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**Research Article**

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# Hydrogen Peroxide Free Radical Scavenging Activities of Leaf, Stem Bark, Root, Flower and Fruit of *Blighia unijugata* Baker (Sapindaceae) Extracts

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**ABSTRACT**

*Inability of antioxidants to scavenge effect of free radicals generated in the body results in 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 study of antioxidant activities of extracts from different parts of the ethno-medicinally utilized 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 MBuFM and MBuFsM respectively), the leaf, stem bark and root extracts were obtained sequentially first with 100% hexane (MBuLH, MBuSH, and MBuRH), and then with equal mixture of ethyl acetate and methanol (MBuLf1, MBuSt1, and MBuRt1). Antioxidant activity evaluations was done using hydrogen peroxide assay. Their 50% inhibitory concentration ($IC_{50}$) values were determined by regression analysis on Microsoft excel. % inhibition of hydrogen peroxide was between 52.28% - 82.41% which is comparable to that of the two standards: Vitamin C and Butylhydroxyl Anisole (BHA) used (58.05%-85.63%). $IC_{50}$ values for the hydrogen peroxide scavenging activities of all the extracts studied gave the following trend: MBuFM>MBuSt1=MBuSH=MBuRt1>MBuLH>MBuFsM=MBuLf1>MBuRH, with their corresponding $IC_{50}$ values being 0.0056 > 0.0057 = 0.0057 = 0.0057 > 0.0059 > 0.0060 = 0.0060 > 0.0064 (mg/ml) respectively. Our results indicate that the five parts studied are potential good sources of free-radical scavenging compounds, which support the ethno-medicinal application of Blighia unijugata as anti-ageing agent.*

**Keywords**: Free radical; Hydrogen peroxide; Antioxidant; Sapindaceae; *Blighia unijugata*

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## 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 medicine [1].

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). The latter have been found to contain certain pharmacological importance to men. Hence, they are of major research interest to scientist. The study of 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 body [2]. 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]. When cells in the body encounter a free radical, the reactive radical may cause destruction in the cell. The primary site of free radical damage is the DNA found in the mitochondria. Hence this free radical generation process disrupts all

levels of cell function resulting in oxidative stress (OS). Oxidative stress 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, cardiovascular diseases [5]. Antioxidants are molecules which can safely react with free radicals and terminate the chain reaction before vital molecules are damaged [3].

*Blighia unijagata* 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. *Blighia unijugata* can be distinguished from the other two species by its leaflets having tufts of hairs in the axils of lateral veins and its fruits which 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 as a vegetable and also in the treatment of fever, nausea and vomiting, leprosy, eyes aches, coughing, headaches, rheumatism, kidney pain and stiffness, dizziness and high blood pressure [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 vomiting [7].
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 extracts [9]. The hypotensive effect, antioxidant activity and antibacterial effect of the active fractions from leaves of *B. unijugata* have been reported [10-12]. Butanol fractions of its leaves also show presence of polyphenols, tannins, flavonoids, saponins, alkanoids, sterols, polyterpenes, reduced sugar, coumarins, quinones and cardiotonic glycosides [11]. Koffuor et al. 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 use [13]. Work done on the proximate and vitamin composition of *Blighia unijugata* leaves suggested the presence of retinol, niacin, ascorbic acid and thiamine [14]. Studies on oils extracted from seeds and aril of *B. unijugata* revealed no clinical toxicity [15]. Modification on the unsaturated methyl esters obtained from the seed oil of *B. unijugata* afforded the isolation of 9, 10-dihydrooctadecanoate [8]. 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. reported the non-toxicity of *B. unijugata* on the histology of liver and kidney of wistar rats in contrast to *B. sapida* [17]. Fowden et al. isolated trans-2- (2-carboxymethylcyclopropyl)glycine and other amino acids from the seeds of *B. unijugata* [18]. Ongarora et al. reported the isolation of two pentacyclic triterpenoids, Friedelin and Epifriedelinol from the chloroform extract of *Blighia unijugata* stem bark [19].
Moronkola et al. reported the essential oil compositions of six different parts of *B.unijugata* which support the ethno-medicinal application of the plant [20]. This research is a comparative study of the antioxidant activities of various parts of *Blighia unijugata* using hydrogen peroxideassay. Our results indicate that the five parts studied are potential good sources of free-radical scavenging compounds, which support the ethno-medicinal application of *Blighia unijugata* as anti-ageing agent which have not been reported earlier in literature.

