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In vitro neuroprotective properties of some commonly consumed green leafy vegetables in Southern Nigeria

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ABSTRACT

Green leafy vegetable is one of the major cuisines in Southern Nigeria and they are not only consumed for their palatability, but also for their nutritional and medicinal properties as reported in folklore. Notable among them are afang (*Gnetum africanum*), editan (*Lasianthera africana*) and utazi (*Gongronema latifolium*). In this study, we investigated the effect of aqueous extracts from afang, editan and utazi leaves on cholinesterases [acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)] and monoamine oxidase (MAO) activities. Fe^{2+} chelating abilities were also determined as an assessment of their neuroprotective potentials *in vitro*. We also assayed for their total phenol contents while the constituent phenolics were characterized using high performance liquid chromatography coupled with diode array detector (HPLC-DAD). The results revealed that the extracts inhibited AChE, BChE and MAO activities and also chelated Fe^{2+} in concentration dependent manner. The HPLC-DAD characterization showed that gallic, caffeic and ellagic acids and rutin were the dominant phenolic compounds in the extracts; nevertheless, utazi had the highest distribution of identified phenolics while afang had the least. The ability of the aqueous extracts of the vegetables to inhibit key enzymes (AChE, BChE and MAO) relevant to neurodegeneration, as well chelate metal ion could help suggest their possible neuroprotective properties. These vegetables could be use as dietary intervention in the management of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.

1. Introduction

Neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) are pathologies of multiple etiologies. Several evidences lay credence to the decline in the brain neurotransmitters such as acetylcholine (ACh) and neuroactive amine, as well as oxidative stress induced by accumulation of metal ions in the brain as some of the key factors in the pathogenesis and progression of these diseases [1]. It has therefore become imperative to develop multidimensional therapeutic approach at the prevention and management of these diseases by focusing attention on these key etiological factors. In this regard, cholinesterase and monoamine oxidase inhibitors, as well as antioxidant therapy, have been the focus of drug design [1,2]; nevertheless, the importance of dietary interventions as complementary approach at prevention and management of these diseases cannot be overemphasized.

Cholinergic neurons make use of ACh as neurotransmitter which is rapidly hydrolyzed by cholinesterases after its release to the synaptive cleft [3,4]. Therefore, cholinesterase inhibitors such as galantamine and physostigmine have been designed for therapeutic management of AD [5]. The activity of Monoamine oxidase (MAO) involves in the breakdown of monoamine neurotransmitters [6]. Due to the important role these neurotransmitters play in neuronal function, MAO has become strategic in the management of several neurodegenerative diseases [6,7].

Green leafy vegetables form major constituent of local diets in Southern Nigeria. They are not only desired for their nutritional benefits, but also for their medicinal properties as reported in folklore. Notable among them are *Gnetum africanum*, *Lasianthera africana* and *Gongronema latifolium*. *G. africanum* is a leafy vegetable desired in different African countries and especially south-eastern Nigeria (where it is commonly referred to as afang) for its nourishment and medicinal properties [8,9]. *L. africana* is known as 'editan' among the south-eastern Nigerians and it is well known for its nutritional and medical benefits [10]. It has been used traditionally for treatment of various diseases such as fever, ulcer and diabetes [11]. Utazi (*G. latifolium*) is another vegetable that is well desired for its nutritive and medicinal properties. Its leaf is consumed as spice and in preparation of soups and stews [12]. Furthermore, the hypolipidermic, hyperglycemic, anti-inflamatory and antioxidant properties of this vegetable has been previously reported [13,14]. In this present study, we investigated the effect of aqueous extracts of afang, editan and utazi leaves on cholinesterases (ChEs)

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and MAO activities. Phenolic constituents of the vegetables were also characterized using HPLC-DAD. The findings of this study are expected to shed some light on possible neuroprotective properties of these vegetables.

2. Materials and methods

2.1. Sample collection and preparation of the extracts

Fresh sample of afang, editan and utazi were harvested from local farmland in Uyo, Nigeria, during the raining season (June) of 2013. The samples were identified and authenticated at Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The leaves were plucked from its trunk, rinsed under running tap water and dried for twenty days at room temperature (under shade) to constant weight. The dried leaves were pulverized using the method of Mukhtar and Turkur [15]. Thereafter, 0.5 g of each pulverized samples was extracted with 100 mL of distilled water and was filtered with Whatman no. 1 filter paper. The filtrate was kept in the refrigerator (≤4^{0}C) for subsequent analysis.

2.2. Chemicals and reagents

Chemicals and reagents used such as gallic acid, quercetin, Folin–Ciocalteau's reagent, semicarbazide and benzylamine, acetylthiocholine and butyrylthiocholine iodide were procured from Sigma-Aldrich, Inc., (St Louis, MO); dinitrophenyl hydrazine (DNPH) from ACROS Organics (New Jersey, USA), methanol and acetic acid were sourced from BDH Chemicals Ltd., (Poole, England). All other chemicals were of analytical grade while the water used for all analysis was glass distilled

