

A Comparative *In Vitro* Study on the Antioxidant and Anti-acetylcholinesterase Properties of Aerial Parts of *Strophanthus preussii* Engl & Pax

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ABSTRACT

Objective: To evaluate and compare the antioxidant and acetylcholinesterase (AChE)-inhibitory properties of aerial parts of *Strophanthus preussii* (leaves, stem and root named as SPL, SPS and SPR, respectively) while catechin served as standard.

Methods: The antioxidant and AChE-inhibitory properties of the methanol extracts of *Strophanthus preussii* were evaluated by standard in vitro methods viz DPPH (2,2-diphenyl-1-picrylhydrazine), nitric oxide (NO), hydroxyl radical (OH[•]) and hydrogen peroxide (H₂O₂) radical scavenging assays as well as reducing power, iron (II) [Fe²⁺]/ascorbate-induced lipid peroxidation (LPO) and AChE inhibition assays. The total phenolic and flavonoid contents of the extracts were also estimated.

Results: High phenolic and flavonoid contents were found in the aerial parts of *Strophanthus preussii*. The amount of phenolic and flavonoid contents followed the order SPL > SPR > SPS at 250–1000 µg/ml. The results revealed that all the extracts showed antioxidant activities in vitro. However, SPL had the highest DPPH, H₂O₂ and OH radical scavenging abilities while the reducing power of the extracts followed the order SPR > SPL > SPS at 1000 µg/ml. In addition, SPL, SPS and SPR significantly inhibited LPO in rat liver by 42%, 23%, 35% and in rat brain by 68%, 31% and 51%, respectively. The LPO inhibitory activities of SPL were statistically similar to the standard. Only SPS produced significant NO scavenging effects among the extracts. The percentage inhibition of AChE activity was significant for SPL and SPR at 750 and 1000 µg/ml.

Conclusion: The leaves and root of *Strophanthus preussii* proved to be potent natural antioxidants and could justify their traditional use in the management of stress-related diseases.

Keywords: Acetylcholinesterase, antioxidant, DPPH, lipid peroxidation, *Strophanthus preussii*

Estudio Comparativo *In Vitro* de las Propiedades Antioxidantes y Anti-Acetilcolinesterásicas de las Partes Aéreas de *Strophanthus Preussii* Engl & Pax

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RESUMEN

Objetivo: Evaluar y comparar las propiedades antioxidantes e inhibitorias de la acetilcolinesterasa (AChE) de las partes aéreas de *Strophanthus preussii* (hojas, tallos y raíces, conocidas por sus siglas en inglés como SPL, SPS y SPR, respectivamente), sirviendo la catequina a modo de estándar.

Métodos: Las propiedades antioxidantes e inhibitorias AChE de los extractos de metanol de *Strophanthus preussii* fueron evaluadas mediante métodos in vitro estándar frente a ensayos de captación de radicales de DPPH (2, 2-difenil-1-picrilhidracil), monóxido de nitrógeno (NO), radical hidroxilo (OH[•]), y peróxido de hidrógeno (H₂O₂), así como ensayos de poder reductor, peroxidación lipídica (POL) inducida por hierro (II) [Fe²⁺]/ascorbato, e inhibición AChE. También se calcularon los contenidos fenólicos y flavonoides totales de los extractos.

Resultados: Se hallaron altos contenido fenólicos y flavonoides en las partes aéreas de *Strophanthus preussii*. La cantidad de contenidos fenólicos y flavonoides siguió el orden SPL > SPR > SPS a 250 – 1000 µg/ml. Los resultados revelaron que todos los extractos mostraron actividades antioxidantes in vitro. Sin embargo, SPL tuvo la capacidad más alta de captación de radicales DPPH, (H₂O₂) y OH[•],

mientras que el poder reductor de los extractos siguió el orden $SPR > SPL > SPS$ a 1000 $\mu\text{g/ml}$. Además, SPL, SPS y SPR inhibieron significativamente la POL en hígados de ratas en 42%, 23%, 35% y en cerebros de ratas en 68%, 31% y 51%, respectivamente. Las actividades inhibitorias POL de SPL fueron estadísticamente similares a la norma. Sólo SPS produjo efectos significativos de captación NO entre los extractos. El porcentaje de inhibición de la actividad de AChE fue significativa para SPL y SPR a 750 y 1000 $\mu\text{g/ml}$.

Conclusión: Las hojas y raíz de *Strophanthus preusii* mostraron ser potentes antioxidantes naturales, y podrían justificar su uso tradicional en el tratamiento de enfermedades relacionadas con el estrés.

