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DPPH free radical scavenging activities of leaf, stem bark, root, flower and fruit of *Blighia unijugata* Baker (Sapindaceae) extracts

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Abstract: Ethno-medicinally, extracts of Sapindaceae *species* in Nigerian folk's medicine have been utilized to address various health disorders for which free radicals have been implicated. The increasing quest for plant based novel compounds of medicinal relevance led to the comparative investigation of the antioxidant activities of extracts of five parts of *Blighia unijugata*.

Samples were collected and authenticated (Department of Botany) at University of Ibadan, Ibadan, Nigeria and separated into leaf, stem bark, root, flower and fruit. Flower and fruit were extracted separately in methanol (labeled MBuFlMeOH and MBuFsMeOH respectively), the leaf, stem bark and root extracts were obtained sequentially first with 100% hexane (MBuLfHex, MBuStHex, and MBuRtHex), and then with equal mixture of ethyl acetate and methanol (MBuLf 1:1, MBuSt 1:1, and MBuRt 1:1). Antioxidant activity evaluations was done using DPPH assay. Their 50% inhibitory concentration (IC₅₀) values were determined by regression analysis on Microsoft excel.

% inhibitions of DPPH for the eight extracts were between 32.26% - 96.56% which is comparable to values obtained for the standards (Vitamin C, Anisole and Vitamin E) used (63.36% - 97.09%). IC₅₀ values for DPPH scavenging activities of the eight extracts studied gave the following trend: MBuLf 1:1>MBuSt 1:1>MBuFs MeOH>MBuRt Hex>MBuRt 1:1>MBuFl MeOH>MBuSt Hex>MBuLf Hex, with

their corresponding IC₅₀ values being 0.0277 > 0.0284 > 0.0301 > 0.0317 > 0.0320 > 0.0342 > 0.0360 > 0.0400 (mg/ml) respectively.

All the five parts studied are potential good sources of free-radical scavenging compounds; this supports the ethno-medicinal application of *Blighia unijugata* as anti-ageing agent.

Keywords: Sapindaceae, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), antioxidant, *Blighia unijugata*, free radical,

INTRODUCTION

Since ancient times, plants have always been used for medicinal purposes, even in modern times. Despite the advances made in orthodox medicine; there has been an increasing interest in herbal medicine¹.

Generally plant produces active components necessary for their major cell metabolism (primary metabolites) and others as defensive mechanism against predators, pest and injury or other environmental stress (secondary metabolites). These secondary metabolites have been found to contain certain pharmacological importance to men. Hence, they are of major research interest to scientist.

Recently, research on free radicals scavenging activities of plants have been on the increase because most common health disorder results from oxidative imbalance caused by reactive oxygen species (ROS), which is typically as a result of inability of the antioxidant in the body to quench or scavenge the effect of free radicals generated in the body. Excess amount of ROS may be harmful, because they can initiate biomolecular oxidative chain reactions in the body².

Free radicals are atoms or group of atoms with an odd (unpaired) number of electrons and are often be formed when oxygen interacts with certain molecules^{3,4}. The primary site of free radical damage is the DNA found in the mitochondria. When reactive radicals are generated in the body, the process disrupts all levels of cell function, resulting in oxidative stress (OS). OS is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses. This oxidative stress results in numerous diseases and disorder such as, ageing, cancers, rheumatoid arthritis, and cardiovascular diseases⁵. Antioxidants are molecules which can safely react with free radicals and terminate the chain reaction before vital molecules are damaged³

Blighia unijugata Baker commonly known as ‘Triangular top’ or Akoko-Isin (South West, Nigeria) belong to the over 2000 species of the sapindaceae family and the 3 *Blighia* species. Members of this family have been widely studied for their pharmacological activities. Insecticides, antioxidant, anti-inflammatory and anti-diabetic properties are the pharmacological activities most commonly described for this family.

Blighia unijugata Baker is a tree indigenous to the forests of West Tropical Africa. It is usually small but sometimes attains 35 meters in height. It is planted for shade and is attractive in appearance, having red or pinkish-yellow fruit. Leaflets of *Blighia unijugata* can be differentiated from other species by tufts of hairs in the axils of lateral veins and its fruits are up to 3 cm long (at least 4 cm long in the other species).

