the phytocompounds that contributed to the disparity in terms of efficacies between the two extracts.

Conclusion

This study concluded that the leaf and stem bark MeOH extracts of *P. africana* had phytocompounds associated with spatial memory-enhancing effects and antioxidant activities in mice treated with scopolamine. Therefore, the null hypotheses formulated in this study were rejected and the study objectives were successfully accomplished.

Authors' contribution

DN, MPN, and CK conceived the research idea. DN performed the experiments, analyzed the data, and drafted the manuscript. MPN and CK supervised the entire project. All authors reviewed the draft manuscript and approved the final version for publication.

Conflict of interests

The authors declare that there is no conflict of interest.

Data availability

All data is included in this manuscript; however, additional information may be obtained from the authors upon request.

Ethical considerations

Authorization for this study was obtained from National Commission for Science, Technology and Innovation (License No: NACOSTI/P/19/150). Ethical issues including plagiarism, double publication, and data fabrication have been completely observed by the authors.

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Supplementary files

Supplementary file 1 contains Figures S1 and S2.

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