Palabras claves: Acetilcolinesterasa, antioxidante, DPPH, peroxidación lipídica, *Strophanthus preusii*

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INTRODUCTION

Free radicals are important in the regulation of signal transduction, gene expression and activation of receptors (1). Common free radicals in biological systems include: reactive oxygen species (ROS) such as superoxide, hydroxyl, hydroperoxyl, peroxy *etc* while reactive nitrogen species (RNS) consist of nitric oxide (NO), peroxyxynitrite *etc*. It is known that excess free radicals is toxic to biological systems (2) and can cause oxidative damage to functional macromolecules such as DNA, proteins and lipids (3). Excess generation of free radicals can lead to cancer, atherosclerosis, neurodegenerative diseases and inflammation (4). Antioxidants from plants can minimize the generation of free radicals (5) and alleviate diseases caused by oxidative stress (6). Medicinal plants are known to contain active constituents that elicit antioxidant properties. Plants such as *Polygonum hyrcanicum*, *Prunella vulgaris* and *Pittosporum viridiflorum* possess interesting biological activities against inflammation, cardiovascular disorder and can mitigate biochemical processes involved in age-related neurodegenerative disorders such as Alzheimer's disease. It is believed that these beneficial effects are, at least in part, due to antioxidant and radical scavenging properties of the plant (7, 8).

Strophanthus preusii Engl & Pax belongs to the Apocynaceae family. The plant is native mainly to Africa with few species in Asia. In ethnomedicine, concoctions from the leaves have been used to cure skin ulcerations, reduce fever and treat gonorrhoea and other sexually transmitted infections. Phytochemical screening revealed the presence of lignans, cytotoxic alkaloids and cardenolide glycosides as major components of the plant (9). There is a dearth of scientific reports on the biological properties of this plant. Therefore, this study was designed to evaluate and compare the antioxidant and acetylcholinesterase-inhibitory activities of the leaves, stem and root methanol extracts of *Strophanthus preusii* in an *in vitro* model.

MATERIALS AND METHODS

Collection of plants: Leaves, stems and roots of the *Strophanthus preusii* (SPL, SPS and SPR, respectively) were harvested from Ikare-Akoko, Ondo State in Nigeria, during the beginning of the rainy season. They were authenticated

at the Department of Botany, University of Ibadan and the voucher specimens were deposited at the herbarium (UI-00164, UI-00165 and UI-00166). Plant materials were washed separately with clean tap water to remove dirty materials and were air dried in the laboratory for several weeks. The dried materials were ground into coarse powder. **Extraction of plants:** About 1 kg of each powdered plant materials was soaked with 2.5 litres of methanol for 96 hours with occasional stirring for cold extraction. The extracts were filtered through muslin cloth and evaporated to dryness at 40 °C with rotary evaporator. The dried extracts were stored in an air-tight container inside a refrigerator until use. **Chemicals and reagents:** 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-deoxy-D-ribose, Folin-Ciocalteu reagent, catechin, 2-thiobarbituric acid (TBA), ethylenediaminetetraacetic acid (EDTA), trichloroacetic acid (TCA), ascorbic acid, ferrous ammonium sulphate, hydrochloric acid, sodium nitroprusside, sulfanilamide and naphthylenediamine dihydrochloride were purchased from Sigma Chemical Company, (Saint Louis, MO, USA). Phosphoric acid, sodium hydroxide (NaOH), aluminium chloride (AlCl_3), sodium nitrite (NaNO_2) and potassium ferricyanide were procured from British Drug Houses (BDH) Chemical Ltd (Poole, UK). Other chemicals were of analytical grade and purest quality available.

Biochemical analysis

Total phenolic content: The total phenolic content of the extracts was determined using the method of Singleton and Rossi (10) with slight modifications. Briefly, 1 mL of Folin-Ciocalteu reagent was added to 1 mL of sample. After three minutes, 1 mL of 15% sodium carbonate (Na_2CO_3) was added and the solution was made up to 5 mL with distilled water. The reaction mixture was placed in a water bath at 40 °C for 20 minutes. The absorbance was measured with a spectrophotometer at 760 nm while catechin was used as standard.

Total flavonoid content: The total flavonoid content was determined with a colorimetric method described by Jia *et al* (11) with some modifications. Briefly, between 10 and 1000 μg of the extract in 1 mL of distilled water was added to 75 μL of 5% NaNO_2 . After five minutes, 150 μL of 10%

aluminium chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) was added, followed by 500 μL of 1 M NaOH and 275 μL of distilled water. The solution was properly mixed and the colour intensity of the mixture read at 510 nm after 15 minutes while catechin served as standard.

Reducing power assay: This was determined according to the method of Oyaizu (12). The extract or standard (100 $\mu\text{g}/\text{mL}$) was mixed with phosphate buffer (pH 6.6) and potassium ferricyanide. The mixture was incubated at 50 °C for 20 minutes and trichloroacetic acid (10%, 2.5 mL) was added to the mixture. A portion of the resulting mixture was mixed with ferric chloride [FeCl_3] (0.1%, 0.5 mL) and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated reductive potential of the extract.

DPPH radical scavenging assay: The DPPH radical scavenging activity of plant extracts was measured as described by Mensor *et al* (13). A portion (1 mL) each of the different concentrations (40–2000 $\mu\text{g}/\text{mL}$) of the extracts or standard in test tubes was added to 1 mL of 1 mM DPPH in methanol. The mixtures were vortexed and incubated in a dark chamber for 30 minutes, after which the absorbance was measured at 517 nm against a DPPH control containing only 1 mL of methanol in place of the extract.