The wood is used for buildings; bark pulp as an enema or is macerated by draught and taken as febrifuge and purgative. Many traditional indications of this plant have been reported. *Blighia*

unijugata is used in the treatment of fever, kidney pain and stiffness, leprosy, eyes aches, coughing, headaches, rheumatism, nausea and vomiting, dizziness, high blood pressure and also as a vegetable^{6,7}.

The seeds, because of their oil content, and the jacket because of its potash content, are burned and the ashes used in making soap. It is also recognized for its sedative and analgesic properties in treatment of rheumatism and the seed infusion is given in case of sickness and vomiting⁷.

Report on ethanol extracts of roots, bark and leaves of *B. unijugata* indicates it has antibacterial activity, which is pronounced against *Staphylococcus aureus*⁸. Phytochemical analysis showed presence of steroids, saponins and tannins in its root, bark and leaf extracts⁹. The hypotensive effect, antioxidant activity and antibacterial effect of the active fractions from leaves of *B. unijugata* have been reported^{10, 11}. Butanol fractions of its leaves also reveal presence of polyphenols, tannins, alkanoids, saponins, reduced sugar, sterols, polyterpenes, flavonoids, coumarins, quinones and cardiotoxic glycosides¹¹. Koffuor *et al.*¹⁴, evaluated the anti-fibroid property of an ethanolic stem bark extract of *Blighia unijugata* on Monosodiumglutamate (MSG)-induced uterine leiomyoma in sprague-dawley rats and concluded it possess anti-fibroid properties and safety for use¹³. Work done on the proximate and vitamin composition of *Blighia unijugata* leaves suggested the presence of retinol, niacin, ascorbic acid and thiamine.

Studies on oils extracted from seeds and aril of *B. unijugata* revealed no clinical toxicity¹⁵. Modification on the unsaturated methyl esters obtained from the seed oil of *B. unijugata* afforded the isolation of 9, 10- dihydrooctadecanoate⁸.

Its fruit has common use as a fish poison. In Jamaica, the fruit serves as a major component of the national dish, ackee (*Blighia sapida*) and codfish. The molluscidal activity of the fruits of *B. unijugata* against *Bulinus globosus* and *Bu. truncatus* has been reported^{7, 16}. Aquaisua *et al.*¹⁷ reported in 2011 the non-toxicity of *B. unijugata* on the histology of liver and kidney of wistar rats in contrast to *B. sapida*.

Fowden, *et al.*¹⁸ isolated trans-2- (2-carboxymethylcyclopropyl) glycine and other amino acids from the seeds of *B. unijugata*. Ongarora *et al.*¹⁹ reported the isolation of two pentacyclic triterpenoids, Friedelin and Epifriedelinol from the chloroform extract of *Blighia unijugata* stem bark.

Moronkola *et al.*²⁰ reported the essential oil compositions of six different parts of *B.unijugata* which support the ethno-medicinal application of the plant.

This research is a comparative study of the antioxidant activities of various parts of *Blighia unijugata* using DPPH method, a standard method for establishing the real antioxidant value in natural product studies, which have not been reported earlier in literature.

EXPERIMENTAL

Chemicals: Distilled Hexane, Ethyl Acetate and Methanol, 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), distilled water.

Plant Collection and Processing: *Blighia unijugata* was collected from University of Ibadan, Nigeria. It was authenticated at Department of Botany, University of Ibadan, Ibadan, Nigeria. Voucher specimen was deposited at the Herbarium, with identification number: UIH-22344. Samples were separated into five different parts (leaf, stem bark, root, flower and fruit). All parts excluding the flower and fruit were air dried; bulky ones were chopped into small pieces and grounded.

The flower and fruit were extracted separately in methanol (labeled MBuFlMeOH and MBuFsMeOH respectively) while the leaf, stem bark and root extracts were obtained sequentially first with 100% hexane (labeled MBuLfHex, MBuStHex, and MBuRtHex respectively), and then with equal mixture of ethyl acetate and methanol (labeled MBuLf 1:1, MBuSt 1:1, and MBuRt 1:1 respectively).

