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RESEARCH ARTICLE

Anticholinesterase and Antioxidant Activities of *Spilanthes filicaulis* Whole Plant Extracts for the Management of Alzheimer's Disease

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Abstract: ***Background***: *Spilanthes filicaulis* is a tropical herb implicated as a memory enhancer in ethnomedicine.

Objective: The study investigated acetyl/butyryl cholinesterase inhibitory and antioxidant activities of different extracts of *S. filicaulis* whole plant and correlated them to its phytochemical constituents.

Methods: The powdered whole plant was successively extracted with *n*-hexane, ethyl acetate and methanol. Acetyl cholinesterase (AChE) and Butyryl cholinesterase (BuChE) inhibitory activity were evaluated by Ellman colorimetry assay. Antioxidant activity was tested using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging, ferric reducing power and nitric oxide scavenging assays. Total phenolic, flavonoid and tannin were estimated using standard methods. Correlation was determined using Quest Graph™ Regression Calculator.

Results: Various extracts exhibited concentration-dependent AChE and BuChE inhibitory activity with ethyl acetate extract being the highest with IC_{50} of 0.77 µg/mL and 0.92 µg/mL for AChE and BuChE respectively. The ethyl acetate extract also showed the highest reducing power when compared with the other extracts. The methanol extract had slightly higher phenolic and flavonoid content and showed the highest DPPH radical scavenging effect. DPPH scavenging, AChE and BuChE inhibition had high correlation with the total flavonoid content with R^2 values of 1.00, 0.800 and 0.992 respectively while nitric oxide scavenging had high correlation with phenolics and tannins with R^2 = 0.942 and 0.806 respectively.

Conclusion: These results show that the extracts of the whole plant of *S. filicaulis* possess significant AChE/BuChE inhibitory and antioxidant properties, mostly due to its flavonoid content, suggesting the possible use of the plant in neurodegenerative diseases such as AD.

Keywords: Alzheimer's disease, anticholinesterase, antioxidant, *Spilanthes filicaulis*, total flavonoids, total phenolic.

1. INTRODUCTION

Alzheimer's disease (AD), sometimes considered an accelerated form of normal aging [1] is a progressive neurodegenerative disorder that has been associated with loss of memory, deterioration of intellectual capacity and spatial disorientation [2] as well as different causative mechanisms [3]. Several pathological and diagnostic changes have been observed in the postmortem brains of AD patients. These changes which include oxidative damage [4], neuronal loss [5], neurotransmitter deficit [6], inflammatory responses [7], and peptide accumulation [8] have been targets for drug development efforts.

Specifically, the pathogenesis of AD is linked to deficiency of the neurotransmitter acetylcholine in the brain, based on the observed correlation of irregularities in the cholinergic system with intellectual and memory impairment [9]. However, in addition to the cholinergic hypothesis, other factors such as oxidative stress and inflammation have been considered and investigated in the etiology of the disease. Thus, cholinesterase inhibitors especially acetylcholinesterase (AChEIs) are being used in the symptomatic relief of symptoms associated with AD [10]. However, Butyrylcholinesterase is also considered an important target in the management of the disease [11]. Current researches have also shown that the mechanism of action of AChEIs is not limited to their effects on the neuron to neuron transmission involving acetylcholine but extends to their protective effects of a cell against free radical toxicity as well as increased production of antioxidants [12]. Thus, agents that combine antioxidant properties with the inhibition of both AChE and BuChE are expected to find usefulness in the management of AD.

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Fig. (1). *S. filicaulis* whole plant growing in its habitat (Ipara Remo, Remo North, Ogun State, Nigeria).

Medicinal plants have been sources of several drugs [13] and several plants from different regions have been screened for their AChE and BuChE inhibitory potential [14-16].

Spilanthes filicaulis, commonly called Creeping Spot flower or African Cress, belongs to the family Compositae and is distributed in Africa, America, Borneo, India, Sri Lanka and Asia which are the tropical and subtropical regions of the world [17]. It is a creeping annual herb with prostrate stems rooting from the nodes. Reproduction is by seeds. They have alternate and ovate leaves. The blade is closely attached to the stem on a short slightly hairy petiole. Ovoid flower heads in short axillary peduncles comprise of the inflorescence. They have yellow ray and disc flowers [18]. The whole plant of *S. filicaulis* is used for the treatment of toothache, stomachache, gastritis and malaria in Babungo, Northwest Region, Cameroon [19]. Also, the entire plant is used for the treatment of chest pain, eczema, guinea worm, stomach problems, headache, cough, and toothache. It is also used as an enema for side pain, to coagulate blood and is rubbed on the skin as a local anesthesia [20].

Previous reports have described the analgesic [21], antimycobacterial [22] and antitumoral [23] activities of extracts of *S. filicaulis*. To explore other potential medicinal uses of this plant, we here describe the AChE and BuChE inhibitory and antioxidant activities of the plant collected in Nigeria (Fig. **1**).

2. MATERIALS AND METHOD

2.1. Chemicals

Folin-Ciocalteu reagent (Fluka Biochemical), vanillin (Fluka Biochemical), potassium ferricyanide (BDH reagents, England), Trichloroacetic acid (Merck chemicals, LTD), sodium nitroprusside (Thomas Baker chemical limited India), 2, 2-diphenyl-1-picrylhydrazyl hydrate (Sigma-Aldrich), Naphthylethylenediamine dichloride (Sigma-Aldrich), acetylthiocholine iodide (Sigma), butyrylthiocholine iodide (Sigma-Aldrich), and 5,5-dinitro-bis-nitrobenzoic acid (Aldrich). All other solvents and reagents used in this study are of analytical grade.

2.2. Plant Collection and Authentication

The whole plants of *Spilanthes filicaulis* were collected from the wild in July, 2018 at Ipara Remo, Remo North, Ogun State and no permission was required for collection. The plant sample was identified and authenticated by Mr. Odewo of the Forest Herbarium Ibadan (FHI), Ibadan, Oyo State. Voucher specimen with FHI number 112015 was then deposited at the Department of Pharmacognosy Herbarium, University of Ibadan. All protocols complied with the plant use ethics of the University of Ibadan.