The inhibition of DPPH was calculated as a percentage using the expression:

$$\% I = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where % I is the percentage inhibition of the DPPH radical, A_{control} is the absorbance of the control and A_{sample} is the absorbance of the test compound.

Hydroxyl radical scavenging assay: The hydroxyl radical scavenging activity of extracts was carried out by the methods described by Halliwell (14). The assay was performed by adding 0.1 mL of EDTA, 0.01 mL of FeCl_3 , 0.1 mL of hydrogen peroxide (H_2O_2), 0.36 mL of deoxyribose, 1.0 mL of plant extract (10–1000 $\mu\text{g}/\text{mL}$), 0.33 mL of phosphate buffer (50 mM, pH 7.4) and 0.1 mL of ascorbic acid in sequence. The mixture was then incubated at 37 °C for one hour. About 1.0 mL of 10% TCA and 1.0 mL of 0.5% TBA were added to develop the pink chromogen which was measured at 532 nm in a spectrophotometer.

Hydroxyl radical scavenging activity (%) =

$$\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where A_{control} is the absorbance of control and A_{sample} is the absorbance of sample solution.

Scavenging of hydrogen peroxide: The ability of the extracts to scavenge hydrogen peroxide was determined according to the methods of Nabavi *et al* (15). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was

determined by absorption at 230 nm using a spectrophotometer. Extracts (0.1–1 mg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by the extracts and standard was calculated as follows:

$$\% \text{ Scavenged } [\text{H}_2\text{O}_2] = \frac{A_o - A_1}{A_o} \times 100$$

Where A_o is the absorbance of the control and A_1 is the absorbance in the presence of the extracts or standard.

Nitric oxide radical scavenging assay: The scavenging effect of the extract on NO radical was measured according to the method of Ebrahimzadeh *et al* (16). Briefly, 1 mL of sodium nitroprusside (5 mM) in phosphate buffered saline (PBS) was mixed with different concentrations of extracts and distilled water. This was incubated at room temperature for 150 minutes after which 0.5 mL of Griess reagent was added. The absorbance of the pink chromophore formed was read at 546 nm in a spectrophotometer. Catechin was used as positive control.

The percentage inhibition was calculated as:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Lipid peroxidation inhibition assay: The lipid peroxidation inhibition assay was determined according to the method described by Liu *et al* (17). The reaction mixtures contained 0.2 mL of rat liver or brain homogenate in varying concentration of 30 mM tris-buffer, 0.38 mL of 0.16 mM ferrous ammonium sulphate, 0.06 mL ascorbic acid and different concentration of the extracts (10–1000 μg) and were incubated for one hour at 37 °C. The resulting thiobarbituric acid reacting substance (TBARS) formed was measured as followed: briefly, an aliquot (0.4 mL) of the reaction mixture was mixed with 1.6 mL of 0.15 M Tris-KCl buffer and 0.5 mL of 30% TCA (to stop the reaction) and placed in a water bath for 45 minutes at 80 °C. After which it was cooled in ice and centrifuged at room temperature for 15 minutes at 3000 rpm to remove precipitates. The absorbance of the clear pink coloured supernatant was measured against blank at 532 nm.

Acetylcholinesterase-inhibitory assay: Acetylcholinesterase (AChE)-inhibitory activity of the extracts was determined using the method of Ellman *et al* (18). Aliquots of homogenates from rat brain were taken and used to measure AChE activity, a marker for cholinergic neurotransmission. Briefly, the AChE activity in the homogenate was measured by adding 2.6 mL of phosphate buffer (0.1M, pH 7.4), 0.1 mL of 5,5'-dithio-bis(2-nitrobenzoic acid) [DTNB] and 0.4 mL of the homogenate. Then 0.1 mL of acetylthiocholine iodide solution was added to the reaction

mixture. The absorbance was read using a spectrophotometer at 412 nm and the changes in absorbance for 10 minutes at two-minute intervals were recorded.

Statistical analysis: Experimental results were expressed as mean \pm standard deviation (SD). All measurements were replicated four times. The results were analysed using one-way analysis of variance (ANOVA). The level of significance used was $p < 0.05$.

RESULTS

Total phenolic and flavonoid contents of *Strophanthus preusii*

The levels of phenolic and flavonoid contents in SPL, SPS and SPR are given in Figs. 1 and 2. There were dose-dependent increases in the levels of flavonoids and total phenolics as the concentration of the three extracts increased.