Evaluation of DPPH radical scavenging activity: Antioxidant activities of the extracts were carried out using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. 3.94mg of DDPH was dissolved in 100 mL methanol to obtain 0.1 mM solution of DPPH. A stock solution of 0.05 mg/ml sample was made and from its other concentrations of 0.025 mg/ml, 0.0125 mg/ml, 0.00625 mg/ml and 0.003125 mg/ml were obtained by serial dilution.

For evaluation, 3 mL of stock solution of DPPH (0.1 mM) was added to 0.5 mL of each concentration of sample. The mixture was left to incubate for 10 minutes and the absorbance¹ was taken at 517 nm. % inhibition was calculated using the formula

$$\%I = 100\left(1 - \frac{A_s}{A_b}\right)$$

Where % I is the percentage inhibition of the DPPH radical, as corresponds to the absorbance of DPPH with the sample and Ab the absorbance of DPPH without sample (blank). For comparison, activities of three know standards: alpha tocopherol (Vitamin E), Ascorbic acid (Vitamin C), and anisole were also evaluated. All tests were run in triplicate and averaged. The sample concentration providing 50% inhibition (IC₅₀) was evaluated from the graph representing the percentage inhibition against sample concentration using regression analysis on Microsoft Excel.

RESULTS AND DISCUSSION

Percentage Yield of Extracts: Eight extracts were obtained from five parts of *B. unijugata* collected; their percentage yields were from 1.50 to 10.44% (Table I). Highest yield was obtained from the leaf (10.44%) and lowest yield was from fruit.

Table I: Yields of extracts obtained from leaf, stem-bark, root, flower and fruit.

S/N	Plant's Part	Weight of Sample (g)	Approximate weight of extract (g)	% Yield of extract
1	Leaf	2,500	MBuLf 1:1 ^a	94.00
			MBuLf Hex ^b	167.00
2	Stem-Bark	2,600	MBuSt 1:1 ^c	10.00
			MBuSt Hex ^d	33.00
3	Root	3,400	MBuRt 1:1 ^e	10.00
			MBuRt Hex ^f	60.00
4	Flower	140	MBuFl MeOH ^g	2.70
5	Fruit	100	MBuFSd MeOH ^h	1.50

^a Ethyl acetate/Methanol (1:1) leaf extract; ^b Hexane leaf extract; ^c Ethyl acetate/Methanol (1:1) stem bark extract; ^d Hexane stem bark extract; ^e Ethyl acetate/Methanol (1:1) Root extract; ^f Hexane root extract; ^g Methanol flower extract; ^h Methanol fruit extract;

DPPH Free Radical Scavenging Activity of Extracts: DPPH is a stable organic radical. The ability of biological compounds to scavenge DPPH radical can be expressed as its magnitude of antioxidant ability³. Result of the DPPH radical scavenging ability of the extract expressed as % inhibition of

DPPH is presented in Table II and Figure I. The free radical scavenging activities for all the eight extracts on DPPH were in this order: 1:1 MBuLf > MBuSt 1:1 > MBuFs MeOH > MBuRt Hex > MBuRt1:1 > MBuFl MeOH > MBuSt Hex > MBuLf Hex: and their corresponding IC₅₀ values are: 0.0277 > 0.0284 > 0.0301 > 0.0317 > 0.0320 > 0.0342 > 0.0360 > 0.0400 (mg/ml) as shown in **Figure 2**. Comparing the two methanol extracts, fruit (0.0301 mg/ml) was more active than flower extract (0.0342 mg/ml).