2.3. Extract and Fractions Preparation

The fresh plant material was rinsed with water, air-dried under shade for 2 weeks and dried in the oven at 40°C for 12 hours and powdered. Powdered plant material (262.44g) was subjected to sequential extraction by maceration for 72h in 4.5L of *n*-hexane to obtain the crude n-hexane extract (c*n*-HE), The marc was air-dried and the procedure repeated with ethyl acetate and methanol solvents to obtain the corresponding crude ethyl acetate extract (cEAE) and crude methanol extract (cMTE) respectively, by filtration and concentration under reduced pressure using rotary evaporator at 40°C.

2.4. Determination of Total Phenolic Content

The total phenolic content of the extracts was determined using the Folin-Ciocalteu reagent (oxidizing agent), following the method described by Singleton [24]. Briefly, 0.5 ml of plant extract (1mg/ml) was mixed with 2.5 ml of 10% Folin-Ciocalteu reagent followed by the addition of 2 ml of 2% Na_2CO_3 and then incubated at 45°C for 15 minutes. The absorbance was measured at 765 nm. The standard used was Gallic acid at varying concentrations of 1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/ml in order to determine the gallic acid equivalent (GAE) of the sample which was calculated from the calibration curve. Distilled water was used as blank. All the experiments were carried out in triplicates.

2.5. Determination of Total Flavonoid Content

The total flavonoid content of the sample was determined using the aluminum chloride colorimetric method [25]. Typ-

ically, 1 ml of sample (1mg/ml) was made up with 3 ml of methanol, mixed with 5.6 ml of distilled water and then 0.2 ml of 10% aluminum chloride and 0.2 ml of 1M potassium acetate was added and left at room temperature for 30 min. The absorbance was measured at 415 nm against the blank. The total flavonoid content was calculated from a calibration curve and the result expressed as mg gallic acid equivalent per gram dry weight. All the determinations were carried out in triplicates.

2.6. Determination of Pro-anthocyanidin Content

The vanillin-HCL method was used for quantitative determination of condensed tannins (proanthocyanidins) [26]. Briefly, to 0.5 ml of extract (1 mg/ml) was added 3 ml of 4% vanillin in methanol and 1.5 ml of Hydrochloric acid (HCL). The mixture was vortexed thoroughly and allowed to stand for 15 min at room temperature. Absorbance was read at 500 nm. A suitable calibration curve was prepared using standard gallic acid solution. All the results were expressed as mg gallic acid equivalents (GAE) per gram of sample.

2.7. DPPH Radical Scavenging Assay

The radical scavenging ability of the plant extracts was determined using the stable radical 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) as described by Sanchez-Moreno [27]. Briefly, to 1 ml of extracts at different concentrations (1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/ml) was added 1ml of 0.1mM DPPH. The reaction was vortexed and left in the dark for 30 min after which the absorbance was read at 517 nm against a DPPH control containing only 1 ml methanol in place of the extract. A control solution of ascorbic acid and 2,6-di-tert-butyl-4-methylphenol (DDM) was also assayed over a concentration range of 1, 0.5, 0.25 and 0.125 mg/ml. The DPPH radical scavenging activity was calculated as the percentage inhibition.

% Inhibition of DPPH radical= $[(A_{control} - A_{test}) / A_{control}]*100$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound).

A_{test} is the absorbance of the test compound.

Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting percentage inhibition against concentration of the extract.

2.8. Reducing Power Antioxidant Assay

The reducing power was determined according to the method of Oyaizu [28]. Typically, 0.2 ml of various concentrations of plant extract were mixed with 0.2 ml of phosphate buffer and 0.2 ml of potassium ferricyanide. The mixture was vortexed and incubated at 50°C for 20 min. After cooling, 0.2 ml of 10% TCA was then added to the mixture and centrifuged at 4500 rpm for 10 min. 100 µl of the upper solution was mixed with 20 µl of ferric chloride solution and 100 µl of distilled water. The absorbance was measured at 700 nm. Control was prepared in a similar manner but without the test sample. Ascorbic acid and DDM at various concentrations were used as standards. The experiments were done in triplicates. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration.

2.9. Nitric Oxide Scavenging Activity Assay

Nitric Oxide scavenging activity was estimated by the use of Greiss Illosvoy reaction [29]. Typically, 2 ml of sodium nitroprusside was mixed with 0.5 ml of phosphate buffer pH 7.4 and 0.5 ml of different concentrations of the extract (1-0.0031 mg/ml) and then incubated at 25°C for 150 min. Absorbance was taken at 540 nm (A0). After the incubation period, 0.5 ml of the incubated mixture was mixed with 1ml of sulfanilic acid reagent and 1 ml of Naphthylethylenediamine dichloride (0.1% w/v), and then incubated at room temperature for 30 min and absorbance was taken at 540 nm (A1). The same reaction mixture without the extract but the equivalent amount of methanol served as the control. Ascorbic acid and DDM at various concentrations were used as standard. All experiments were done in triplicates. The percentage inhibition was linearized against the concentrations of each extract and standard antioxidant. The percentage of nitrite radical scavenging activity of the extracts and standard was calculated using the following formula:

% nitrite radical scavenging activity = $[(A_{control} - A_{test}) / A_{control}]*100$ where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound). A_{test} is the absorbance of the test compound.

