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In vitro neuroprotective potentials of aqueous and methanol extracts from *Heinsia crinita* leaves

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Abstract

This study was designed to determine the neuroprotective potentials of aqueous and methanol extracts from *Heinsia crinita* leaves in vitro. The total phenol and flavonoid contents of the extracts were determined using colorimetric method while phenolic characterization of the leaf was analyzed via high performance liquid chromatography-diode array detector (HPLC-DAD). The effects of the extracts on Fe^{2+}-induced lipid peroxidation in rats' brain homogenate, monoamine oxidase (MAO), Na^+/K^+-ATPase, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities were also assessed. The aqueous extract had higher total phenol and flavonoid contents than the methanol extract. HPLC-DAD revealed that quercetin ellagic, chlorogenic and caffeic acids were the most abundant phenolic compounds in the leaves. The aqueous extract had higher inhibitory effects on MAO, AChE and BChE activities while there was no significant difference between their Fe^{2+}-induced lipid peroxidation inhibitory effects. Furthermore, both extracts stimulated Na^+/K^+-ATPase activity; however, methanol extract had higher stimulatory effect. The neuroprotective properties of *H. crinita* leaves could be associated with its inhibitory effects on Fe^{2+}-induced lipid peroxidation and modulation of MAO, Na^+/K^+-ATPase, AChE, and BChE activities. Therefore, *H. crinita* leaves could be used as a functional food and dietary intervention for the management of some neurodegenerative diseases. Nevertheless, the aqueous extracts exhibited better neuroprotective properties.

Keywords: *Heinsia crinita*; Neurodegeneration; Oxidative stress; Malondialdehyde; Polyphenols

1. Introduction

Oxidative stress has been implicated in some neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's diseases (PD). Free radical-induced neurodegeneration in brain cells is usually caused by high levels of polyunsaturated fatty acid, low antioxidant capacity, high lipid content of myelin sheaths, high consumption of metabolic oxygen and lipid peroxidation in the cell membrane [1,2]. In addition, elevated levels of reactive oxygen species (ROS) can also induce oxidative damage in the nerve cells which can lead to neuronal injury and radical-induced cell death [3].

Furthermore, increase in monoamine oxidase (MAO) activity has been linked to the excessive production of free radicals, oxidative stress, neuronal injury and hydrolysis of neuro-active amines such as dopamine, serotonin etc. [4]. However, there are growing evidences that the inhibition of MAO activity could play a neuroprotective role in some neurodegenerative conditions [5]. Therefore the use of MAO inhibitors could be a good

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therapeutic strategy in the management/treatment of some neurodegenerative conditions such as AD and PD. Furthermore, several reports have revealed that decrease in the activities of cholinesterases (AChE and BChE), and stimulation of Na^+/K^+-ATPase activity relevant to the regulation of neurotransmitters and synaptic responses could help to improve cognitive and neuronal functions [6,7]. However, increase in AChE and BChE activities could lead to deficits in cholinergic neurotransmitters in AD patients, while decrease in Na^+/K^+-ATPase activity can induce glutamate neurotoxicity in PD [7–9]. Hence, inhibition of AChE and BChE activities and stimulation of Na^+/K^+-ATPase activity could be good therapeutic strategies in the management and/or treatment of AD and PD. Interestingly, previous report has established that cholinesterase inhibitors can also increase the activity of Na^+/K^+-ATPase [10].

Vladimir-Kneevic et al. [11] reported that consumption of medicinal plants can improve cognitive functions in neurodegenerative conditions. The use of dietary antioxidants and bioactive compounds from plants and plant extracts has also been established for the treatment/and or management of some neurodegenerative diseases. *Heinsia crinita* also known as bush apple (locally referred to as "atama" in Southern-Nigeria) is a shrub with dense crown, bisexual flowers and conspicuous leafy calyx lobes with edible fruits. The leaves are consumed either as vegetable in preparation of local cuisine or as component of alcoholic concoction for the treatment of some diseases such as bacterial infections, diabetes, hypertension and infertility [12,13]. However, to the best of our knowledge, the neuroprotective properties of *H. crinita* leaf extracts have not been reported. Therefore, this study was designed to investigate the neuroprotective potentials of aqueous and methanol extracts from *H. crinita* leaves via their effects on Fe^{2+}-induced oxidative stress in rats' brain and enzymes (MAO, AChE, BChE and Na^+/K^+-ATPase) linked to neurodegenerative diseases such as Alzheimer's diseases (AD) and Parkinson disease (PD).