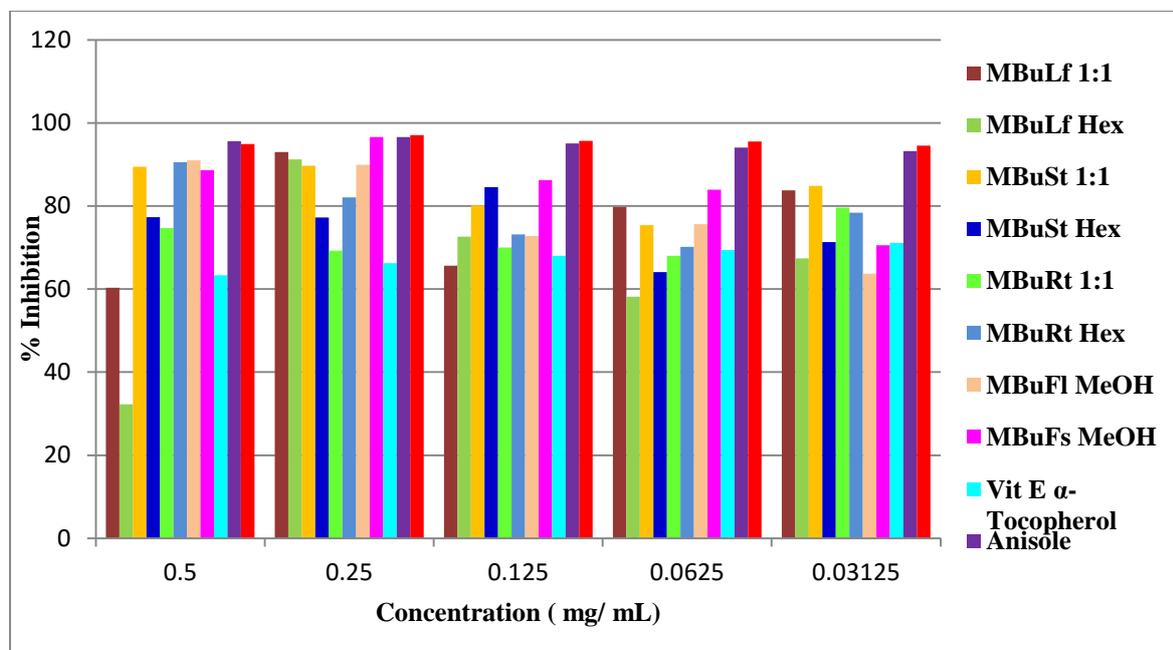


Figure I: DPPH scavenging results of *B. unijugata* extracts and standard antioxidants (%)