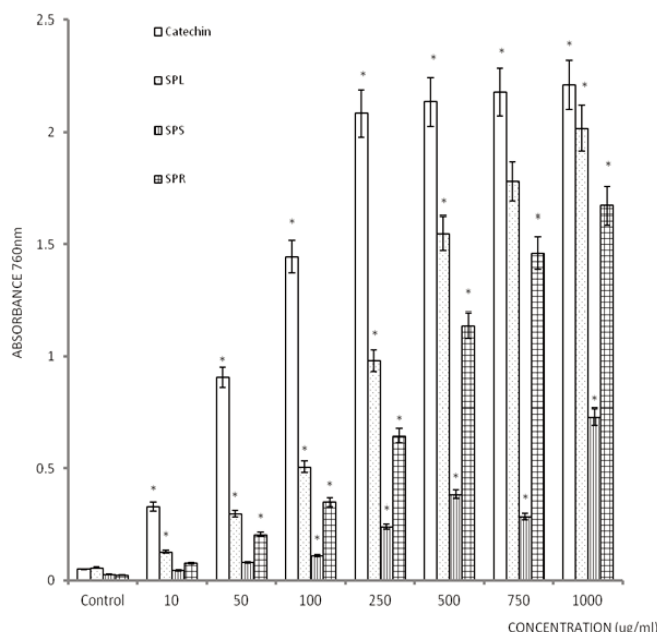


Fig. 1: Total phenolic content in the leaves, stem and root extracts of *Strophanthus preusii*. *Dose-dependent and significant increase from control ($p < 0.05$)

Importantly, the phenolic and flavonoid contents followed the order SPL > SPR > SPS from 10–1000 $\mu\text{g/mL}$. At 1000 $\mu\text{g/mL}$, the total phenolic content of SPL was statistically similar to the catechin (Fig. 1).

Reducing power, DPPH and nitric oxide scavenging effects of extracts of *Strophanthus preusii*

Table 1 shows the DPPH radical scavenging activity of SPL, SPS and SPR. At a concentration of 500 $\mu\text{g/mL}$, the scavenging activity of SPL, SPS and SPR were 86.7%, 13.4% and 69.8%, respectively, while catechin was 40.8%. Thus, SPL and SPR exhibited significant DPPH radical scavenging activity than catechin. The DPPH radical sca-

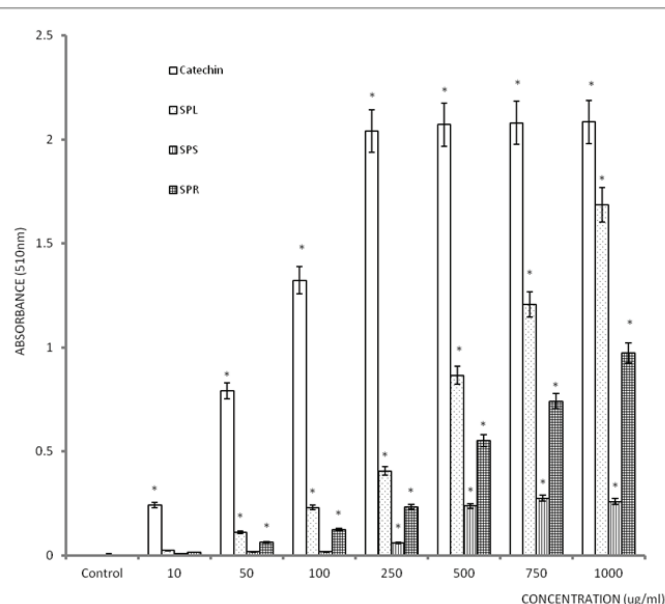


Fig. 2: Total flavonoid content in the leaves, stem and root extracts of *Strophanthus preusii*. *Dose-dependent and significant increase from control ($p < 0.05$)

Table 1: The scavenging activities of *Strophanthus preusii* leaves, stem and root extracts on DPPH radical *in vitro*

Concentration ($\mu\text{g/mL}$)	DPPH scavenging activity (%)			
	Catechin	SPL	SPS	SPR
Control	0.000	0.000	0.000	0.000
10	19.2 \pm 1.07*	11.5 \pm 0.12*	2.65 \pm 0.35*	10.2 \pm 1.03*
50	32.9 \pm 2.31*	13.1 \pm 0.04*	3.21 \pm 0.52*	18.0 \pm 1.52*
100	39.9 \pm 1.21*	20.8 \pm 0.94*	2.42 \pm 0.21*	29.4 \pm 1.31*
250	31.1 \pm 3.48*	50.7 \pm 2.57*	8.03 \pm 1.16*	41.1 \pm 1.41*
500	40.8 \pm 3.22*	86.7 \pm 4.40*	13.4 \pm 2.43*	69.8 \pm 3.35*
750	52.3 \pm 1.17*	88.3 \pm 3.86*	41.1 \pm 2.32*	66.7 \pm 5.58*
1000	88.3 \pm 3.08*	88.3 \pm 2.11*	42.4 \pm 1.80*	76.8 \pm 3.30*

Data are expressed as mean \pm SD (n = 4). *Significantly different from control ($p < 0.05$)

DPPH = 2,2-diphenyl-1-picrylhydrazine, SPL = *Strophanthus preusii* leaves, SPS = *Strophanthus preusii* stem, SPR = *Strophanthus preusii* root

vening activity of extracts of *Strophanthus preusii* and catechin were in the following order: SPL = catechin > SPR > SPS at 1000 $\mu\text{g/mL}$.