2. Materials and methods

2.1. Sample collection

Fresh sample of *H. crinita* leaves was purchased from Akure main market, Akure, Nigeria. The sample was identified and authenticated at the Department of Biology, Federal University of Technology, Akure, Nigeria by A. A. Sorungbe. The sample was deposited at the university herbarium with voucher no FUTA/BIO/135. The leaves were separated from the stem, air dried at room temperature and pulverized using laboratory blender. The pulverized sample was sieved in Willey 60 mesh size and stored in the refrigerator. The powder was analyzed via HPLC-DAD. Unless stated otherwise, all other chemicals and reagents used were of analytical grades and the water was glass distilled. Kenxin (Model KX3400C) refrigerated centrifuge was used while JENWAY UV-visible spectrophotometer (Model 6305; Jenway, Barlo World Scientific, Dunmow, United Kingdom) was used to measure absorbance.

2.2. Preparation of extracts

The methanol and aqueous extracts were prepared by macerating 5 g of the powdered sample in 100 mL of absolute methanol and distilled water for 16 h respectively. The extracts were filtered (filter paper Whatman No. 2) and centrifuged at 4000 rev/min for 10 min to obtain clear supernatant. Supernatant from the methanol extract was evaporated under a vacuum at 45 °C until about 90% of the filtrate was evaporated. Thereafter, both samples were lyophilized to obtain dry extracts which were kept in the refrigerator (≤4 °C) in sealed vials for further analysis.

2.3. Determination of total phenol content

The total phenol content was determined according to the method described by Singleton et al. [14]. Briefly, diluted extract were oxidized with 2.5 mL of 10% Folin–Ciocalteau's reagent (v/v) and neutralized with 2.0 mL of 7.5% sodium carbonate. The mixture was incubated for 40 min at 45 °C and the absorbance was measured at 765 nm using UV–visible spectrophotometer. The total phenol content was subsequently calculated using gallic acid as standard and expressed as gallic acid equivalent (GAE) based on the dry weight of the sample.

2.4. Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by Meda et al. [15]. Briefly, 0.5 mL of the extracts were mixed with 0.5 mL of absolute methanol, 50 μL of 10% $AlCl_3$, 50 μL of 1 mol/L potassium acetate, and 1.4 mL of distilled water. The solution was incubated at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm. The total flavonoid content was calculated using quercetin as standard and expressed as quercetin equivalent (QE) based on the dry weight of the samples.

2.5. Quantification of phenolic compounds by HPLC-DAD

Reverse phase chromatography analyses were carried out under gradient conditions using 1% formic acid and acetonitrile as the mobile phase and C_{18} column (4.6 mm × 150 mm) as the stationary phase. A composition gradient of 13% acetonitrile was run for 10 min. The composition gradient was subsequently increased and varied with respect to time as described by Adedayo et al. [16] with slight modifications. The powder that was obtained from *H. crinita* leaves and the mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. The extract was analyzed at a concentration of 20 mg/mL. The flow rate was set at 0.6 mL/min while the injection volume used for the analysis was 40 μL. Appropriate wavelengths were used to determine gallic acid (254 nm), catechin (280 nm), epicatechin (280 nm), chlorogenic acid (325 nm), caffeic acid (325 nm), ellagic acid (325 nm), quercetin (365 nm), quercitrin (365 nm), rutin (365 nm) and kaempferol (365 nm). Stock solutions of

reference standards for the flavonoids (0.030–0.250 mg/mL) and phenolic acids (0.050–0.450 mg/mL) were prepared using the mobile phase. Chromatographic peaks were confirmed by comparing the retention time of the samples with reference standards by DAD spectra (200–500 nm). All chromatographic operations were carried out at ambient temperature and in triplicates. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve [17].