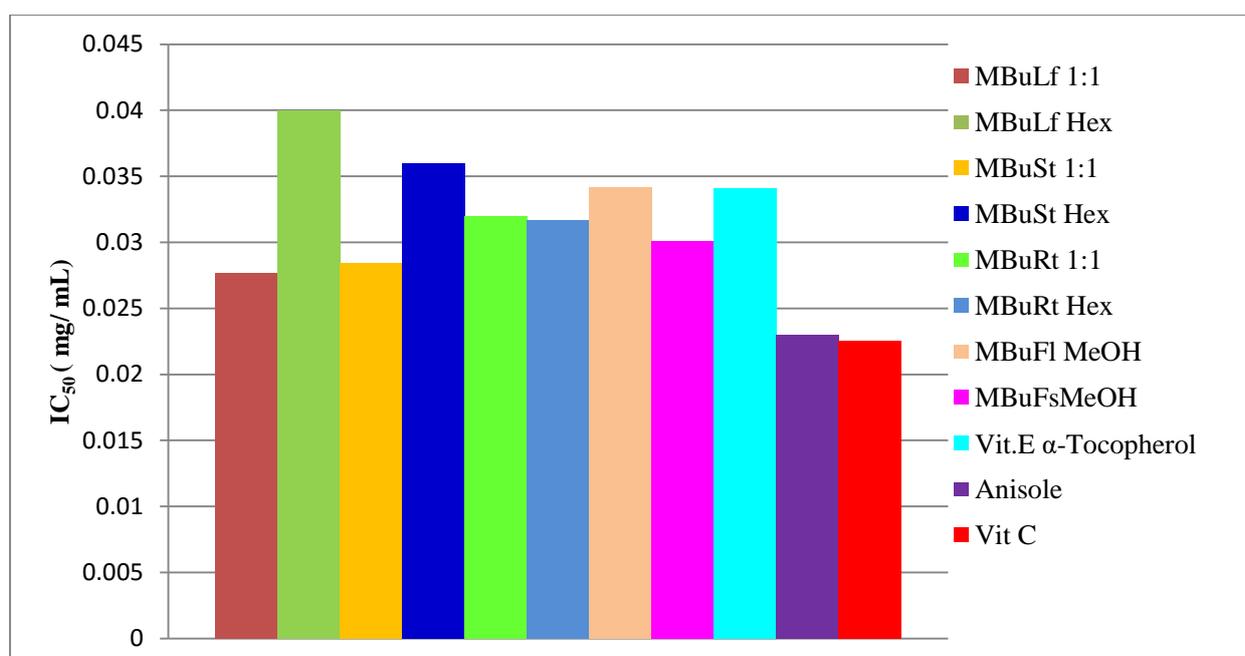


Figure II. IC₅₀ values for DPPH scavenging activity of *B. unijugata*

Table II. Results of DPPH scavenging activity of various parts of *Blighia unijugata*

Plant Part	% Inhibition at 0.1 mg/ml	% Inhibition at 0.05 mg/ml	% Inhibition at 0.025 mg/ml	% Inhibition at 0.0125 mg/ml	% Inhibition at 0.00625 mg/ml	IC ₅₀ mg/ml x10 ⁻²
MBuLf 1:1 ^a	60.28	92.94	65.67	79.78	83.73	2.77
MBuLf Hex ^b	32.26	91.19	72.64	58.15	67.4	4.00
MBuSt 1:1 ^c	89.47	89.67	80.16	75.35	84.87	2.84
MBuSt Hex ^d	77.29	77.22	84.57	64.13	71.34	3.60
MBuRt 1:1 ^e	74.66	69.27	70.01	68.00	79.61	3.20
MBuRt Hex ^f	90.6	81.94	73.18	70.22	78.35	3.17
MBuFl MeOH ^g	90.98	89.84	72.79	74.92	63.77	3.42
MBuFSd MeOH ^h	88.52	96.56	86.23	83.93	70.49	3.01
Vit E ⁱ	63.36	71.81	68.00	69.38	71.07	3.41
Anisole	95.59	96.59	95.09	94.09	93.29	2.3
Vit C ^j	94.89	97.09	95.69	95.49	94.48	2.25

^a Ethyl acetate/Methanol (1:1) leaf extract; ^b Hexane leaf extract; ^c Ethyl acetate/Methanol (1:1) stem bark extract; ^d Hexane stem bark extract; ^e Ethyl acetate/Methanol (1:1) Root extract; ^f Hexane root extract; ^g Methanol flower extract; ^h Methanol fruit extract; ⁱ Vitamin E (α -tocopherol); ^j Vitamin C (Ascorbic Acid)

Leaf extracts, 1:1 Stem extract, Fruit extract and the three standards used showed maximum % inhibition at 0.25 mg/ml sample concentration. While maximum % inhibition for the root extracts, hexane extract of stem, and flower extract show maximum % inhibition at 0.125 mg/ml, 0.03125 mg/ml, 0.5 mg/ml and 0.5 mg/ml respectively. Of all the part of *B. unijugata*, fruit methanol extract showed the highest % inhibition of 96.56% which is above that of vitamin E (71.87%) and comparable to that of Anisole and vitamin C which showed maximum % Inhibition of 96.59% and 97.09% respectively.

Intra solvent system comparison of the different parts showed that for the 1:1 Solvent system (i.e. Ethyl acetate: Methanol) used, DPPH free radical scavenging activities was in the order Leaf > Stem > Root: having IC₅₀ values as 0.0277 mg/ml > 0.0284 mg/ml > 0.0320 mg/ml respectively. While the hexane extracts gave the following order: Root > Stem > Leaf: 0.0317 mg/ml > 0.0360 mg/ml > 0.0400 mg/ml

CONCLUSION

Our results indicate that the five parts studied are potential good sources of free-radical scavenging compounds. MBuLf 1:1 gave the highest activity for DPPH free radical scavenging potential, while MBuLfHex gave the least free-radical scavenging potential. This study supports the ethno-medicinal application of *Blighia unijugata* as anti-ageing agent.

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