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PRELIMINARY INVESTIGATION OF RADICAL SCAVENGING POTENTIAL OF BLACK-PLUM *VITEX DONIANA* SEED

Eze-Steven, P.E[1]., Edeh, F.O[1]. and *Obeagu Emmanuel Ifeanyi[2]

[1]Applied Biochemistry Department, Enugu State University of Science and Technology, Agbani, Enugu State, Nigeria.
[2]Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria

Email : emmanuelobeagu@yahoo.com

ABSTRACT

Highly reactive molecules (NO, $O2^{-}$, HOO^{-}) generated in biological systems are associated with initiation of degerative diseases. The antioxidants can reduce this risk. Fruits are one of the most important sources of antioxidants such as vitamins and phenolic phytochemicals. *Vitex* plants are wild tropical plant which has found wide application in traditional medicine and human food in the west and central African regions. This study was conducted to test radical scavenging activity of fruits of *Vitex doniana* and to compare the values with results of other scholars. The free radicals deactivating ability were measured using DPPH and BHA scavenging assay. The extracts demonstrated good scavenging activities against DPPH and BHA assays which were not significantly different at ($p<0.05$). Thus these results suggest that extract *Vitex doniana* may serve as potential source of natural antioxidant for food and pharmaceutical application.

Keywords: *RADICAL, SCAVENGINGPOTENTIAL, BLACK-PLUM, Vitex doniana SEED*

Introduction

Free radicals such as the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated through endogenous processes such as metabolism, respiration and phagocytosis. They are also generated by exogenous systems such as pesticides, some pollutants, organic solvents and during radiation.

However, the generation of these free radicals is normally balanced by an equivalent production of antioxidants through our natural antioxidant defence mechanism, which are the enzymatic antioxidants (superoxide dismutase, glutathione peroxidase, quinonereductase and catalases) and the non-enzymatic antioxidant (ascorbic acid, a-tocopherol, melatonin, β-carotene) obtained from the diet (Chun-Weng, 2011). However when the generation of free radicals overwhelm the antioxidant capacity of the biological defence system; it gives rise to oxidative stress. When oxidative stress occurred, it eventually leads to several deteriorating effects to our cellular bio-molecules such as DNA damage, lipid peroxidation, tissue injury and protein degradation (Chun-Weng, 2011). Therefore, oxidative stress is increasingly recognized for their contribution to a number of diseases such as cancer, arthritis, neurodegenerative disorders, atherosclerosis and aging (Praveen and Awang, 2007). This concept is supported by increasing evidence that oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants oppose this and lower risk of disease.

Recently, there is considerable interest in finding out about the antioxidants that are present in plants. Plants are the potential source of natural antioxidants that can protect against oxidative stress and therefore, have a main role to protect against injuries from lipid peroxidation. Carotenoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, to copherols, tocotrienols, polyphenols such as phenolics acids, flavonoids, proanthocyanidins, among others, are some of the antioxidants produced by plants for their survival (Praveen and Awang, 2007; Jimoh, 2008). Black–plum (*Vitexdoniana*) of the family *Verbanaceae* is a tree crop that grows in open woodland and savannah regions of tropical Africa and some East African countries including Uganda, Kenya and Tanzania and high rainfall areas. It produces fruits which are black, edible, sweet and mealy. It is frequently eaten as a snack and sold in local markets. The fruits are approximately 3cm long and contain one to four seeds. Fresh fruits cannot be stored for long time periods (Ruffo*et al*., 2012). The fruit is green when mature and changes to dark brown when fully ripe, with the pulp surrounding a hard stone containing 1–4 seeds. The fruits are also referred to as black-plum or African olive (Glew*et al*., 2007). It has been reported that syrup similar to honey was produced from the fruit and that physicochemical and sensory results showed that it can be substituted for other syrups as a nutritive sweetener. There are also reports of its potential use in the

production of wine and jam (Okigbo, 2011).

1.1 Aim And Objectives

The study was done to investigate the radical scavenging activity of black plum grown in Udi Area of Enugu State with the view of recommending the seeds as alternative source of antioxidants that can scavenge free radicals.

Materials and Methods

Materials

Sample Collection

The sample used was the seed of (*Uchakiri*) *Vitexdoniana* known as black-plum and was bought at Eke Market Agbani, Enugu State. It was brought to the Enugu State University of Science and Technology (ESUT) where it was identified and authenticated by Prof.Eze of the Department of Applied biology and Biotechnology.

Method

Reagent Preparation

10g Aluminum trichloridemonohydride

Aluminumtrichloridemonohydride (10g, 100ml) was prepared by weighing exactly 10.0g of AlCl3 into a 100ml volumetric flask using Harvard trip balance. Measuring cylinder (50ml) was used to measure 20ml of distilled water which was used to dissolve the pellet in a 100ml volumetric flask, after which an additional quantity of distilled water was added to make up to the mark. The AlCl3 solution was transferred to a 100ml china reagent bottle.