2.3. Enzyme assays

2.3.1. Cholinesterases inhibition assay

The acetylcholinesterase (AChE) activity was determined in a reaction mixture containing 200 μL of AChE solution (EC 3.1.1.7) in 0.1 M phosphate buffer, pH 8.0, 100 μL of a solution of 5,5′-dithio-bis(2-nitrobenzoic) acid (DTNB) 3.3 mM in 0.1 M phosphate buffered solution, pH 7.0, containing NaHCO3 (6 mM), extracts (0–100 μL) and 500 μL of phosphate buffer, pH 8.0. After incubation for 20 min at 25 °C, 100 μL of 0.05 mM acetylthiocholine iodide was added as the substrate. AChE activity was determined by monitoring changes in the absorbance at 412 nm for 3 min. Hundred microliter of butyrylthiocholine iodide was used as a substrate to assay for BChE activity, while all the other reagents and conditions remained the same

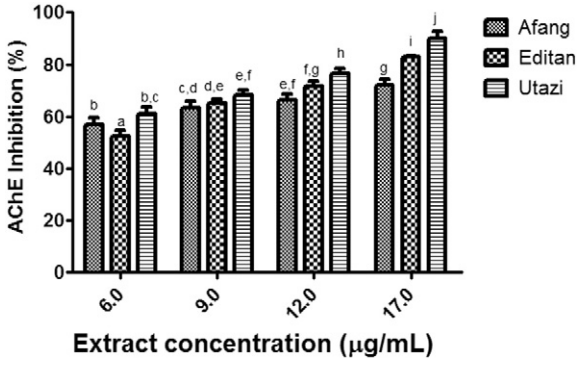

Fig. 1. Percentage acetylcholinesterase (AChE) inhibitory effect of aqueous extracts of *Gnetum africanum* (Afang), *Lasianthera africana* (Editan) and *Gongronema latifolium* (Utazi) leaves. Bars not sharing a common superscript letter are significantly different at P < 0.05.

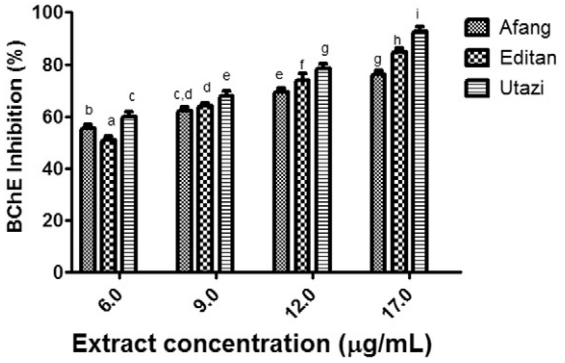

Fig. 2. Percentage butyrylcholinesterase (BChE) inhibitory effect of aqueous extracts of *Gnetum africanum* (Afang), *Lasianthera africana* (Editan) and *Gongronema latifolium* (Utazi) leaves. Bars not sharing a common superscript letter are significantly different at P < 0.05.

[16]. The percentage inhibitory effect of the extracts on AChE and BChE activities was subsequently calculated as:

$$\text{Percentage Inhibition} = [(\text{Abs}_{\text{ref}} - \text{Ab}_{\text{ext}})/\text{Abs}_{\text{ref}}] * 100$$

Where, Abs_{ref} is the absorbance without sample and Abs_{extr} is the absorbance of the extract.

2.3.2. MAO inhibitory assay

The effect of the extracts on MAO activity was measured according to a previously reported method [17], with slight modification. In brief, the mixture contained 0.025 M phosphate buffer (pH 7.0), 0.0125 M semicarbazide, 10 mM benzylamine and 75 μL of MAO solution (EC 1.4.3.4) and 0–100 μL of the extracts. After 30 min, acetic acid was added and boiled for 3 min water bath followed by centrifugation. The resultant supernatant (1 mL) was mixed with equal volume of 2,4-DNPH and 1.25 mL of benzene (absolute) was added after 10 min of incubation at room temperature. After separating the benzene layer, equal volume of 0.1 N NaOH was added. Alkaline layer was decanted and heated at 80°C for 10 min. The orange–yellow color developed was measured at 450 nm in a spectrophotometer. The percentage inhibitory effect of the extracts on MAO activity was subsequently calculated as:

$$\text{Percentage Inhibition} = [(\text{Abs}_{\text{ref}} - \text{Ab}_{\text{ext}})/\text{Abs}_{\text{ref}}] * 100$$

Where, Abs_{ref} is the absorbance without sample and Abs_{extr} is the absorbance of the extract.

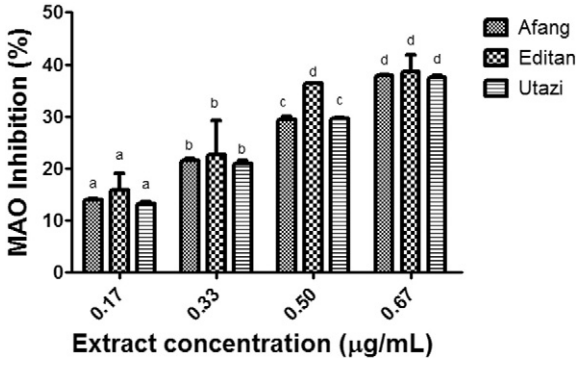

Fig. 3. Percentage monoamine oxidase (MAO) inhibitory effect of aqueous extracts of *Gnetum africanum* (Afang), *Lasianthera africana* (Editan) and *Gongronema latifolium* (Utazi) leaves. Bars not sharing a common superscript letter are significantly different at P < 0.05.