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# LEAVES OF *PICRALIMA NITIDA* (PILE PLANT) COULD HAVE FREE RADICAL SCAVENGING ACTIVITY

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**Abstract:**

*Earlier investigations have shown that there are a number of plants which shows antioxidant activity. This study evaluated the free radical scavenging activities of the crude methanol extract of Picralima nitida leaves. Picralima nitida commonly called Pile plant is a shrub with wide applications in West African folk medicine. The methanol extract of P. nitida was subjected to in-vitro antioxidant evaluation using DPPH (2,2-diphenyl-1-picrylhydrazyl) and BHA (butylated hydroxyl anisole) as standards. Results indicated that BHA exhibited higher level of scavenging activity (IC50350.28µg/ml) as compared to the methanolic extracts of the sample (IC50402.50µg/ml). This is indicative that P. nitida leaves extract have free radical scavenging activity.*

*Keywords: Free radicals, Picralima nitida, Pile plant, Scavengers, Antioxidants, extract.*

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## INTRODUCTION:

Free radicals such as the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated through endogenous processes like respiration and phagocytosis. They are generated by exogenous systems such as pesticides, some pollutants, organic solvents and during radiation [1]. Generation of free radicals are balanced by equivalent generation of antioxidant through the body's natural antioxidant defence mechanisms.

Antioxidants, such as dietary phytochemicals (examples as polyphenols, flavonoids, coumarins, and terpenoids) including molecules like ascorbic acid (Vitamin C), alpha-tocopherol (Vitamin E), may be obtained from diets [2].

Antioxidants are present within cells and contribute to cellular defence mechanisms by chelating transition metals and preventing them from catalysing the production of free radicals in cells [4]. Oxidation reactions produce free radicals which can start chain reactions [5] that normally cause damage or death to the cell. However, antioxidants which terminate these chain reactions by removing free radicals include thiols, ascorbic acid or polyphenols, glutathione, vitamin A, vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases [6]. Insufficient levels of the antioxidants or inhibition of the antioxidant enzymes, cause oxidative stress [7]. Antioxidants are widely used in dietary supplements though have not been suggested so helpful in dealing with oxidative stress and excess of them may be harmful [8]. Antioxidants also have other industrial uses, such as food preservatives, cosmetics and to prevent rubber and gasoline degradation [9]. Oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species [10].

Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that collaborate to prevent damage to cellular components such as DNA, proteins and lipids [11]. Generally, antioxidants systems either prevent these reactive species from being formed or remove them before they can damage vital components of the cell [12]. However, reactive oxygen species also have useful cellular roles, such as redox signalling. Thus, the function of antioxidant systems is not to remove oxidants entirely, but to keep them at an optimum level [13]. The reactive oxygen species produced in cells include hydrogen peroxide, hypochlorous acid, and free radicals such as hydroxyl radical and superoxide anion, peroxynitrite and peroxynitrous acid [14]. The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules. It is produced from hydrogen peroxide in metal-catalysed redox reactions such as the Fenton reaction [15]. These oxidants damage cells by starting up chemical chain reactions such as lipid peroxidation, oxidizing DNA or proteins. Damage to DNA can cause mutation while damage to proteins causes enzyme inhibition, denaturation and degradation [16]. Superoxide anion is produced as a by-product of several steps in the electron transport chain [17]. Peroxide is produced from the oxidation of reduced flavoproteins, such as complex I of electron transport chain. In plants (algae and cyanobacteria), reactive oxygen species are also produced during photosynthesis [18], especially under high light intensity [19]. This effect is offset by large amounts of iodide and selenium in algae and cyanobacteria [20].

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyse the breakdown of the superoxide anion into oxygen and hydrogen peroxide [21]. They are an important antioxidant defence in nearly all cells exposed to oxygen except *Lactobacillus plantarum* and related lactobacilli [22]. Superoxide dismutase plays a critical role in reducing the oxidative stress implicated in atherosclerosis and other life-threatening diseases, reducing internal inflammation and lessening pain associated with conditions such as arthritis [23]. Hypochlorous acid is produced by myeloperoxidase from the reaction between hydrogen peroxide and chloride ion; this potent oxidant contributes to the antimicrobial activity of phagocytes [24].

However, it has been proven that either chronic or prolonged production of hypochlorous acid by the myeloperoxidase-hydrogen peroxide-chloride ion system contributes to tissue damage and the initiation and propagation of vascular diseases [25]. Furthermore, low density lipoprotein oxidized by this system accumulates in macrophages and exerts pro-inflammatory effects on monocytes and endothelial cells [26]

Another crucial enzyme among the antioxidant enzyme system is thioredoxin reductase; a member of the nucleotide pyridine disulphide oxidoreductase family, which includes glutathione reductase, alkyl hydroperoxide reductase and lipoamide dehydrogenase [27]. Thioredoxin reductase catalyses the disulphide reduction of oxidized thioredoxin using NADPH via the FAD molecule and the redox-active cysteine residues [28].