1.0M Sodium Hydroxide

Sodium Hydroxide (1.0M, 100ml) was prepared by weighing exactly 4.0g of NaOH into a 500ml volumetric flask using Harvard trip balance. Measuring (100ml) cylinder was used to measure 20ml of distilled water which was used to dissolve the pellet in a 100ml volumetric flask, after which an additional quantity of distilled water was added to make up to the mark. The NaOH solution was transferred to a 100ml china reagent bottle.

0.5M Sulphuric Acid

Sulphuric acid (0.5M, 250ml) was prepared by measuring exactly 7.0ml of Sulphuric acid into a 250ml volumetric flask using a 50ml Pyrex measuring cylinder. About 30ml of distilled water was used to dilute it before an additional quantity of distilled water was used to make up to the mark. The acid solution was stored in 250ml china reagent bottle.

Sample Preparation

Preliminary Work

The sample (the seeds of *Vitex doniana*) was separated from pebbles and microbial infected ones by hand-picking. The sample was washed with tap water, air-dried using an open pan before grinding it whole together with the pulp coat using a manual Japan-engine grinder. The grinding was repeated severally to obtain the finest surface area and kept in air-tight plastic container at 25^0C prior to use. *Vitex doniana* flour (3.0g) was weighed into a clean empty storage bottle using the OHAUS trip balance and100ml of methanol was added using pyrex measuring cylinder. The mixture was vortexed for 24 hours so as to break the bioactive components of the sample before filtering the residue using Whatman No.4 filter paper. The residue was resoaked with another 100ml of methanol overnight to re-extract the active components. The two different

filtrates were combined in a beaker heat to evaporate the solvent at 400C. After the evaporation, the filtrate was re-dissolved with 100ml of methanol and stored at 40C in a storage bottle prior to use.

Sample Analysis

Dpph Radical Scavenging Assay (Dpph)

The DPPH radical scavenging activity of *Vitexdoniana* extract was assayed by the DPPH radical scavenging activity assay. DPPH solution was prepared by dissolving 6 mg of DPPH in 100 ml of methanol. To 0.3ml of various concentration of the extracts (200, 400, 600, 800, 1000 µg/ml), 6x10-5mM, 2.7mlml of methanolic solution containing DPPH solution. The mixture was shaken vigorously and left to stand for 60mins in the dark (until stable absorbance values were obtained). The reduction of the DPPH radical was determined by measuring the absorbance at 517nm wavelength. The radical scavenging activity was calculated at a percentage of DPPH dis-colouration using the equation:

% RSA = [(ADPPH - As)/ ADPPH X 100]

Where: **As**say the absorbance of the sample extract and **A** is the absorbance of the DPPH solution. The extract concentration providing 50% of radical scavenging activity (EC50) was calculated from the graph of RSA (%) against extract concentration. BHA and DPPH were used as standard.

RESULTS

Table 1: Standard curve of BHA for the determination of radical Scavenging Activity of *Vitexdoniana*

S/No	1	2	3	4	5	6
Conc. (µg/ml)	0	200	400	600	800	1000
Vol. of extract (ml)	0	0.5	1.0	1.5	2.0	2.5
Vol. of methanol (ml)	2.5	2.0	1.5	1.0	0.5	0.0
Total vol. (ml)	2.5	2.5	2.5	2.5	2.5	2.5

Table 2: Standard curve of DPPH for the determination of Radical Scavenging Activity of *Vitexdoniana*

S/NO	1	2	3	4	5	6
Conc. (µg/ml)	0	200	400	600	800	1000
Vol. of extract (ml)	0	0.2	0.4	0.6	0.8	1.0
Vol. of methanol (ml)	1.0	0.8	0.6	0.4	0.2	0.0
Total vol. (ml)	1.0	1.0	1.0	1.0	1.0	1.0

Table 3: Results of Concentration and Absorbance of Standard Solutions (DPPH and BHA) and *Vitexdoniana*

S/No	Conc. of extract (µg/ml)	ABSORBANCE (nm)	% RSA of *Vitex doniana*	ABSORBANCE (nm)	% RSA of *Vitex doniana*
		DPPH		BHA	
1	0.00	0.00	0.00	0.000	
2	200	0.539	65.6	0.311	80.20
3	400	0.229	85.4	0.275	82.50
4	600	0.206	86.9	0.238	84.80
5	800	0.110	93.0	0.187	88.10
6	1000	0.106	93.2	0.154	90.20