The ferrous reducing power of SPL, SPS and SPR are shown in Fig. 3. At 500 $\mu\text{g/mL}$, the absorbance of SPL, SPS and SPR and catechin were 0.854, 0.633, 1.902 and 2.209, respectively. A higher absorbance indicates a higher reducing power, hence SPL, SPS and SPR showed higher reducing activity at higher concentrations (750 and 1000 $\mu\text{g/mL}$). The ferrous reducing capacities of SPR (2.722 and 2.914) were significantly higher ($p < 0.05$) than catechin (2.215 and 2.222) at 750 and 1000 $\mu\text{g/mL}$, respectively.

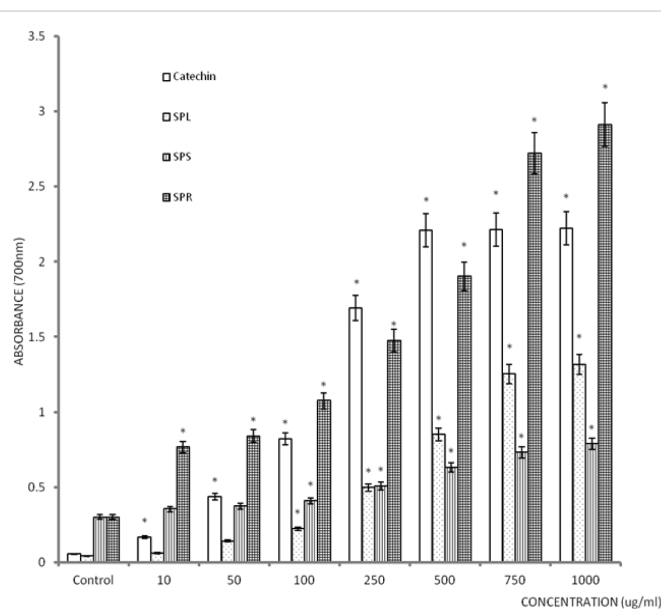


Fig. 3: The reducing power of the leaves, stem and root extracts of *Strophanthus preusii*.

*Dose-dependent and significant increase from control ($p < 0.05$)

The NO scavenging activities of extracts of *Strophanthus preusii* are shown in Fig. 4. Of all the extracts, only SPS elicited dose-dependent and significant scavenging of NO *in vitro*. The NO scavenging effect of SPS was better than catechin from 250–1000 µg/mL.

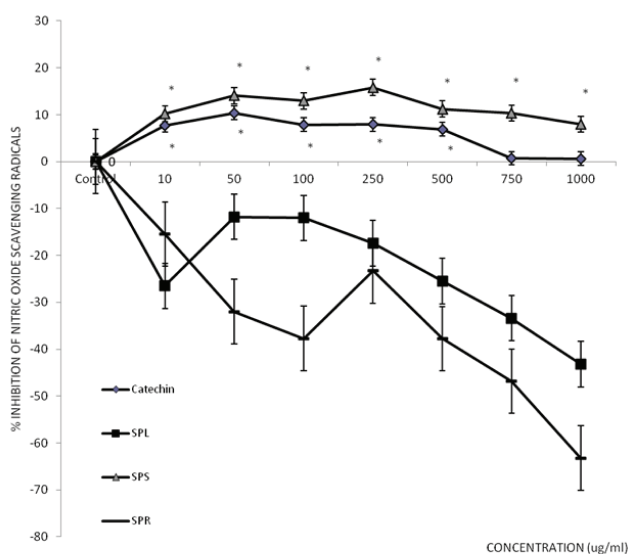


Fig. 4: The nitric oxide scavenging effect of the leaves, stem and root extracts of *Strophanthus preusii*.

*Significantly different from control ($p < 0.05$)

Inhibition of lipid peroxidation, hydroxyl radical and H_2O_2 scavenging effects of extracts of *Strophanthus preusii*

The H_2O_2 scavenging activity of SPL, SPS and SPR is given in Table 2. *Strophanthus preusii* leaves, stem and root were

Table 2: The scavenging activities of *Strophanthus preusii* leaves, stem and root extracts on hydrogen peroxide radical *in vitro*

Concentration (µg/mL)	Percentage inhibition (%)			
	Catechin	SPL	SPS	SPR
Control	0.000	0.000	0.000	0.000
10	13.1 ± 1.22*	1.90 ± 0.53*	10.2 ± 1.41*	18.5 ± 2.91*
50	26.9 ± 2.16*	6.12 ± 0.41*	28.0 ± 3.22*	27.3 ± 2.78*
100	54.5 ± 1.58*	24.8 ± 1.94*	34.8 ± 1.26*	35.2 ± 3.82*
250	71.5 ± 3.28*	56.6 ± 1.45*	42.2 ± 1.60*	41.5 ± 2.01*
500	78.7 ± 3.99*	74.9 ± 5.25*	68.2 ± 2.80*	68.9 ± 3.47*
750	88.2 ± 2.83*	73.9 ± 3.28*	66.7 ± 3.19*	69.7 ± 2.15*
1000	82.4 ± 3.20*	78.1 ± 3.61*	64.3 ± 2.29*	72.1 ± 3.02*