**Aim and Objectives**

The objective of this study was to investigate possible antioxidant potential of *Picralima nitida*. The aim was to prevent oxidative damages arising from disease states or aging process.

## MATERIALS AND METHODS:

**Sample Collection**

Leaves of *Picralima nitida* were bought from Eke Market, Agbani, Enugu State and conveyed to Biochemistry laboratory of Enugu State University of Science and Technology, Agbani, in a black polyethene bag where it was identified and authenticated by a Botanist: Prof. Eze of the Department of Applied Biology and Biotechnology.

**Standard Reagent Preparation**

**Preparation of DPPH (2, 2-diphenyl-1-picrylhydrazyl)**

One gram (1g) of DPPH was weighed and transferred to a beaker. The weighed sample was dissolved in methanol and the volume made up to 100ml mark.

Electric magnetic stirrer was used to ensure that the mixture dissolved properly.

**Preparation of BHA (Butylated hydroxyl anisole)**

One gram (1g) of BHA was weighed using a weighing balance and transferred into a beaker. Weighed sample was later dissolved in methanol and volume made up to 100ml of volumetric flask.

**Free Radical Scavenging Assay**

The method used was that described by [29] and [30].

**Procedures for Free Radical Scavenging Assay**

The stock of the sample is 1g/10ml of methanol. A measurement of 1ml of the stock solution was taken and made up to 10ml with 9ml of methanol. Various concentration of *Picralima nitida* extract were added to different test tubes in different volumes; 0.2, 0.4, 0.6, 0.8 and 1.0ml respectively. Another 1ml of water was added to each of the test tubes. Using 5ml pipette, 2ml of DPPH was added to each of the test tubes and shook properly and left to stand in the dark for 60 minutes. Same procedure applied BHA as standard sample. The absorbance was measured at 517nm using UV spectrophotometer.

The free radical scavenging activity was calculated at a percentage DPPH discoloration using the equation:

% RSA =
(ADPPH – Asample)/ADPPH × 100

Where Asample is the absorbance of the sample extract and ADPPH is absorbance of DPPH solution.

The extract concentration providing 50% of reducing power activity was calculated from the graph of RSA(%) against extract concentration. BHA and DPPH were used as standard.

## RESULTS:

**Table 1: Absorbance of Methanolic leave extracts of *Picralima nitida* at wavelength of 517nm at various concentrations**

| Concentration(µg/ml) | Absorbance of sample |
|---|---|
| 0 | 0 |
| 200 | 0.110 |
| 400 | 0.168 |
| 600 | 0.111 |
| 800 | 0.106 |
| 1000 | 0.067 |

**Table 2: Absorbance of the standard (BHA) at wavelength of 517 nm at various concentrations**

| Concentration(µg/ml) | Absorbance of BHA |
|---|---|
| 0 | 0 |
| 200 | 0.149 |
| 400 | 0.103 |
| 600 | 0.087 |
| 800 | 0.068 |
| 1000 | 0.061 |