2.10. Anti-cholinesterase Colorimetry Assay

The acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities of the different fractions were determined by using a modified method of Ellman [30]. The plant extract was prepared in a stock solution of 2% Tween 20 in a buffer and was used for the cholinesterase inhibition assay, while Physostigmine hemisulfate salt (Eserine) and Donepezil prepared in buffer were used as the reference compound (positive control). Typically, 240 µl of buffer (50mMTris-HCL, pH 8.0) was mixed with 20 µl of varying concentrations of the test sample (1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/ml) and 20 µl of the enzyme preparation. The mixture was incubated for 30 min at 37°C after which 20 µl of 10 mM DTNB was added. The reaction was then initiated by the addition of 20 µl of 25 mM ATChI. The rate of hydrolysis of ATChI was then determined spectrophotometrically by measuring the change in absorbance per minute (ΔA/min) due to the formation of the yellow 5-thio-2-nitrobenzoate anion read at 412 nm every 30 s for 4 min. Assay reactions with plant extracts were all performed in triplicate at concentrations of 1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/ml. A solution of buffer was used as a negative control. The percentage inhibition of AChE by various extracts was calculated relative to inhibition by eserine, with the extract concentration producing 50% inhibition (IC_{50}). The percentage inhibition (I%) of plant extract was obtained using the formula:

$I(\%)=[(Vo-Vi)/Vo]*100$

where: I(%)=Percentage inhibition

Vi =enzyme activity in the presence of extract.

Vo = enzyme activity in the absence of extract.

Table 1. Total phenolic, total flavonoid and total tannin content of the extracts of *S. filicaulis*.

Assays	*n*-Hexane Extract	Ethyl Acetate Extract	Methanol Extract
% Yield	2.971	2.518	20.26
Total phenolics (mg GAE/g)	8.56 ± 0.02	8.86 ± 0.03	9.42 ± 0.02
Total flavonoids (mg GAE/g)	6.22 ± 0.01	8.59 ± 0.01	8.72 ± 0.01
Total tannins (mg GAE/g)	2.20 ± 0.06	9.72 ± 0.07	16.01 ± 0.02

Data are expressed as Mean ± SEM (n=3). GAE: Gallic Acid Equivalent.

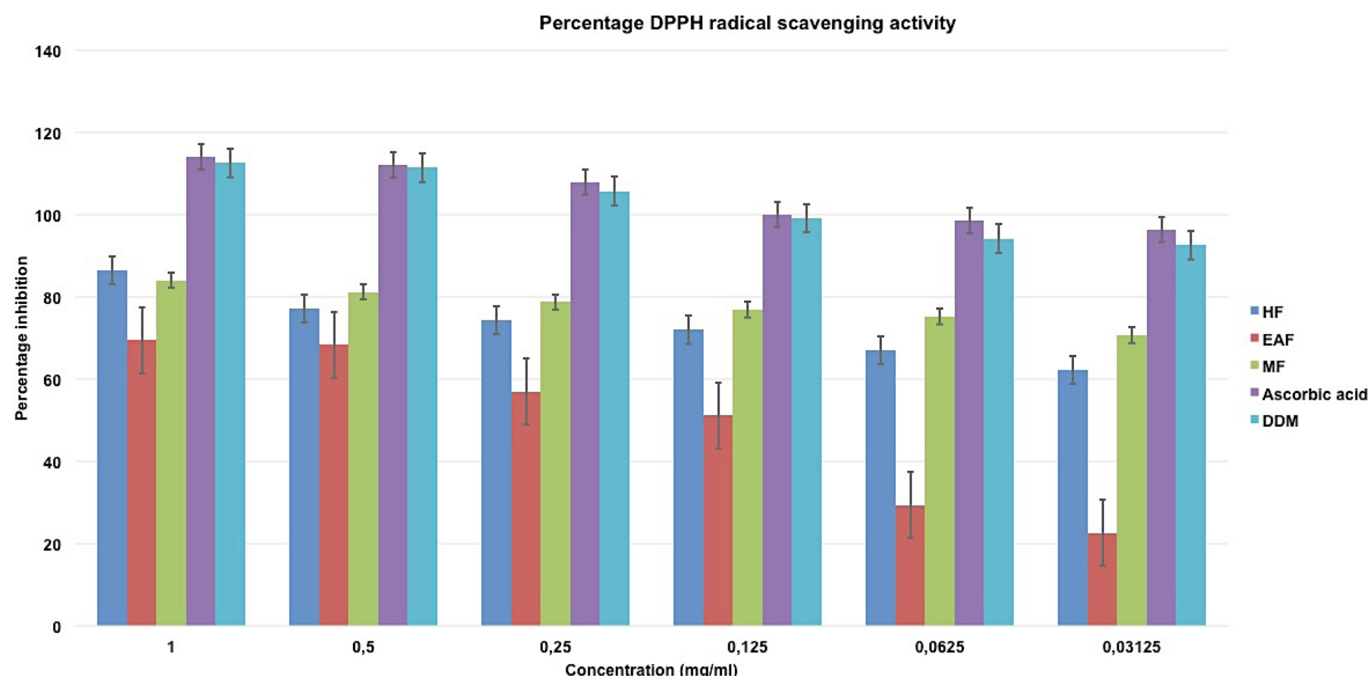

Fig. (2). Percentage DPPH radical scavenging activity of extracts of *S. filicaulis*. Data are expressed as Mean ± SD (n=3). HF: *n*-Hexane extract, EAF: Ethyl acetate extract, MF: Methanol extract, DDM: 2,6-di-tert-butyl-4-methylphenol.

2.11. Statistical Analysis

Statistical analysis of parametric data for IC_{50} was carried out using graph pad prism software. All analyses were performed in triplicate and data are reported as mean ± standard error of the mean of at least three independent experiments. Correlation and regression analysis of activities (Y) versus the total phytochemical content (X) was carried out using the online Quest Graph™ Linear, Logarithmic, Semi-Log Regression Calculator [31].

3. RESULTS

3.1. Determination of Total Phenolic, Total Flavonoid and Total Tannin Content

Three solvents of increasing polarity, n-hexane, ethyl acetate and methanol were used for the sequential extraction of the whole plant of *S. filicaulis*. The polar solvent, methanol had the highest percentage yield of 20.26% (Table **1**). The phenolic content of the various extracts as well as total flavonoids and total pro-anthocyanins was quantified against gallic acid using standard methods. The methanol extract appears to be the richest, with the highest amount of phenolics, flavonoids and tannins when compared with other less polar extracts. Flavonoids accounted for a small portion of the total phenolics and were detected across the three fractions (Table **1**).