2.6. Handling of experimental animals

Adult male wistar strain albino rats (weighing between 180 and 210 g) were purchased from the animal breeding colony of Animal Production and Health Department, Federal University of Technology, Akure. Handling of the animals was in accordance with the Guide for Care and Use of Laboratory Animals prepared by the National Academy of Science which was published by the National Institute of Health (USA) [18]. The rats were allowed to acclimatize for 14 days and maintained at room temperature under laboratory conditions with access to standard animal feed and water ad libitum.

2.7. Lipid peroxidation assay

The rat was decapitated under mild anesthesia (diethyl ether) and the brain tissue was isolated and placed on ice and weighed. The tissue was subsequently homogenized in cold saline (1/10, w/v) with about 10-up and -down strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenate was centrifuged for 10 min at 3000 × *g*. The pellets obtained were discarded while the supernatant was kept for lipid peroxidation assay [19]. One hundred microliter (100 μL) of the supernatant was mixed with a reaction mixture containing 30 μL of 0.1 mol/L Tris–HCl buffer (pH 7.4), different concentrations of extract and 30 μL of freshly prepared $FeSO_4$ solution (250 μmol/L). The volume was made up to 300 μL with distilled water before incubation at 37 °C for 1 h. The chromogen was developed by adding 300 μL of 8.1% sodium dodecyl sulphate (SDS), 600 μL of acetic acid/HCl mixture (pH 3.4) and 600 μL of 0.8% TBA. The reaction mixture was incubated at 100 °C for 1 h. The TBARS produced was measured at 532 nm [20] and calculated as the percent of MDA (Malondialdehyde) produced (% Control) using the MDA standard curve.

2.8. Enzyme inhibition assay

2.8.1. Monoamine oxidase (MAO) inhibition assay

Different concentrations of the extracts were prepared according to the methods of Green and Haughton [21] and Turski et al. [22], with slight modification. The reaction mixture contained 0.025 mol/L phosphate buffer of pH 7, 0.0125 mol/L semicarbazide, 10 mmol/L benzylamine (pH 7), 0.67 mg of enzyme and extract in a total reaction volume of 2 mL. After 30 min, 1 mL of acetic acid was added and boiled for 3 min and then centrifuged. The resultant supernatant (1 mL) was mixed with equal volume of 0.05% 2, 4-DNPH and 2.5 mL of absolute benzene. The resultant solution was incubated at room temperature for 10 min. After the incubation, the benzene layer was isolated and mixed with equal volume of 0.1 N NaOH. Alkaline layer was decanted and heated at 80 °C for 10 min. The orange-yellow color formed was measured at 450 nm.

2.8.2. Na^+/K^+-ATPase activity assay

The Na^+/K^+-ATPase activity was measured according to the method described by Wyse et al. [23]. The assay mixture consist of 50 μL of Na^+/K^+-ATPase substrate buffer (pH 7.4) containing 30 mmol/L of Tris–HCl, 0.1 mmol/L of EDTA, 50 mmol/L of NaCl, 5 mmol/L of KCl, and 6 mmol/L of $MgCl_2$ (pH 7.4), extract (50 μL) and 50 μL of supernatant in the presence or absence of 50 μL of 1 mmol/L ouabain in a final volume of 200 μL. The reaction was initiated by the addition of 50 μL ATP to a final concentration of 3 mmol/L. After incubation for 30 min at 37 °C, the reaction was terminated by the addition of 70 μL of 50% (w/v) trichloroacetic acid (TCA). The amount of inorganic phosphate (Pi) released was quantified as described by Fiske and Subbarow [24] using a reaction mixture that contained 100 μL of ammonium molybdate (50 mmol/L), 40 μL of reaction mixture from first grid and 10 μL of ascorbic acid (8%). Different concentrations (0, 4, 8, 10, 20, 40 nmol/L) of NaH_2PO_4 (1 mmol/L) were used to make a calibration curve of inorganic phosphate. Specific Na^+/K^+-ATPase activity was calculated by subtracting the ouabain-insensitive activity from the overall activity (in the absence of ouabain) and expressed in nmol of Pi/mg of protein/min.