## EXPERIMENTAL SECTION

### Chemicals

Distilled Hexane, Ethyl Acetate and Methanol, Hydrogen Peroxide, Sodium bihydrogen phosphate, Sodium hydrogen phosphate, Sodium Chloride, Potassium chloride, 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 MBuFM and MBuFsM respectively) while the leaf, stem bark and root extracts were obtained sequentially first with 100% hexane (labeled MBuLH, MBuSH, and MBuRH respectively), and then with equal mixture of ethyl acetate and methanol (labeled MBuLf1, MBuSt1, and MBuRt1 respectively).

**Evaluation of Hydrogen Peroxide Scavenging Ability of the Extracts**

The ability of the *Blighia unijugata* extracts to scavenge hydrogen peroxide was determined using the modified method described by Babu et al. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). 3 mL of different concentrations of the extract (0.1 to 0.003125 mg/ml) dissolved in phosphate buffer were added to a hydrogen peroxide solution (0.5 mL, 40 mM). After 10 min of incubation, absorbance of hydrogen peroxide was measured at 230 nm using UV spectrophotometer against a blank solution containing the phosphate buffer with hydrogen peroxide only. Butylhydroxyl Anisole (BHA) and Ascorbic acid were used as positive control. Test were run in triplicate and averaged. The percentage of hydrogen peroxide scavenged of both *Blighia unijugata* extracts and standard used were calculated using the formula:

$$\%[\text{Hydrogen peroxide}]\ \text{Scavenged} = 100\left(1 - \frac{As}{Ab}\right)$$

Where $A_b$ is the absorbance of the control and $A_S$ is the absorbance in the presence of the sample of *Blighia unijugata* extracts or standards.

## 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 1). Highest yield was obtained from the leaf (10.44%) and lowest yield was from fruit.

**Table 1: 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 | MBuLH | 94 | 10.44 |
| | | | MBuLf1 | 167 | |
| 2 | Stem-Bark | 2,600 | MBuSH | 10 | 1.65 |
| | | | MBuSt1 | 33 | |
| 3 | Root | 3,400 | MBuRH | 10 | 2.06 |
| | | | MBuRt1 | 60 | |
| 4 | Flower | 140 | MBuFM | 2.7 | 1.92 |
| 5 | Fruit | 100 | MBuFsM | 1.5 | 1.5 |

**Hydrogen Peroxide Free Radical Scavenging Activity of Extracts**

Hydrogen peroxide, though a weak oxidizing agent is less toxic, when allowed to permeate into cell membrane it may results in generation of hydroxyl radicals in the cell which may results in many toxic effect. It is therefore imperative for cells to get rid of them [21]. The hydrogen peroxide scavenging activity of the various parts of *Blighia unijugata* and standard is as shown in Table 2 and Figure 1. All the eight extracts showed comparable hydrogen peroxide scavenging activities to that of BHA and Ascorbic acid which are known standards.