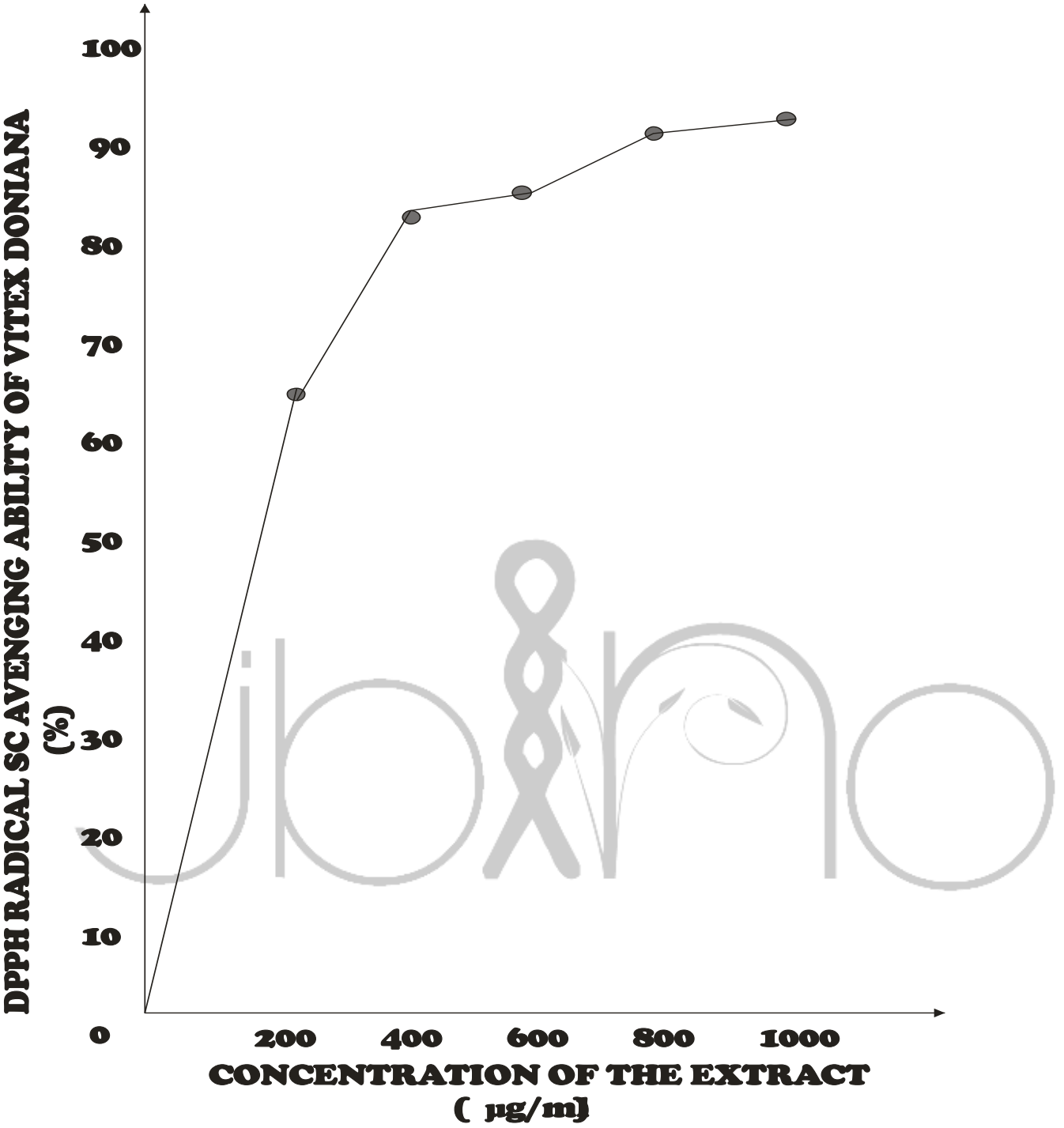

Figure 1: Standard curve of DPPH radical scavenging activity (%) against concentration of *Vitex doniana* extract (µg/ml)