3.2. DPPH Scavenging Activity

The antioxidant activity of all the extracts was determined using three spectrophotometric methods; DPPH radical scavenging, ferric reducing power and nitric oxide scavenging ability.

The percentage DPPH radical scavenging activity of extracts of *S. filicaulis* is as shown in Fig. (**2**). In the DPPH assay, the methanol extract was the best scavenger with an IC_{50} value of 0.007 µg/mL. followed by the ethyl acetate extract and then the hexane extract (Table **2**).

3.3. Ferric Reducing Power

The reducing power of the methanol, ethyl acetate and n-hexane extracts of *Spilanthes filicaulis* is shown in Fig. (**3**). The greatest reducing power was observed in ethyl acetate fractions relative to other fractions.

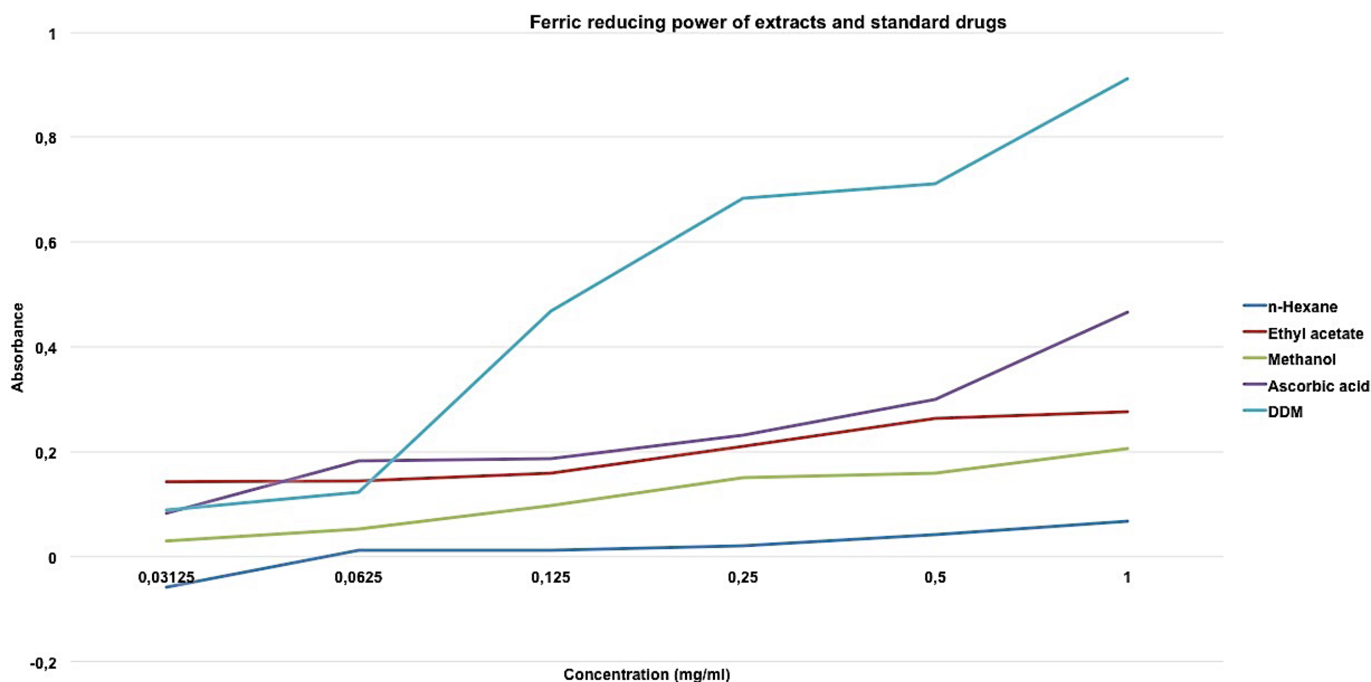

Fig. (3). Reducing power of various extracts of *S. filicaulis* and reference drugs.