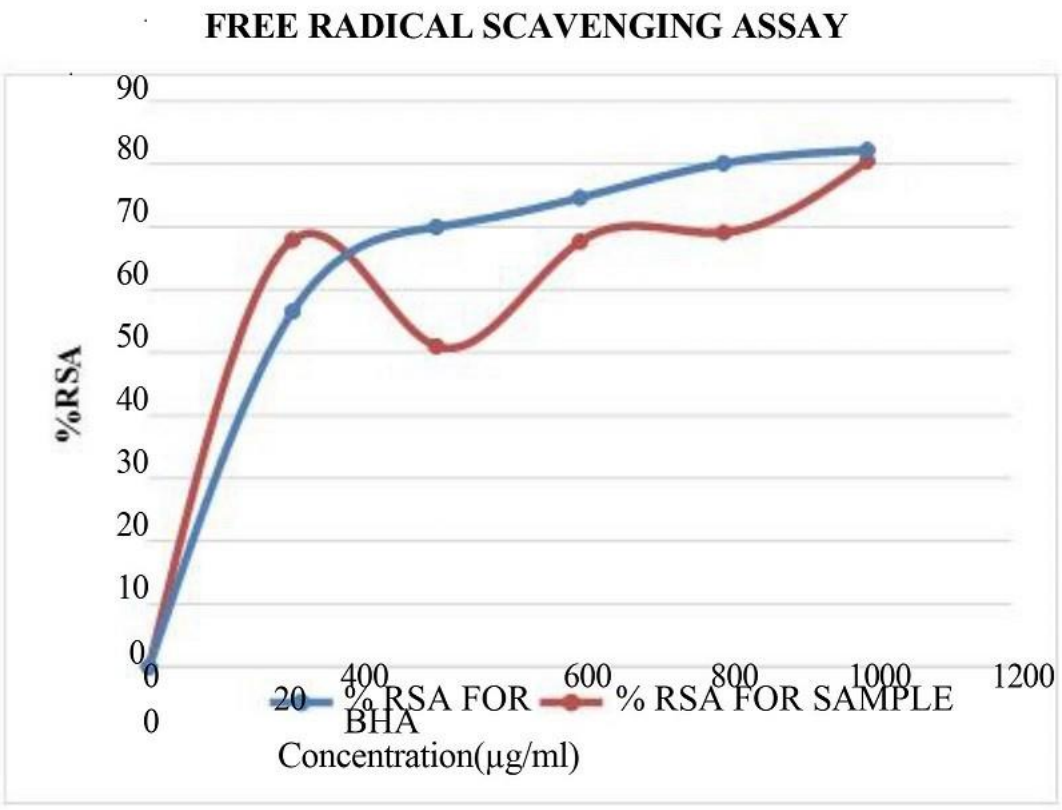
**Table 3: Results of Percentage Radical Scavenging Activity (%RSA) Of The Sample (*Picralima nitida*)**

| Concentration(µg/ml) | % RSA |
|---|---|
| 0 | 0 |
| 200 | 67.93 |
| 400 | 51.02 |
| 600 | 67.63 |
| 800 | 69.09 |
| 1000 | 80.46 |

**Table 4: Results of percentage radical scavenging activity (%RSA) of BHA**

| Concentration(µg/ml) | % RSA |
|---|---|
| 0 | 0 |
| 200 | 56.55 |
| 400 | 69.97 |
| 600 | 74.63 |
| 800 | 80.17 |
| 1000 | 82.21 |

**FREE RADICAL SCAVENGING ASSAY**



The graph of radical scavenging activity of *P. nitida* (%RSA plotted against concentration). The blue curve is the dose response for radical scavenging ability of butylated hydroxylanisole (BHA) while the red curve is that of the plant *P. nitida*.

## DISCUSSION:

The most common definition of free radicals is "molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals". Free radicals are uncharged, very reactive, and short-lived molecules. Human beings contain 10,000 – 20,000 free radicals which attack each individual cell of our body. Many of these radicals are beneficial in that they work for immune cells responsible for killing bacterial cells and toning of smooth muscles, which in turn regulate the normal working of blood vessels and internal organs. Uncontrolled generation of free radicals in our body may lead to various ill effects such as autoimmune diseases, heart and neurodegenerative diseases, cancers, etc.

The free radicals are produced during ATP generation through mitochondria. They are generally divided into two well-known entities: reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS collectively form all the radical and non-radical (oxidants) entities. Radicals are more reactive and less stable than their non-radical counterparts. Non-radical derivatives or oxidants can be easily converted into free radicals by various reactions in living organisms. The body has various enzymes and antioxidants to combat the damage incurred by oxidative stress. Antioxidants are chemicals that bind with free radicals and nullify their effect from causing damage to biological molecules. Radical scavenging is one of the mechanisms of antioxidants activities and the DPPH assay provides a convenient, rapid and easy method to evaluate antioxidants and radical scavengers. It is based on the ability of 2, 2-diphenyl-1-picrylhydrazyl (DPPH), a stable free radical to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron which is responsible for the absorbance at a wavelength of 517nm. It accepts an electron donated by an antioxidant compound. The DPPH is decolorized and this effect can be quantitatively measured from the changes in the absorbance. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract. DPPH assay is known to give accurate information concerning the antioxidant ability of tested compounds

As result indicates, the methanolic extracts of *P. nitida* exhibited lower level of scavenging activity as compared to the BHA (Butylated hydroxyl anisole), though their activities are comparable and close to each other and are likely to exhibit similar scavenging activity. The gap between the synthetic antioxidant and *P. nitida* shows the radical scavenging ability of *P. nitida*. It is observed that *P. nitida* is a good radical scavenger and could act as effectively as a primary antioxidant against free radicals.

## CONCLUSION:

Methanolic extracts of *Picralima nitida* leaf exhibit potent free radical scavenging and antioxidant activity as close to the standard BHA (IC50350µg/ml), therefore could be used as a good supplement to different natural antioxidants within the body, thus acting as a cure to oxidative stress but comparatively less than BHA. Further investigations should be done on this plant with standard equipment under regulated environment.

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