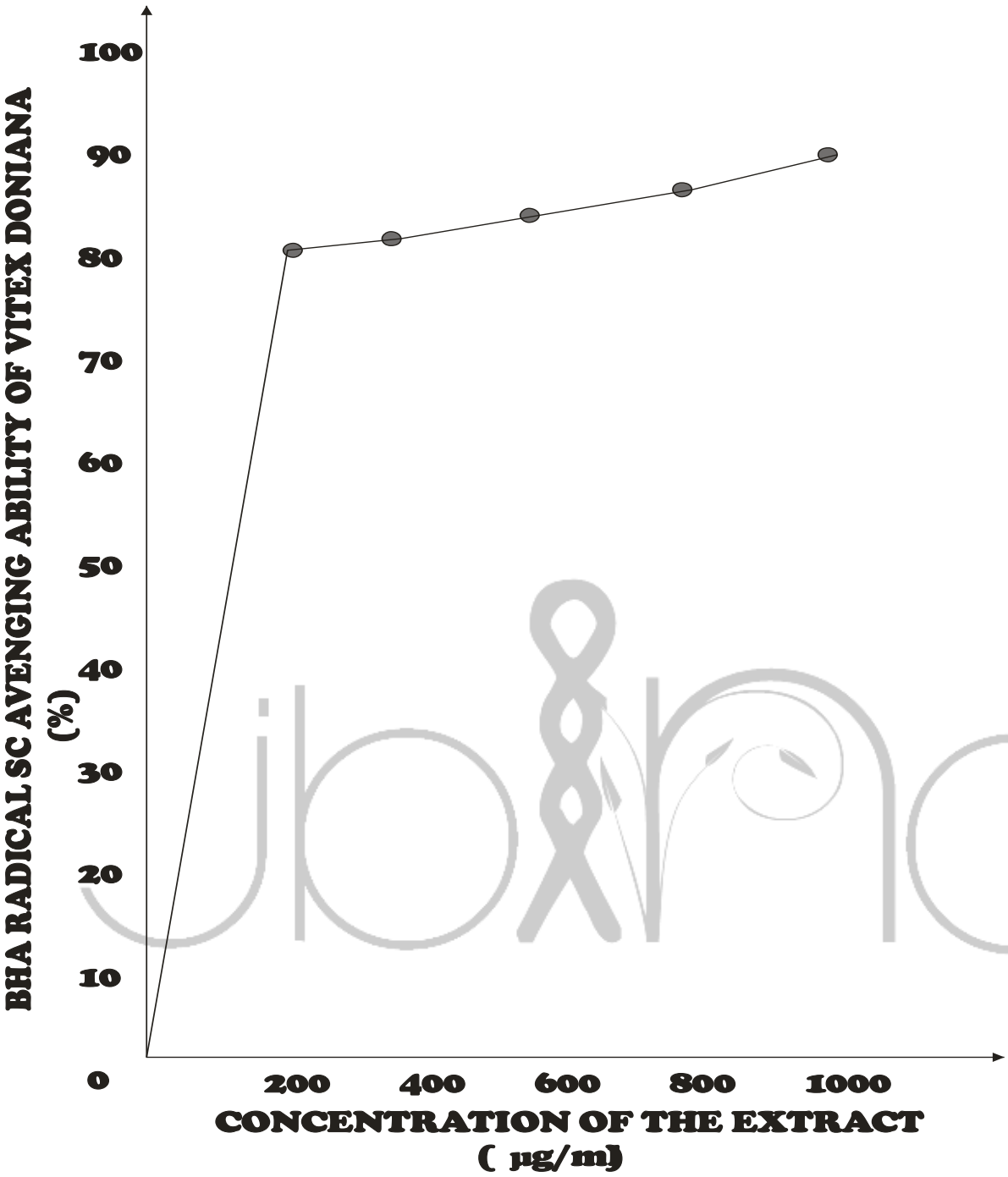

Figure 2: Standard curve of DPPH radical scavenging activity (%) against concentration of *Vitexdoniana* extract (µg/ml)

Discussion

Radical scavenging activity is strongly correlated with the total phenolic contents of *Vitex doniana* extracts. HighR2 values for DPPH radical scavenging activity (0.758-0.995) indicate that at least 76% of the free radical scavenging activities detected can be attributed to total phenolic contents.DPPH method measured the primary antioxidant activity of plant extracts because it is one of the most effective methods for evaluating the concentration of radical scavenging materials actively by a chain-breaking mechanism. The reduction capability of DPPH was determined by the decrease in its absorbance at 520nm induced by antioxidants. Results were reported as IC50, which was defined as the amount of antioxidant required to inhibit 50% of DPPH free radical under experimental conditions (Ismail *et al*, 2010). The extract that required the lowest concentration to positive DPPH test suggest that the sample (*Vitex doniana*) was a high free radical scavenger. In this study, the IC50 result showed that the methanol seed extract scavenged 50% DPPH radicals at the lowest sample concentration. Our findings was in agreement with Ochieng and Nandwa, (2010) who reported that Phenolic compound s generally exhibited significant scavenging effects against the DPPH free radical. The extraction procedure does affect the antioxidant activity of the extracts. Radical scavenging activity is strongly correlated with the total phenolic contents of *Vitex doniana* extracts.

Conclusion

In this study, the extract of Seeds of *Vitex doniana* extracts has shown remarkable scavenging activities and thus proved it traditional uses in the management of diseases associated with oxidative stress. The lowest IC50 of the methanolic seeds extracts suggest to us that this extracts may have more potent free radical scavenging ability. This provides a complementary preventive value and supports their gaining popularity as under-utilized botanical food supplement. *Vitex doniana* (ripe fruits) demonstrated better RSA.

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