2.8.3. Acetylcholinesterase inhibition assay

The AChE inhibitory ability of the extracts was assessed by a modified colorimetric method of Perry et al. [25]. The AChE activity was determined in a reaction mixture containing 200 μL of AChE solution (EC 3.1.1.7, 0.1 mol/L phosphate buffer pH 8.0), 100 μL of 5,5′-dithio-bis(2-nitrobenzoic) acid (DTNB 3.3 mmol/L), different concentration (0–100 μL) of the extract and 500 μL of phosphate buffer (pH 8.0). After incubation for 20 min at 25 °C, acetylthiocholine iodide (100 μL of 0.05 mmol/L solution) was added as the substrate, and AChE activity was determined from the changes in absorbance at 412 nm which was read for 3 min at room temperature. The AChE inhibitory activity was expressed as percentage inhibition.

2.8.4. Butyrylcholinesterase (BChE) inhibition assay

Inhibition of BChE was assessed by a modified colorimetric method of Ellman et al. [26]. The BChE activity was determined in a reaction mixture containing 200 μL of BChE solution (0.415 U/mL in 0.1 mol/L phosphate buffer, pH 8.0), 100 μL of a solution of 5,5′-dithiobis(2-nitrobenzoic) acid (3.3 mmol/L in 0.1 mol/L phosphate-buffered solution, pH 7.0) containing $NaHCO_3$ (6 mmol/L), extract (0–100 μL), and 500 μL of phosphate buffer, pH 8.0. After incubation for 20 min at 25 °C, butyrylthiocholine iodide (100 μL of 0.05 mmol/L solution) was added as the substrate, and BChE activity was determined from

Table 1
The total phenol and flavonoid contents and EC_{50} values of modulation of Fe^{2+}-induced MDA production, MAO, Na^+/K^+ ATPase, AChE and BChE activities of *Heinsia crinita* leaves extracts.

Parameter (unit)	Aqueous extract	Methanol extract
Total phenol content (mg GAE/g)	8.77 ± 0.20[a]	5.51 ± 0.09[b]
Total flavonoid content (mg QE/g)	14.47 ± 1.29[a]	5.57 ± 0.21[b]
Fe^{2+}-induced lipid peroxidation (mg/mL)	3.06 ± 0.04[a]	3.01 ± 0.03[a]
Monoamine oxidase (mg/mL)	4.03 ± 0.07[a]	6.76 ± 0.05[b]
Na^+/K^+ ATPase (mg/mL)	4.05 ± 0.05[a]	3.33 ± 0.03[b]
Acetylcholinesterase (mg/mL)	32.11 ± 0.12[a]	33.67 ± 0.22[b]
Butyrylcholinesterase (mg/mL)	30.4 ± 0.52[a]	32.95 ± 0.43[b]

Values represent means of triplicate readings. Values with same superscripts are not significantly different at $P > 0.05$.

Table 2
Phenolic acid and flavonoid composition of *Heinsia crinita* leaves.

Parameter	mg/g	%
Gallic acid	43.51 ± 0.03	4.35
Catechin	9.46 ± 0.01	0.09
Chlorogenic acid	72.90 ± 0.01	7.29
Caffeic acid	80.51 ± 0.02	8.05
Ellagic acid	116.74 ± 0.01	11.61
Epicatechin	64.39 ± 0.03	6.43
Rutin	54.27 ± 0.02	5.42
Quercitrin	39.15 ± 0.01	3.91
Quercetin	44.26 ± 0.02	4.43
Kaempferol	7.52 ± 0.03	0.57

Values represent means ± standard deviation of triplicate readings.

the changes in absorbance at 412 nm which was read for 3 min at room temperature.

2.9. Data analysis

The results of triplicate readings of the experiments were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to analyze the mean and the post hoc treatment was performed using Duncan multiple test. Significance was accepted at $P \leq 0.05$. EC_{50} (extract concentration causing 50% enzyme/antioxidant activity) was determined using non-linear regression analysis.