Data are expressed as mean ± SD (n = 4); *Significantly different from control ($p < 0.05$)

SPL = *Strophanthus preusii* leaves, SPS = *Strophanthus preusii* stem, SPR = *Strophanthus preusii* root

found to be efficient scavengers of H_2O_2 radical from 250–1000 µg/mL. At 500 µg/mL, H_2O_2 scavenging activities of SPL, SPS and SPR were 74.9%, 68.2% and 68.9, respectively while catechin was 78.7%. The H_2O_2 radical scavenging capacity of all extracts was very high and comparable to the standard.

The lipid peroxides scavenging activities of SPL, SPS and SPR in rat liver and brain homogenates are given in Tables 3 and 4. At a concentration of 1000 µg/mL, the

Table 3: The scavenging activities of *Strophanthus preusii* leaves, stem and root extracts on iron/ascorbate-induced lipid peroxidation in rat liver homogenate *in vitro*

Concentration (µg/mL)	Percentage inhibition of lipid peroxidation (%)			
	Catechin	SPL	SPS	SPR
Control	0.000	0.000	0.000	0.000
10	14.4 ± 1.36*	11.8 ± 1.09*	0.2 ± 0.08	13.2 ± 1.68*
50	17.6 ± 1.81*	19.6 ± 1.72*	1.75 ± 0.11*	19.8 ± 1.40*
100	20.2 ± 2.95*	18.3 ± 0.94*	2.03 ± 0.20*	14.9 ± 1.05*
250	24.8 ± 2.57*	26.5 ± 1.51*	9.40 ± 1.91*	19.7 ± 1.03*
500	31.9 ± 3.73*	42.8 ± 2.32*	16.3 ± 1.63*	23.8 ± 1.79*
750	37.2 ± 2.91*	48.9 ± 1.79*	18.0 ± 2.57*	32.9 ± 2.02*
1000	39.5 ± 1.92*	41.7 ± 2.76*	23.8 ± 2.51*	34.9 ± 1.56*

Data are expressed as mean ± SD (n = 4); *Significantly different from control ($p < 0.05$)

SPL = *Strophanthus preusii* leaves, SPS = *Strophanthus preusii* stem, SPR = *Strophanthus preusii* root

scavenging activities of SPL, SPS and SPR in rat liver were 41.7%, 23.8% and 34.9%, while in the rat brain, they were 67.8%, 30.5%, and 50.5%, respectively. The lipid peroxides scavenging activities of SPL and SPR in rat liver and brain were statistically similar to catechin.

Table 4: The scavenging activities of *Strophanthus preusii* leaves, stem and root extracts on iron/ascorbate-induced lipid peroxidation in rat brain homogenate *in vitro*

Concentration ($\mu\text{g/mL}$)	Percentage inhibition of lipid peroxidation (%)			
	Catechin	SPL	SPS	SPR
Control	0.000	0.000	0.000	0.000
10	6.57 \pm 0.41*	12.7 \pm 0.42*	13.1 \pm 1.41*	6.40 \pm 0.22*
50	16.5 \pm 1.46*	14.9 \pm 0.66*	11.1 \pm 0.32*	5.30 \pm 0.38*
100	28.4 \pm 1.23*	52.2 \pm 2.43*	17.1 \pm 1.76*	26.2 \pm 1.16*
250	54.4 \pm 2.50*	54.2 \pm 1.24*	11.3 \pm 0.34*	21.0 \pm 1.21*
500	59.4 \pm 1.59*	61.8 \pm 1.73*	24.2 \pm 0.64*	24.0 \pm 2.08*
750	60.1 \pm 1.48*	66.3 \pm 3.72*	30.9 \pm 2.73*	43.2 \pm 1.18*
1000	63.9 \pm 2.80*	67.8 \pm 3.34*	30.5 \pm 2.27*	50.5 \pm 2.27*

Data are expressed as mean \pm SD (n = 4); *Significantly different from control ($p < 0.05$)

SPL = *Strophanthus preusii* leaves, SPS = *Strophanthus preusii* stem, SPR = *Strophanthus preusii* root

Table 5 shows the OH scavenging activity of SPL, SPS and SPR. At concentration of 500 $\mu\text{g/mL}$, the scavenging activities of SPL, SPS and SPR were 85.3%, 86.8% and

Table 5: The scavenging effects of *Strophanthus preusii* leaves, stem and root extracts on hydroxyl radical *in vitro*