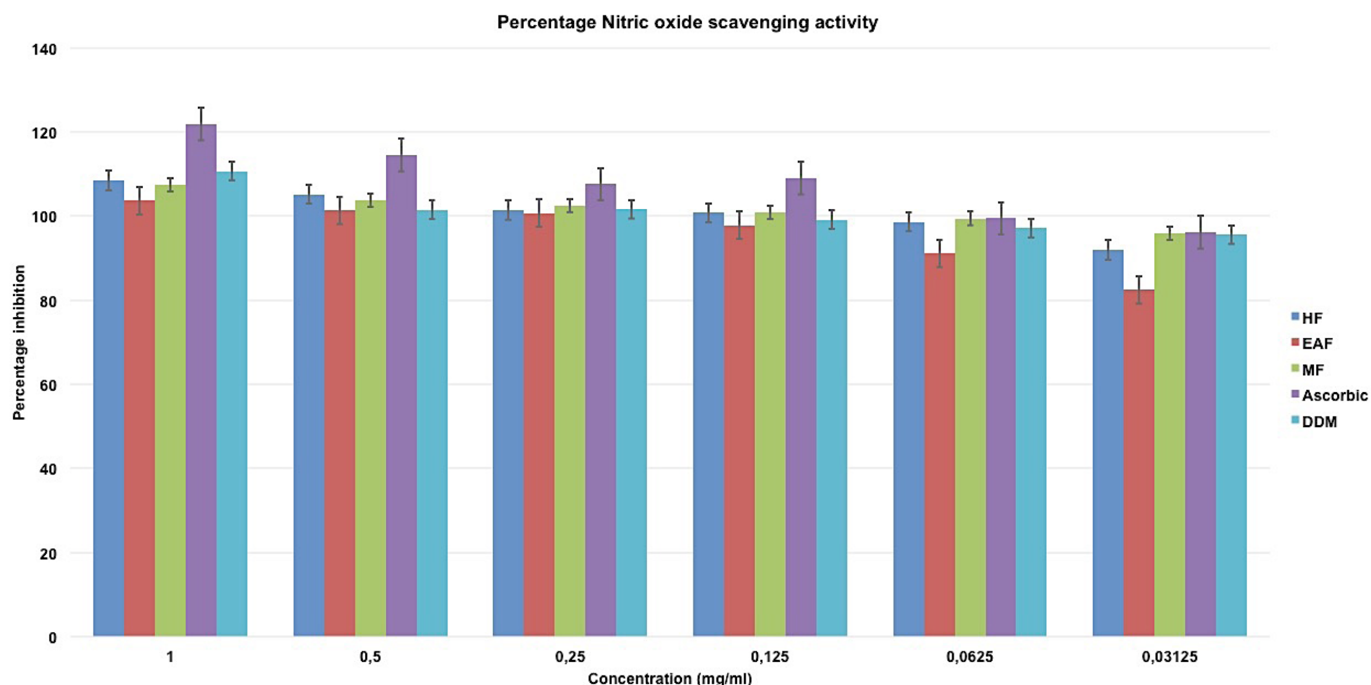

Fig. (4). Percentage inhibition for the Nitric Oxide scavenging antioxidant activity. HF= Hexane extract, EAF= Ethyl acetate extract, MF= Methanol extract, Ascorbic= Ascorbic acid, DDM= 2,6-di-tert-butyl-4-methylphenol. Data are expressed as Mean ± SD (n=3).

Table 2. IC_{50} values for DPPH and NO scavenging activity of extracts of *S. filicaulis.*

Extract/Drug	IC_{50} Values (µg/mL)	
	DPPH Scavenging	Nitric Oxide Scavenging
N-Hexane	1.747	0.005
Ethyl acetate	0.103	0.026
Methanol	0.007	0.167
Ascorbic acid	0.205	0.330
DDM	0.182	0.982

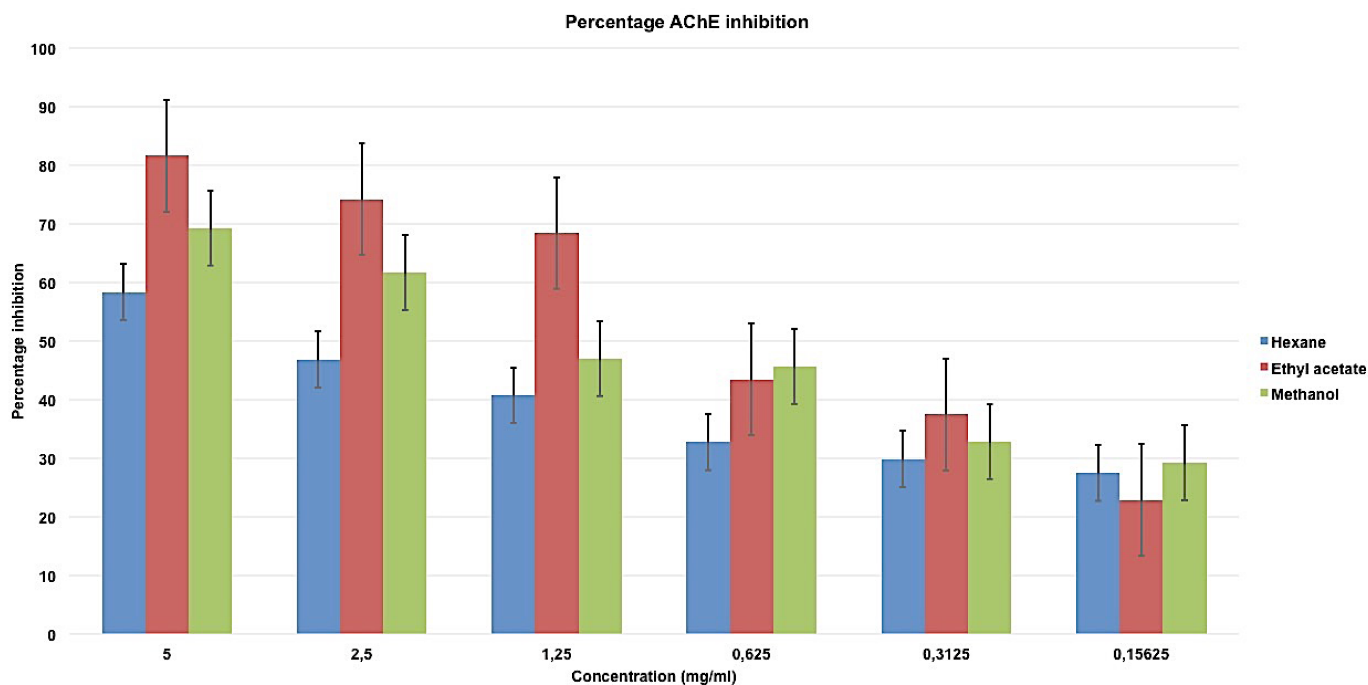

Fig. (5). Percentage AChE inhibition of *S. filicaulis* extracts Data are expressed as Mean ± SD (n=3).