3. Results and discussion

3.1. Phenolic composition

The results of total phenol and flavonoid contents of the extracts presented in Table 1 showed that the aqueous extract had higher total phenol (8.77 mg GAE/g) and flavonoid (14.47 mg QE/g) contents than methanol extract (total phenol = 5.51 mg GAE/g; total flavonoid = 5.57 mg QE/g). Furthermore, the phenolic characterization of *H. crinita* leaves revealed the presence of gallic acid (43.51 mg/g), catechin (9.46 mg/g), chlorogenic acid (72.90 mg/g), caffeic acid (80.51 mg/g), ellagic acid (116.74 mg/g), epicatechin (64.39 mg/g), rutin (54.27 mg/g), quercitrin (39.15 mg/g), quercetin (44.26 mg/g) and kaempferol (7.52 mg/g) (Fig. 1 and Table 2).

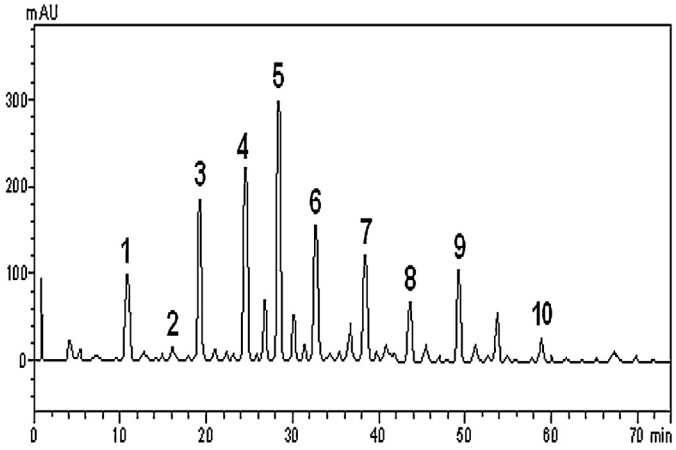

Fig. 1. Representative high performance liquid chromatography profile of powdered *Heinsia crinita*. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), epicatechin (peak 6), rutin (peak 7), quercitrin (peak 8), quercetin (peak 9) and kaempferol (peak 10).

3.2. Inhibition of malondialdehyde production

The ability of the extracts to inhibit Fe^{2+}-induced lipid peroxidation in rats' brain homogenate is presented in Fig. 2. The result revealed that Fe^{2+} caused a significant increase in MDA content in the rats' brain. However, the addition of the extracts in a dose-dependent manner (0.78–3.5 mg/mL) caused a significant decrease in MDA levels (aqueous extract = 67.9–61.1%; methanol extract = 50.0–79.8%). Our findings revealed that the extracts were able to inhibit the production of MDA in rats' brain. MDA is a toxic chemical capable of causing oxidative damage to the brain cells and has been implicated in the pathogenesis and progression of some neurodegenerative conditions such as AD and PD [27]. Previous studies have shown that Fe accumulates in the brain of AD patients and animal models. Moreover Fe can initiate Fenton reaction which could lead to the production of OH radicals [28]. These radicals are capable of inducing oxidative stress/damage to membrane lipids, DNA, proteins and other electron rich biomolecules. The inhibition of Fe^{2+}-induced lipid peroxidation by the extracts from *H. crinita* leaves agrees with previous studies on plant extracts [29]. However, there was no significant ($P > 0.05$) difference between the Fe-induced inhibitory abilities of aqueous and methanol extracts.

3.3. Inhibition of enzymes linked to some neurodegenerative diseases

The effects of the extracts on MAO, Na^+/K^+-ATPase, AChE and BChE activities are presented in Figs. 3–6 respectively with their EC_{50} values in Table 1. The extracts were able to modulate enzyme activities in a dose dependent manner. The aqueous extract (4.03 mg/mL) had higher MAO inhibitory activity than the methanol extract (6.79 mg/mL). The inhibition of MAO activity by the extracts indicates their therapeutic potential in the treatment/management of AD and PD. The decrease in MAO activity could consequently, increase the level of amine neurotransmitters such as dopamine and serotonin