Concentration ($\mu\text{g/mL}$)	Percentage inhibition of hydroxyl radical (%)			
	Catechin	SPL	SPS	SPR
Control	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
10	46.1 \pm 3.22*	56.2 \pm 1.27*	59.7 \pm 1.67*	22.9 \pm 1.11*
50	55.1 \pm 1.74*	73.5 \pm 2.37*	69.8 \pm 1.66*	39.9 \pm 2.79*
100	61.9 \pm 0.41*	78.6 \pm 2.03*	70.1 \pm 2.11*	44.4 \pm 3.19*
250	70.4 \pm 1.59*	82.1 \pm 2.69*	83.6 \pm 1.46*	57.1 \pm 1.40*
500	76.6 \pm 1.19*	85.3 \pm 1.06*	86.8 \pm 1.25*	52.8 \pm 1.02*
750	81.4 \pm 2.02*	85.7 \pm 2.32*	84.7 \pm 2.19*	45.4 \pm 2.52*
1000	85.1 \pm 2.05*	87.4 \pm 2.54*	83.8 \pm 2.71*	54.6 \pm 1.83*

Data are expressed as mean \pm SD (n = 4); *Significantly different from control ($p < 0.05$)

SPL = *Strophanthus preusii* leaves, SPS = *Strophanthus preusii* stem, SPR = *Strophanthus preusii* root

52.8%, respectively, while catechin was 76.6%. The OH scavenging activity of the extracts followed the order SPL > SPS > SPR at 1000 $\mu\text{g/mL}$.

Anti-acetylcholinesterase effects of extracts of *Strophanthus preusii*

The ability of extracts of *Strophanthus preusii* to inhibit brain AChE activity is given in Table 6. The extract SPS did not produce significant ($p > 0.05$) effect on AChE activity *in vitro* from 10–1000 $\mu\text{g/mL}$. However, at 1000 $\mu\text{g/mL}$, SPL, SPR and catechin significantly ($p < 0.05$) inhibited brain AChE activities by 62.5%, 53.1% and 43.8%, respectively, relative to control values.

Table 6: The effect of *Strophanthus preusii* leaves, stem and root extracts on the activities of brain acetylcholinesterase (AChE) *in vitro*

Concentration ($\mu\text{g/mL}$)	AChE activities (nmol/mg protein)			
	Catechin	SPL	SPS	SPR
Control	0.32 \pm 0.05	0.32 \pm 0.05	0.32 \pm 0.05	0.32 \pm 0.05
10	0.35 \pm 0.12	0.37 \pm 0.15	0.41 \pm 0.11	0.28 \pm 0.07
50	0.30 \pm 0.10	0.30 \pm 0.06	0.36 \pm 0.09	0.35 \pm 0.10
100	0.29 \pm 0.12	0.28 \pm 0.17	0.38 \pm 0.04	0.41 \pm 0.13
250	0.27 \pm 0.09	0.29 \pm 0.10	0.42 \pm 0.25	0.29 \pm 0.10
500	0.28 \pm 0.14	0.26 \pm 0.09	0.40 \pm 0.22	0.27 \pm 0.15
750	0.20 \pm 0.05*	0.16 \pm 0.02*	0.35 \pm 0.10	0.19 \pm 0.03*
1000	0.18 \pm 0.03*	0.12 \pm 0.03*	0.29 \pm 0.08	0.15 \pm 0.05*

Data are expressed as mean \pm SD (n = 4); *Significantly different from control ($p < 0.05$)

SPL = *Strophanthus preusii* leaves, SPS = *Strophanthus preusii* stem, SPR = *Strophanthus preusii* root

DISCUSSION

Polyphenols are the most abundant antioxidants in the plant kingdom. The antioxidant activity of polyphenolic compounds is believed to be mainly due to their redox properties (19), which play an important role in neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Flavonoids are the most ubiquitous group of plant secondary metabolites and have good antioxidant potential. Flavonoids have been shown to possess antimutagenic and antimalignant effect (20). Furthermore, flavonoids have a chemopreventive role in cancer and neurodegenerative disorders through their effect on signal transduction in cell proliferation and angiogenesis (21). Results obtained in the present study revealed that the levels of phenolic compounds and flavonoids in SPL and SPR were very high. Our findings strongly suggest that the phenolics are important components of *Strophanthus preusii* and some of its pharmacological effects could be attributed to the presence of these valuable constituents.

In this study, the *in vitro* radical scavenging capability of *Strophanthus preusii* also revealed its strong antioxidant potential. Results showed that the DPPH scavenging activities of SPL, SPS and SPR were concentration dependent. DPPH, a purple coloured bleaching solution, is an important source of free radical and is frequently used to measure the electron donating ability of antioxidants (22). The extent of colour change is proportional to the strength and concentration of the antioxidant. In this study, SPL and SPR exhibited a strong DPPH radical quenching activity that is positively correlated with their high phenolic and flavonoid contents. Hence, SPL and SPR may act as health promoting agents through an antioxidative mechanism since they successfully neutralize the DPPH radical by donating hydrogen in an *in vitro* model. The mutagenic capacity of free radicals is due to the direct interactions of hydroxyl radicals with DNA and therefore plays an important role in cancer formation and neurodegenerative disorders (23).