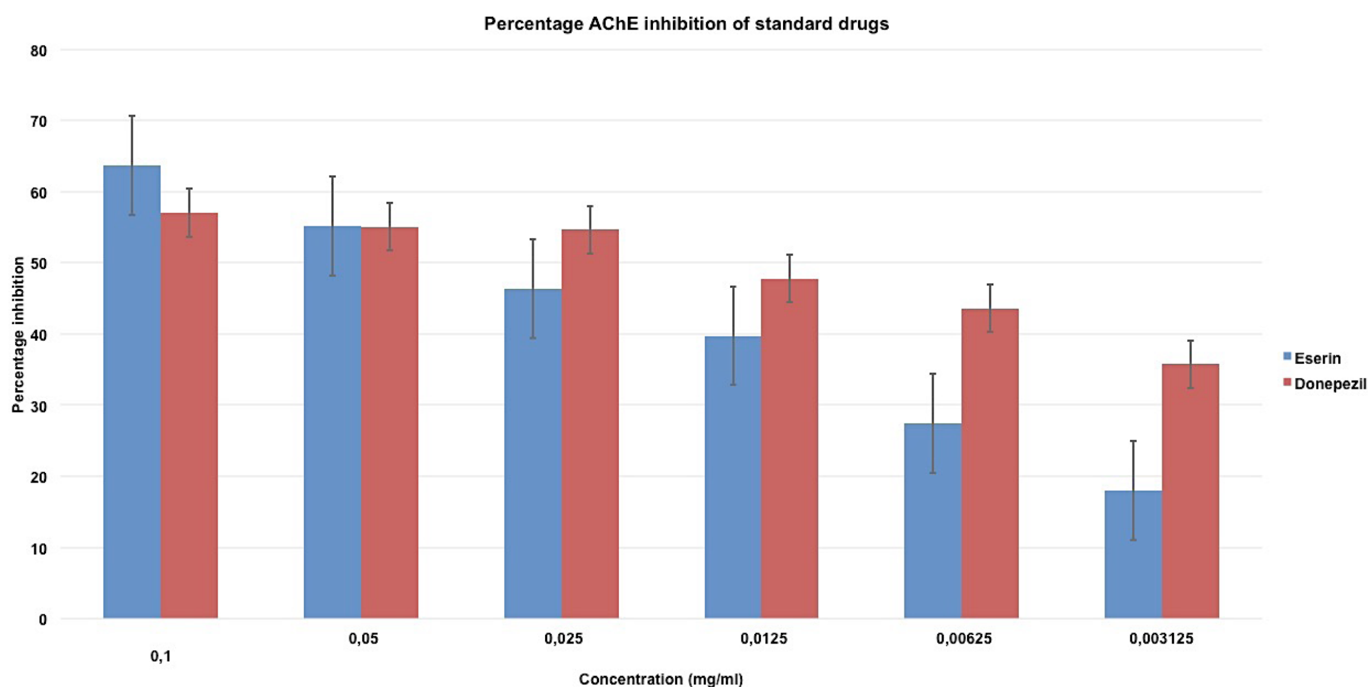

Fig. (6). Percentage AChE inhibition of standard drugs (Eserine and Donepezil). Data are expressed as Mean ± SD (n=3).

Table 3. IC_{50} values for the AChE and BuChE activity of extracts of *S. filicaulis.*

Extracts	AChE	BuChE
	IC_{50}(µg/mL)	
n-Hexane	3.27	6.59
Ethyl acetate	0.77	0.92
Methanol	1.79	1.20
Eserine	0.05	0.01
Donepezil	0.04	0.004

Data are expressed as Mean ± SD (n=3).

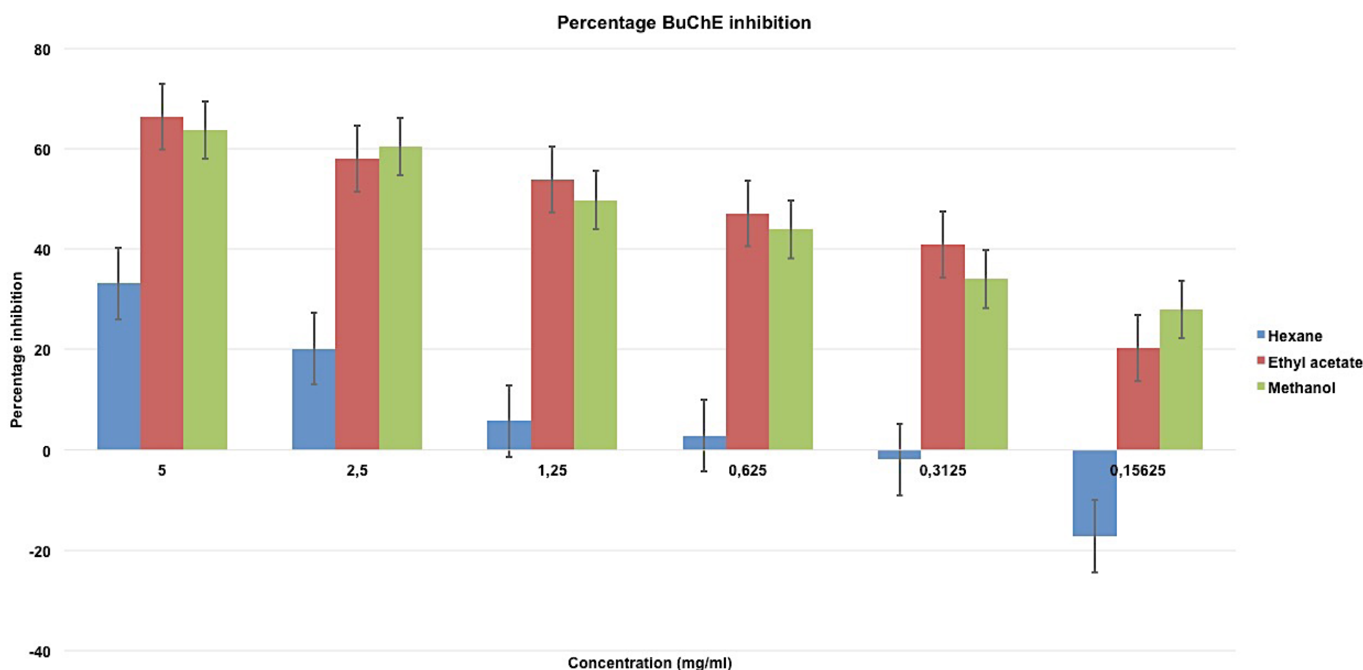

Fig. (7). Percentage BuChE inhibition of *S. filicaulis* extracts, Data are expressed as Mean ± SD (n=3).