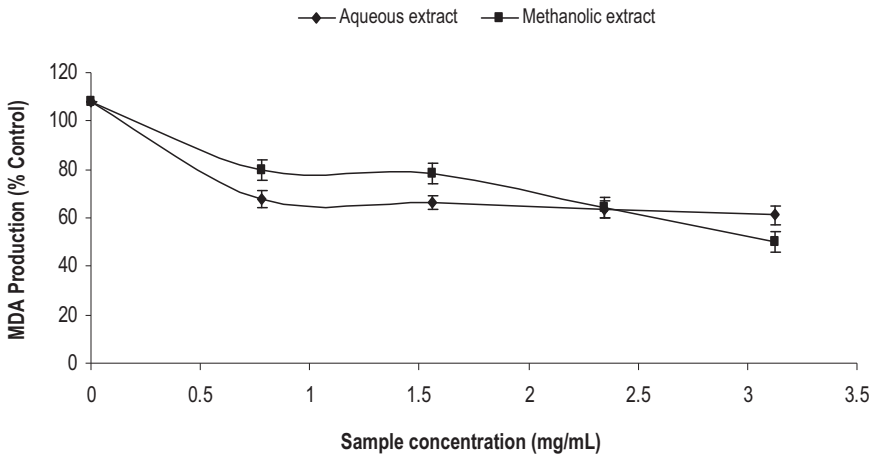

Fig. 2. Inhibition of Fe^{2+} induced lipid peroxidation in rat brain by aqueous and methanolic extract from Heinsia crinita leaves. Values represent mean ± standard deviation ($n = 3$).

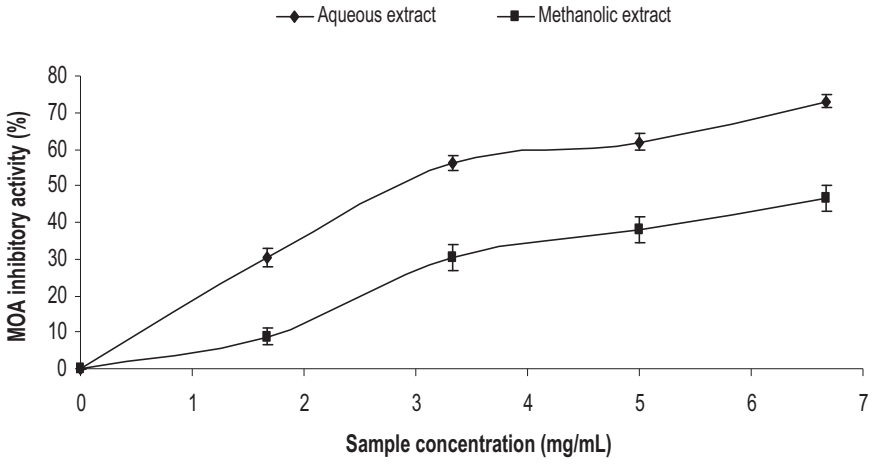

Fig. 3. Inhibition of monoamine oxidase activity by aqueous and methanolic extract from *Heinsia crinita* leaves. Values represent mean ± standard deviation ($n = 3$).

[4,30], and also prevent the release of ROS from the degradation of amine [31]. The inhibitory effect of the extracts could be attributed to their phenolic contents. Previous report has shown that flavonoids can inhibit MAO activity due to their structural similarities with synthetic MAO inhibitors [32]. The higher inhibitory effects observed in the aqueous extract correlates with the high flavonoid content when compared to the methanol extract.

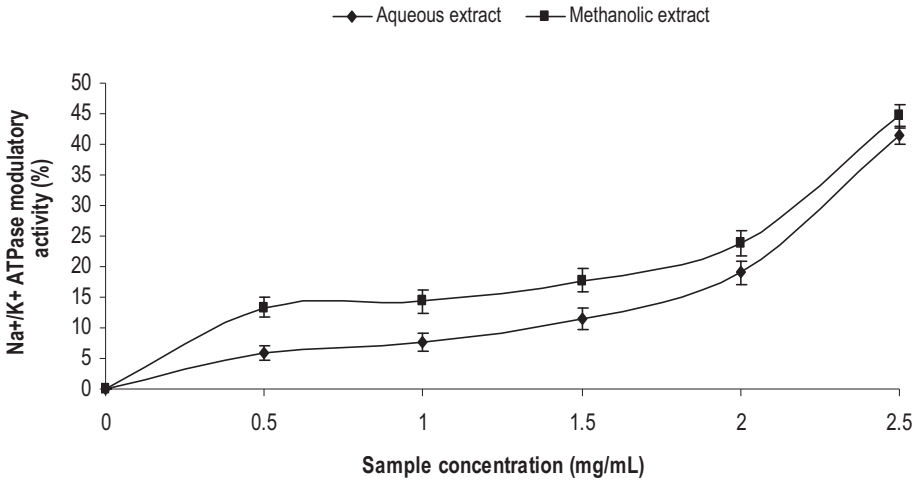

Fig. 4. Stimulation of Na^+/K^+ ATPase activity by aqueous and methanolic extract from *Heinsia crinita* leaves. Values represent mean ± standard deviation ($n = 3$).

