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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.
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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

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