**Table 2: Results of hydrogen peroxide 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 | $IC_{50}$ ($\times 10^{-2}$) mg/ml |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| MBuLf1 | 52.28 | 69 | 72.18 | 78.82 | 75.8 | 0.6 |
| MBuLH | 72.49 | 76.11 | 77.98 | 77.22 | 79.09 | 0.59 |
| MBuSt1 | 67.17 | 73.94 | 78.75 | 79 | 81.42 | 0.57 |
| MBuSH | 70.23 | 77.14 | 78.18 | 78.55 | 82.21 | 0.57 |
| MBuRt1 | 68.94 | 76.39 | 79.62 | 80.52 | 80.19 | 0.57 |
| MBuRH | 69.07 | 74.83 | 76.29 | 81.43 | 66.28 | 0.64 |
| MBuFsM | 61 | 71.73 | 76.57 | 79.94 | 75.55 | 0.6 |
| MBuFM | 70.38 | 75.52 | 79.68 | 80.55 | 81.32 | 0.56 |
| BHA | 58.05 | 69.47 | 79.41 | 80.71 | 78.94 | 0.58 |
| Vit C- | 73.21 | 78.64 | 83.23 | 85.63 | 84.05 | 0.53 |

% inhibitions for the hydrogen peroxide scavenging ability of *Blighia unijugata* were between 52.28% - 82.41% which is comparable to that of the standard used (58.05%-85.63%). The1 leaf extracts, hexane and1 root extracts, fruit extract and the two standards used has maximum % inhibition at 0.0125 mg/ml.

Hexane Leaf extract, stem extracts and flower extract at 0.00625 mg/ml. The $IC_{50}$ (Figure 2) reveals extract from the flower had the highest hydrogen peroxide scavenging ability and others in the following order MBuFM > MBuSt1 = MBuSH = MBuRt1 > MBuLH > MBuFsM = MBuLf1 > MBuRH; and their corresponding $IC_{50}$ values are: 0.0056 > 0.0057 = 0.0057 = 0.0057 > 0.0059 > 0.0060 = 0.0060 > 0.0064 (mg/ml) respectively.

MBuFM show highest and MBuRH shows the lowest Hydrogen peroxide scavenging activity respectively based on their $IC_{50}$.

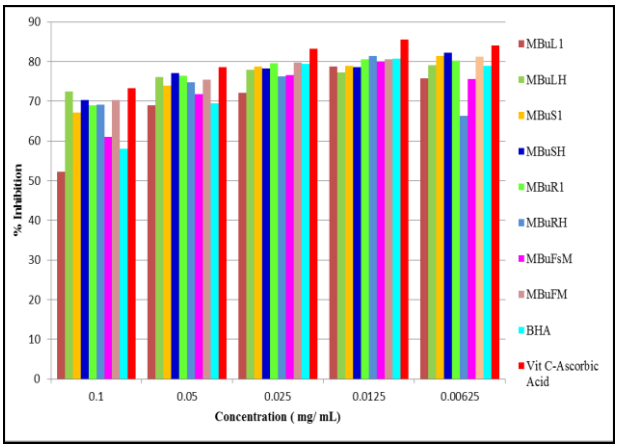


**Figure 1: Hydrogen peroxide scavenging results of *B. unijugata* extracts and standard antioxidants (%)**

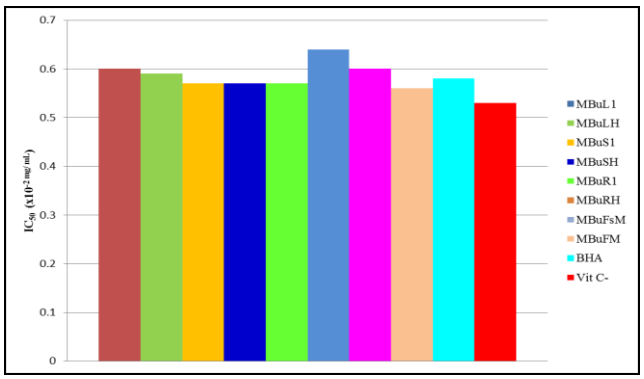


**Figure 2: $IC_{50}$ values for hydrogen peroxide scavenging activity of *B. unijugata***

## CONCLUSION

Our results indicate that the five parts studied are potential good sources of free-radical scavenging compounds. This study supports the ethno-medicinal application of *Blighia unijugata* as anti-ageing agent.

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