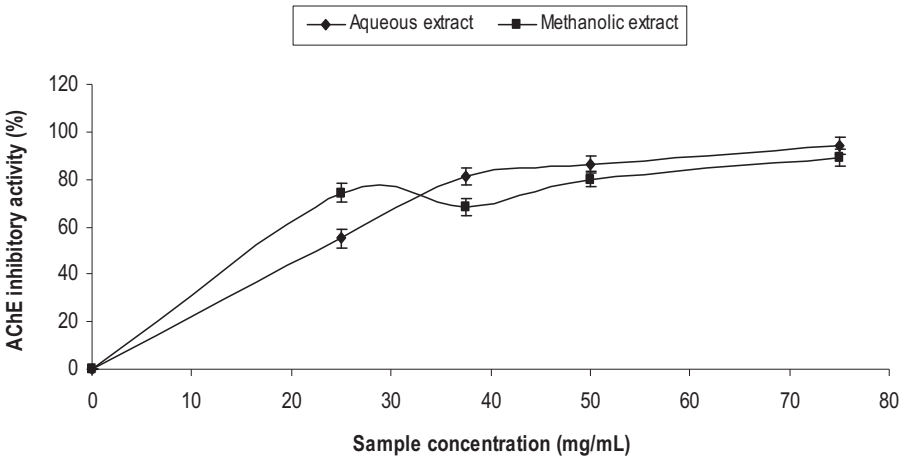

Fig. 5. Inhibition of acetylcholinesterase activity by aqueous and methanolic extract from *Heinsia crinita* leaves. Values represent mean ± standard deviation ($n = 3$).

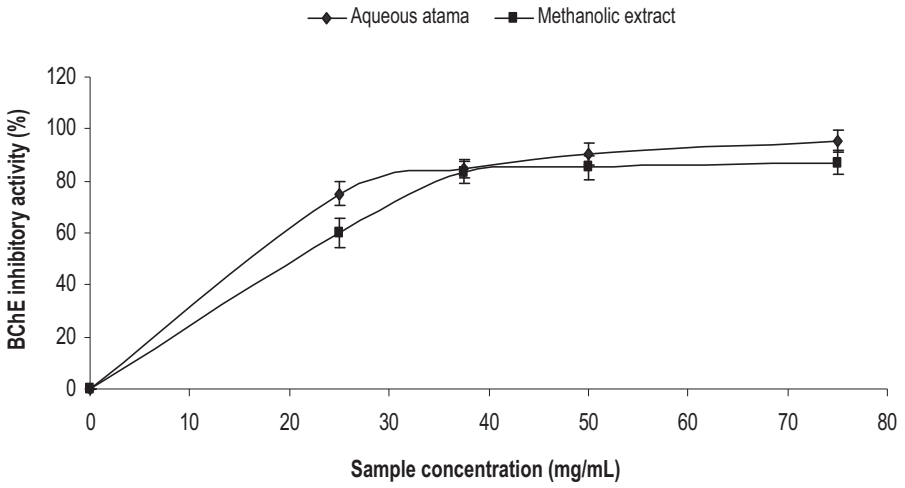

Fig. 6. Inhibition of butyrylcholinesterase activity by aqueous and methanolic extract from *Heinsia crinita* leaves. Values represent mean ± standard deviation ($n = 3$).

