Food Bioscience 1 (2013) 48–56

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/fbio

Polyphenolic extract of *Blighia sapida* arilli prevents N-nitrosodiethylamine-mediated oxidative onslaught on microsomal protein, lipid and DNA

Hussein Oyelola Bukoye Oloyede[a,b], *Taofeek Olakunle Ajiboye*[a,*], *Yesirat Omonike Komolafe*[a], *Amodu Kayode Salau*[c]

[a]*Antioxidants, Free Radicals, and Toxicology Research Laboratory, Biochemistry and Nutrition Unit, Department of Chemical Sciences, Fountain University, Osogbo, Nigeria*

[b]*Nutritional Biochemistry Research Laboratory, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria*

[c]*Phytomedicine Research Laboratory, Biochemistry and Nutrition Unit, Department of Chemical Sciences, Fountain University, Osogbo, Nigeria*

ARTICLE INFO

Article history:
Received 11 July 2012
Received in revised form
15 February 2013
Accepted 6 March 2013

Keywords:
Blighia sapida
N-nitrosodiethylamine
Reactive oxygen species
Protein carbonyl
DNA fragmentation

ABSTRACT

Polyphenolic extract of *Blighia sapida* arilli was evaluated for reactive oxygen species (ROS) scavenging and detoxification potentials in rat microsomes. ROS scavenging potentials of the polyphenolic extract of *B. sapida* (0.2–1.0 mg/mL) was investigated using DPPH radical, superoxide anion radical, hydrogen peroxide, hydroxyl radical and ferric ion reducing system. The detoxification of ROS was evaluated in N-nitrosodiethylamine-induced redox imbalance in rat microsomes. *B. sapida* polyphenolic extract (1.0 mg/mL) scavenged DPPH, superoxide anion radical, hydrogen peroxide, and hydroxyl radical by 60, 67, 63, and 57%, respectively, while ferric ion was significantly reduced. N-nitrosodiethylamine-mediated decrease in the activities of ROS detoxifying enzymes was significantly ($P<0.05$) attenuated. N-nitrosodiethylamine-mediated elevation in the concentrations of oxidative stress biomarkers: malondialdehyde, conjugated dienes, lipid hydroperoxides, protein carbonyl and percentage DNA fragmentation were significantly ($P<0.05$) lowered by *B. sapida* polyphenolic extract. Overall, the polyphenolic extract of *B. sapida* arilli elicited ROS scavenging and detoxification potentials and prevented lipid peroxidation, protein oxidation and DNA fragmentation.

1. Introduction

Reactive oxygen species (ROS) derived free radicals such as superoxide anion ($O_2^{\bullet-}$), hydroxyl ($HO^{\bullet}$), peroxyl ($RO_2^{\bullet}$), and alkoxyl ($RO^{\bullet}$) radicals, as well as O_2-derived non-radical species such as hydrogen peroxide (H_2O_2) perturb cellular functions through oxidation of cellular macromolecules (proteins, lipids and DNA). They are generated in response to both endogenous and exogenous stimuli (Ushio-Fukai & Nakamura, 2008). Regardless of the origin, increased ROS production or oxidative stress has two consequences: activation of specific signal transduction pathways and damage to cellular components leading to adaptive and maladaptive molecular responses respectively (Covarrubias, Hernandez-Garcia, Schnabel, Salas-Vidal, & Castro-Obregon, 2008). ROS act as signaling molecules mediating cell growth and differentiation under normal physiological conditions, whereas at higher concentrations, they induce cell death, apoptosis and

*Corresponding author. Mobile: +2348035844608.
E-mail address: ajiboyeyong@yahoo.com (T.O. Ajiboye).

2212-4292/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fbio.2013.03.003

senescence (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006). Accumulative ROS production is suggested to stimulate oncogenesis via alterations in redox regulated signaling pathways suggesting that the redox state plays a critical role in signal transduction, cellular proliferation, differentiation and apoptosis (Chandra, Samali, & Orrenius, 2000; Valko et al., 2006).

Multiple pathways involved in ROS-induced cell death have been proposed. ROS can cause direct injury to proteins (carbonylation), lipids (lipid peroxidation), and nucleic acids (DNA fragmentation), leading to misregulation of wide variety of enzymatic process and growth factors that can result in cell death (Stadtman & Levine, 2000). Lipid peroxidation-mediated cell death has been linked with the activation of spingomyelinase and release of ceramides, which activate apoptosis (Fruhwirth & Hermetter, 2008). Nucleic acid oxidation has been linked with physiologic and premature aging as well as DNA strand breaks, leading to necrosis and/or maladaptive apoptosis (Auten, Whorton, & Nicholas, 2002). The magnitude of these changes and the cell's ability to repair this damage determines whether the effects are adaptive or maladaptive. Chemical compounds (both natural and synthetic) known to have free radical scavenging properties could effectively protect against this damage only upon their absorption. Among these numerous compounds includes the polyphenols and flavonoids.