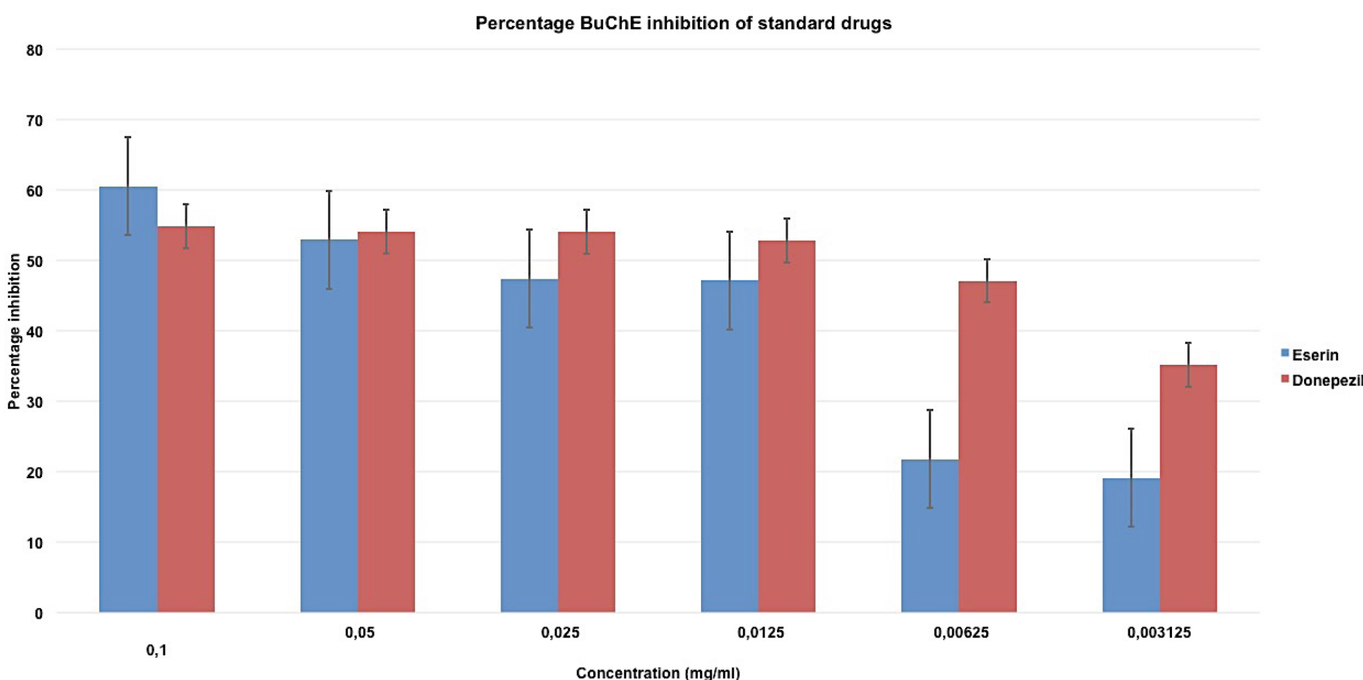

Fig. (8). Percentage BuChE inhibition the standard drugs (Eserine and Donepezil). Data are expressed as Mean ± SD (n=3).

Table 4. Correlation of antioxidant and anticholinesterase activities with total phenol, total flavonoid and total proautocyanidin content of *S. filicaulis.*

Correlation Values (R^2)			
Activities	**Total Phenolics**	**Total Flavonoids**	**Proautocyanidin**
DPPH scavenging	0.636	1.000	0.831
NO scavenging	0.942	0.404	0.806
AChE inhibition	0.194	0.800	0.396
BUChE inhibition	0.545	0.992	0.757

3.4. Nitric Oxide Scavenging Activity

The hexane extract showed slightly higher nitric oxide scavenging activity than the other extracts with a percentage inhibition of 108.40 ± 0.039% and an IC_{50} value of 0.005 µg/mL (Fig. **4** and Table **2**).

3.5. AChE and BuChE Inhibitory Activity

The percentage inhibition of the various extracts and standard drugs on AChE and BuChE is shown in Figs. (**5-8**) while the IC_{50} values are presented in Table **3**. All th extracts showed a dose-dependent inhibition with the ethyl acetate extract showing the highest activity with the lowest IC50 of 0.77 µg/mL and 0.92 µg/mL for AChE and BuChE respectively followed by the methanolic extract having an IC50 of 1.79 µg/mL and 1.20µg/mL respectively in comparison to the standards eserine and donepezil having an IC50 of 0.05 µg/mL and 0.04 µg/mL respectively for AChE and 0.01 and 0.004 for BuChE.

3.6. Correlation of Activities with Phytochemical Content

The correlation between the IC_{50} values of antioxidant (DPPH and nitric oxide scavenging) as well as cholinesterase (AChE and BuChE) is presented in Table **4**. The DPPH scavenging had high correlation with phenolic, flavonoid and tannin content (R^2 0.636, 1.000 and 0.831 respectively) while the nitric oxide scavenging correlated well with the phenolic and tannin content (R^2 = 0.942 and 0.806). The acetyl and butyrylcholinesterase inhibition, however, showed the highest correlation with the flavonoid content, R^2 = 0.800 and 0.992 respectively.

4. DISCUSSION

Spilanthes filicaulis is a tropical plant that has found wide use in ethnomedicine and as food flavourant [32]. Different extracts of the whole plant of *S. filicaulis* collected from Nigeria were evaluated for their total phenolics, total flavonoids, and total tannin content as well as their reducing antioxidant power, free radical scavenging capacity, nitric oxide scavenging ability and cholinesterase (acetyl and butryl) inhibitory actions.

Phenolics are widely distributed in the tissues of plants and importantly serve as antioxidants [33]. Researches over the years have correlated the phenolic content of plants to their antioxidant capacity [34-37]. In fact, gallic acid and butylated hydroxytoluene are examples of phenolic compounds with proven antioxidant activity [38]. The phenolic content in the different extracts of *S. filicaulis* is reported in Table **1**. The amount of phenolic compounds in the methanol extract was slightly higher than in the ethyl acetate and hexane extracts. The higher content of total phenols in the methanol extract may account for its better DPPH radical scavenging effect.