Furthermore, the effects of the extracts on Na^+/K^+-ATPase activity revealed that there was an increase in the activity of the enzyme. The methanol extract (13.2–44.6%) had better Na^+/K^+-ATPase stimulatory ability compared to aqueous extract (5.7–41.5%). Increase in Na^+/K^+-ATPase activity is an important therapeutic target in the management of some neurodegenerative diseases [33,34]. This is because Na^+/K^+-ATPase is required for memory function, neuronal-ion balance and transmission of messages in the synaptic cleft. Reduction of Na^+/K^+-ATPase activity could lead to cognitive impairment and neuronal damage characterized by necrosis and apoptosis [35]. Several reports have indicated decrease in Na^+/K^+-ATPase activity in some neurological disorders such as AD, epilepsy, depression and cerebral ischemia [10,36]. In this study, the extracts stimulated Na^+/K^+-ATPase activity which could be due to their phenolic constituents. This result agrees with that of Subash et al. [37], that pomegranate improved Na^+/K^+-ATPase activity. Furthermore, Javorková et al. [38] reported that there is a relationship between polyphenols and increase in Na^+/K^+-ATPase activity. Moreover flavonoids such as quercetin and rutin have previously been reported to be potent modulators of Na^+/K^+-ATPase activity [37,39]. It is important to note that oxidative stress caused by overproduction of ROS could reduce the activity of Na^+/K^+-ATPase [40] which consequently affects the depolarization of neurons and induce oxidative damage to the nerve cells [41]. Therefore, the ability of the extracts to stimulate Na^+/K^+-ATPase activity and inhibit lipid peroxidation in isolated rat brain tissue homogenate could be of great therapeutic importance in the management of several neurodegenerative diseases.

The AChE inhibitory activity of the extracts revealed that the aqueous extract had higher inhibitory effect (32.11 mg/mL) than methanol extract (33.67 mg/mL). Similarly, the aqueous extract (30.4 mg/mL) also had higher inhibitory effect on BChE activity than the methanol extract (32.95 mg/mL). Cholinesterases (AChE and BChE) catalyze the rapid breakdown of acetylcholine (ACh) and butyrylcholine (BCh) into acetate and choline. Moreover, this could lead to cholinergic deficit and cognitive impairment. Hence, inhibition of cholinesterases could be a good therapeutic target for the treatment and management of AD as it can increase the concentration of ACh and BCh in the synaptic cleft and consequently improve communications between nerve cells in the brain [42]. Our findings revealed that *H. crinita* leave extracts had inhibitory effects on AChE and

BChE activities which is consistent with earlier studies on some fruits and vegetables [37,43]. In addition, inhibition of BChE activity is therapeutically important in the management of AD; increase in BChE activity could lead to increase in the production of neurotoxic plaques which is a risk factor in the pathogenesis of AD [44–46]. The aqueous extract had higher inhibitory effects than methanol extract and this could be due to the higher total phenol and flavonoid contents that was observed in the aqueous extract. Phenolic acids and flavonoids are known to have structural similarities with synthetic cholinesterase inhibitors such as donepezil, rivastigmine and prostigmine [47,48]. Moreover, molecular docking studies have shown that flavonoids such as quercetin, quercitrin, rutin and kaempferol which were identified in *H. crinita* leaves have aromatic rings (B-rings) and hydroxyl groups that could bind to the peripheral anionic site of cholinesterases thereby blocking the natural substrate from binding to the site [47–49]. Furthermore, according to the report of Roseiroa et al. [48], the methoxyl groups of caffeic acid could bind to the tryptophan residue on the active site of the enzyme thereby reducing its activity. Interestingly, several reports have shown that dietary polyphenols can cross blood–brain barrier, either in their natural form or as metabolites [50,51].

4. Conclusion

This study revealed that aqueous and methanol extracts from *H. crinita* leaves modulates the activities of enzymes (MOA, AChE, BChE and Na^+/K^+-ATPase) linked to neurodegenerative diseases, as well as inhibit Fe^{2+}-induced lipid peroxidation in isolated rat brain; these biological activities could be part of the possible mechanisms by which the extracts exert their neuroprotective abilities and could be linked to their phenolic constituents. However, the aqueous extract showed higher neuroprotective properties than the methanol extract. These findings have given a clue on the dietary and medicinal importance of *H. crinita* leaves as an alternative/complementary therapy for the treatment/management of neurodegenerative diseases. Nevertheless, further *in vivo* and clinical experiments are recommended.

Conflict of interest

All authors declare no conflict of interest.

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