Hydroxyl radicals can be generated by biochemical reaction in which superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which can subsequently produce extremely reactive hydroxyl radicals in the presence of divalent metal ions, such as iron and copper. Therefore, removal of the hydroxyl radical is possibly one of the most effective defences of a living body against various diseases. The results obtained in this study demonstrate that extracts of *Strophanthus preusii* (SPL and SPS) had appreciable hydroxyl radical scavenging activity when compared with standard antioxidant (catechin) and could serve as an anti-cancer agent by inhibiting the interaction of hydroxyl radical with DNA. The ability of these extracts to quench hydroxyl radicals might directly be related to the prevention of lipid peroxidation. Hydrogen peroxide is a reactive oxygen radical and, at high concentration, may become toxic and damage cells when it is converted into hydroxyl radical which can initiate lipid peroxidation and DNA mutations (24). The result from this study confirms the potency of extracts from *Strophanthus preusii* (SPL, SPS and SPR) to effectively scavenge H_2O_2 radical *in vitro*. The H_2O_2 radical scavenging activities of SPL and SPS were very high and statistically similar to catechin.

Lipid peroxidation involves the formation and propagation of lipid radicals with numerous deleterious effects, such as destruction of membrane lipids, metabolic disorders and inflammation. Production of malondialdehyde (MDA) is the end product of LPO. This process is initiated by hydroxyl and superoxide radicals, leading to the formation of peroxy radicals that ultimately propagate chain reactions in lipids. Thus, antioxidants which are capable of scavenging hydroxyl, superoxide or peroxy radicals could prevent LPO. Results from this study showed that extracts from aerial parts of *Strophanthus preusii* inhibit LPO in rat liver and brain homogenates. Importantly, SPL and SPR protected against LPO induced by iron (II) $[Fe^{2+}]$, considerably reduced MDA content in a concentration-dependent manner in rat liver and brain. *Strophanthus preusii* leaves had the greatest inhibiting activity (67.8% in rat liver homogenate and 41.7% in rat brain homogenate) when compared to the activity of standard (catechin; 63.9% in rat liver homogenate and 39.5% in rat brain homogenate).

Nitric oxide is an important intracellular and inter-cellular regulator of multiple biological functions, including macrophage-mediated cytotoxicity, neurotransmission and smooth muscle relaxation (25). Initially, NO was thought to have only beneficial effects, but it is now known that over-production of NO is closely associated with different pathological diseases, such as chronic inflammation, autoimmune diseases and cancer (26). The NO radicals play an important role in inducing inflammatory response and their toxicity multiplies when they react with superoxide radicals to form peroxynitrite which damages the biomolecules (27). In this study, measurement of NO radical scavenging activity was

based on the principle that sodium nitroprusside in an aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated using a Griess reagent. Scavengers of NO act against oxygen, leading to reduced production of nitrite ions which can be monitored at 546 nm. The results obtained show that of the three extracts of *Strophanthus preusii* tested, only SPS effectively scavenged NO radical *in vitro*; SPS showed dose-dependent NO scavenging activities which were higher than catechin in all the tested concentrations.

In reducing power assay, the presence of reductants (antioxidants) would result in the reduction of iron (III) $[Fe^{3+}]$ to Fe^{2+} by donating an electron which serves as a significant reflection of antioxidant activity (28). The amount of Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability (29). All the three extracts of *Strophanthus preusii* showed substantial electron donating capacity and reduced Fe^{3+} to Fe^{2+} . The reducing ability of the extracts and catechin was in the order SPR > catechin > SPL > SPS from 500–1000 $\mu g/mL$. In some neurological disorders such as Alzheimer's disease, there is progressive memory loss which may lead to dementia. The main treatment strategy is to maintain adequate levels of acetylcholine at neurotransmission sites (30). Thus, the inhibition of AChE, an enzyme that breaks down acetylcholine, prevents the hydrolysis of acetylcholine, thereby maintaining normal memory function. In the present study, SPL and SPR inhibited AChE by 63% and 53%, respectively *in vitro*. Both extracts (SPL and SPR) could be considered as strong inhibitors of AChE.

CONCLUSIONS

The extracts of *Strophanthus preusii* present both antioxidant and anti-acetylcholinesterase activities which may render them potentially useful as an adjuvant in the treatment of cognitive diseases such as Alzheimer's disease. The activities reported in the present work show that these extracts (SPL and SPR) may contribute to increase the levels of acetylcholine in cholinergic neurons. Our results clearly show that SPL had the strongest antioxidant and anti-acetylcholinesterase activities among the three extracts. The potent antioxidant activities of the extracts of *Strophanthus preusii* may be due to their high phenolic and flavonoid contents.

AUTHORS' NOTE

The authors declare that they have no competing interests.

Authors' contributions: OAA was involved in the conception and design, interpretation of data, drafting and revising of the manuscript while AO made significant contribution to acquisition of data and analysis. Both authors read and approved the final manuscript.

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