Flavonoids and other polyphenols play an active role in quenching of free radicals due to their redox properties [39,40]. Flavonoids occur ubiquitously in medicinal plants as glycosides containing several phenolics hydroxyl groups. These hydroxyl groups confer radical scavenging ability on flavonoids thus making them effective antioxidants [41]. Our study revealed the presence of flavonoids in the three extracts of *S. filicaulis* with the methanol extract having a slightly higher concentration than the others (Table **1**). Reports have also shown that part of the mechanism of action of flavonoids is through inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2 as well as their effects on membrane permeability [42], Thus, the presence of flavonoids in the extracts will contribute to the various medicinal uses of *S. filicaulis*

Tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes mainly with proteins, polysaccharides, alkaloids, nucleic acids and minerals [43], They are known to be useful in the treatment of several ailments including cancer, ulcers and wound [44]. Specifically, tannins have been found useful in neurodegenerative diseases [45-47]. In our study, the level of tannin was significantly high in the methanol extract when compared with the other extracts (Table **1**).

Reducing power has been in association with antioxidant activity and may serve as a meaningful observation of the antioxidant activity [48]. From the experiment, the presence of the reducing agent caused the reduction of Fe^{3+}/ferricyanide complex to the ferrous form, giving different shades of green and blue. Thus, the concentration of Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Fig. (**3**) shows the reducing power of various extracts of *S. filicaulis* with the ethyl acetate fraction having the greatest reducing power. It has been reported that the reducing power of plants might be due to their hydrogen-donating ability [49]. Therefore, the ethyl acetate extract might have a higher quantity of reductone which will react with the free radicals to block radical chain reactions.

The radical scavenging potential of the different extracts was evaluated using DPPH. Usually, freshly prepared DPPH solution has a deep purple colour, which gradually fades in the presence of an antioxidant, with maximum absorption at 517 nm. This implies that antioxidants provide hydrogen atoms or donate an electron to quench DPPH free radicals thus converting DPPH to colourless (2,2-diphenyl-1-hydrazine) product with reduced absorbance. Thus, the more rapidly an extract/compound can decrease the absorbance, the more potent it is as an antioxidant. DPPH enjoys some preference in the assay for free radical scavenging because it is usually unaffected by side reactions such as enzyme inhibition and metal chelation [50]. Also, free radical scavenging is a known mechanism by which antioxidants prevents lipid peroxidation [51]. The percentage of DPPH radical scavenging activity of extracts of *S. filicaulis* is as shown in Fig. (**2**) with the IC_{50} for the various extracts reported in Table **2**. The results showed that methanol extract with an IC_{50} of 0.007 µg/mL had the highest scavenging activity. This correlated well with the phenolic and flavonoid content of the extract.

Nitric oxide (NO) is an important chemical mediator that is involved in the regulation of various physiological processes and is generated by endothelial cells, macrophages, neurons, *etc*. [52]. Nitric oxide is produced by phagocytes and endothelial cells can yield more reactive species like peroxynitrite which is then decomposed to form OH radical. This implies that several diseases are as a result of excess

concentration of NO [53] and agents which can reduce NO in the system will have beneficial effects. In our study, all the extracts showed significant reduction of NO with the hexane extract showing slightly higher scavenging activity than the other extracts with an IC_{50} value of 0.005 µg/mL (Fig. **3** and Table **3**). This might be due to the presence of phenolics in all the extracts.

The loss of neurotransmitter acetylcholine in the brain is important in the pathogenesis of AD and increasing the level of acetylcholine in the brain can be achieved by inhibiting the activity of the enzyme acetylcholinesterase (AChE) and butrylcholinesterase (BuChE), which break down the respective neurotransmitter. Currently, most of the approved and effective therapies for cognitive dysfunction in AD are cholinesterase inhibitors which function by inhibiting ACh degradation [54-56]. The use of cholinesterase inhibitors with dual ability to inhibit both AChE and BuChE is believed to produce better clinical results especially in AD patients [55, 57]. Several cholinesterase inhibitors have been developed based on compound templates from medicinal plants [58]. These inhibitors differ in their mechanism of inhibition, reversibility and selectivity for AChE and BuChE. From our study, we observed that all the extracts showed a dose-dependent inhibition of both enzymes in relation to the standard drugs (Figs. **5-8**), with the ethyl acetate extract having the best activity with an IC50 of 0.77 µg/mL and 0.92 µg/mL for AChE and BuChE respectively followed by the methanolic extract having an IC50 of 1.79 µg/mL and 1.20µg/mL respectively in comparison to the standards eserine and donepezil (Table **4**).

R^2 values have been used as an indicator between phytochemical constituents and activities of medicinal plants, with a high value being indicative of good correlation [59]. Numerous biological activities such as antioxidant, anti-inflammatory, antimicrobial have also been associated with the presence of phenolics flavonoids and tannins in plant extracts [60-64]. In this study, the DPPH scavenging and cholinesterase activity had a strong correlation with the flavonoid content (R^2 1.00 (DPPH), 0.800 (AChE), 0.992 (BuChE)) while the nitric oxide scavenging had a high correlation with phenolics and proautocyanidin (R^2 0.942 and 0,806 respectively). This indicates that the phenolic compounds in the plant are playing a role in the various observed activities.

On the whole, the antioxidant property of medicinal plants has been known to contribute to their therapeutic potentials [65-69] and because of the direct link between oxidative stress and neurodegeneration, this is especially true for AD. Thus, both the antioxidant and anticholinesterase properties of *S. filicaulis* provide a justification for the inclusion of the plant in therapies used as a memory enhancer in ethnomedicine.

CONCLUSION

The present research shows that *Spilanthes filicaulis* had good anticholinesterase and antioxidant activities which could be exploited in the management of neurodegenerative conditions such as AD.

LIST OF ABBREVIATIONS

AChE	=	Acetylcholinesterase
ATChI	=	Acetyl thiocholine iodide
BuChE	=	Butyrylcholinesterase
BuChI	=	Butryl thiocholine iodide
DDM	=	2,6-di-tert-butyl-4-methylphenol
DPPH	=	2, 2-diphenyl-1-picrylhydrazyl hydrate
DTNB	=	5,5-dinitro-bis-nitrobenzoic acid
FHI	=	Forest Herbarium Ibadan
GAE	=	Gallic acid equivalents
TCA	=	Trichloroacetic acid

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

All experiments complied with international guidelines and was approved by the plant use ethics of the University of Ibadan.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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