Polyphenols and flavonoids are widely distributed in plants most especially cereals, fruits and vegetables (Krishnaswamy & Polasa, 2001). Polyphenols and flavonoids have been shown to have health benefits by several researchers. Wise use of fruits, medicinal plants, and vegetables requires investigations into the phytochemicals and antioxidants as well as the possible medicinal properties and prospective products, such as nutraceuticals and phytomedicines. In recent years, we have shown the antioxidants and cytoprotective effects of some medicinal plants and alluded the protective role to the polyphenolic and flavonoid constituents of the plants (Ajiboye et al., 2010a, b; Ajiboye, Salawu et al., 2011; Ajiboye, Yakabu, & Oladiji, 2011; Ajiboye, 2011).

Blighia sapida (König), also known as ackee, is a tropical to subtropical plant in the soapberry family (Sapindaceae) and indigenous to equatorial Africa. It is cultivated in the West Indies, Central and South America, and Florida for its edible yellow fruit arils. It is commonly known as Ackee, in Nigeria it is called Gwanja Kusa (Hausa), Isin (Yoruba) and Okpu (Igbo). It is an evergreen tree of about 33–40 ft (10–12 m) with a dense crown spreading branches. It is rather handsome, usually with a short trunk of 6 ft (1.8 m) in circumference. The bark is gray and nearly smooth (Morton, 1987). The flower is small, greenish-white and about 5 mm long (Purseglove, 1987). Owonubi (1986) reported that the fleshy pulp is usually eaten raw, made into soup or fried in oil.

Phytochemical investigation of the extracts of *B. sapida* shows the presence of some groups of phytochemicals such as saponins, reducing sugar, phytosterols, and polyamide (Antwi, Martey, Donkor, & Nii-Ayitey, 2009). Isolation of six principal groups of compounds: triterpenes, steroids, and their glycosides (collectively called saponins), sesquiterpenes, quinines, alkaloids, and polyphenols have also been reported (Asprey & Hornton, 1955; Balogun & Fetuga, 1988; Garg & Mitra, 1967; Stuart, Roberts, & Whittle, 1975). Recently, Parkinson (2007) ascribed the high antioxidant and high total phenolic content of the ackee pods to six known polyphenols.

In furtherance of our investigations into the possible antioxidant and cytoprotective mechanism present in fruits, medicinal plants and vegetables, this study investigates the reactive oxygen specie scavenging and detoxification potentials of *B. sapida* arillil's polyphenolic extract in rat microsomes.

2. Materials and methods

2.1. Materials

2.1.1. Experimental animal

Healthy, 2 month, male albino rat (*Rattus norvegicus*) of Wistar strain, weighing 158 g was obtained from animal house of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria.

2.1.2. Plant materials

Fresh and ripe *B. sapida* fruits were collected at Ido-Osun, Osogbo, Nigeria in November 2011. The plant sample was authenticated and deposited in the Herbarium unit of Forestry Research Institute of Nigeria, Ibadan, Oyo state with Voucher no: FHI. 109506.

2.1.3. Chemicals and assay kits

Diphenylamine, 5,5′-Dithio-bis(2-nitrobenzoic acid), guanidine hydrochloride, *N*-ethyl-maleimide (NEM), and salicylic acid were procured from Research Organics, Cleveland, Ohio, USA. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-red) and glucose 6-phosphate dehydrogenase (Glc-6-PD) were products of Randox Laboratories Ltd., Co. Antrim, United Kingdom. All other reagents used were supplied by Sigma-Aldrich Inc., St. Louis, USA.

2.2. Methods

2.2.1. Preparation of *Blighia sapida* arillil's polyphenolic extract

The polyphenolic extract of *B. sapida* arilli was prepared as described by Ajiboye et al. (2013) with slight modification. Briefly, the arilli of *B. sapida* fruits was nipped from the black shiny seeds and freeze-dried using lypophilizer (LTE SCIENTIFIC LTD, Greenfield, Oldham OL37ET). The lyophilized arillis (500 g) were homogenized, exhaustively and successively extracted using hexane, ethyl acetate and methanol. The filtered extracts were concentrated under reduced pressure using rotatory evaporator and kept frozen.

2.2.2. Qualitative and quantitative determination of total phenolics and flavonoids

2.2.2.1. *Qualitative phytochemical screening*. A preliminary phytochemical screening was done on the polyphenolic extract of *B. sapida* arilli to test for the presence of phenolics and flavonoids using the procedure described by Sofowora (1993).