Antioxidant Properties of Some Nigerian Green Leafy Vegetables

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Abstract- The anti-oxidant properties of acetone extracts of Ocimum citriodorum, Portulaca oleracea, Senna alata, Gnetum africanum and Vernonia amygdalina were studied using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. The result showed that the extracts from Portulaca oleracea had 95 % Gnetum africanum 92 % Vernonia amygdalina 89 %, Senna alata 86 % and Ocimum citriodorum 75 % of 2,2 –diphenyl-1-picrylhydrazyl inhibition respectively. Thus, the extracts of the five vegetables showed strong antioxidant properties.

Index Terms- antioxidant properties, green leafy vegetables, DPPH-scavenging method, acetone extracts.

I. INTRODUCTION

Antioxidants are molecular entities that inhibit the oxidation of the other molecules. They operate by reacting directly with the oxidative species, chelating with metals and also by catalyzing the activity of other molecules that will function as antioxidants. Oxidation is a chemical reaction that transfers electrons or hydrogen from one substance to an oxidizing agent. Although oxidation reaction reactions are crucial for life, they cause damage to tissues because free radical and reactive oxygen species produced during the oxidation process, in turn, attack the cells, tissues and other components of the system, thereby causing damage or death to the cells. (Sies et al., 2005).

Many studies have shown that oxidation activities of free radicals and reactive oxygen species are the causes of several diseases like Neuro degenerative disease, Cancer, Aging and Coronary heart diseases (Zheng and Wang, 2001). The oxidation of double bonds in unsaturated oils and the oxidation of plastic polymers are also the negative consequences to check excessive oxidation that may bring negative consequences to reactive systems.

Classification based on the uses and the systems by which antioxidants are grouped falls into two major types, namely: biological system antioxidants and non-biological system antioxidants. Biological system antioxidants are the ones that operate mainly in biological systems as their environment. These include all the antioxidants that are synthesized in the living system and those that are synthesized in the living system but are consumed by the system. Examples of such antioxidants are uric acid, phenol derivatives, anthocyanins, flavonoids and tannin. On the other hand, Non-Biological System Antioxidants are the antioxidants that are useful outside the living system. They are mainly used in industries as preservatives on foods, gasoline, plastic polymers and as anti-knocking agents in internal combustion engines. They include N, N-di-2-butyl-1, 4-phenylenediammine, 2, 6-di-tert-butyl-4-methylphenol, turbine oil etc.

Antioxidants can also be classified based on their solubility potentials or the solvent by which it can be dissolved. They are classified into hydrophilic and hydrophobic antioxidants. Hydrophilic Antioxidants are groups of antioxidants that are capable of dissolving in polar solvents like water and ethanol. For this reason, they only react with oxidants that are present in the cell cytosol and blood plasma. Typical examples are ascorbic acid, and uric acid (Sies et al., 2005), while Hydrophobic Antioxidants are antioxidants that are soluble in lipids. Consequently, they are absorbed in the cell membrane from lipid per-oxidation in all the biological system. These include Vitamin E (tocopherol), Vitamin A (retinol), and carotene. They have characteristic long chain structure (Sies et al., 2005).

Antioxidants have a wide range of application in pharmacology, nutrition and in many industries like the petroleum, plastic paint and polymer and where they are used for corrosion control. In medicine and pharmacology, antioxidants are used in various capacities. Some are used for the treatment of disease while others are used for the preservation of drugs. Vitamin C an antioxidant is useful for the treatment of diseases like scurry and for stimulation of co-enzyme in the body, (Sies et al, 2005), similarly, Vitamin A is used for the treatment of night blindness, and Vitamin E used for the preservation of processed drugs for long shelf life.

In nutrition, antioxidants serve as the major nutrient requirement in the body. They are useful in the prevention of diseases and help in promoting total wellness of the body. Green leafy vegetables are inescapable and indispensable components of our African diets and for this reason; any investigations into the anti-oxidant properties of some of these leafy vegetables should be welcomed.

The main objective of the investigation is to determine the antioxidant properties of the following leafy vegetables, the green leafy vegetables of concern in this study are Bitter leaf (Vernonia amygdalina), Lemon basil (Ocimum citriodorum), Candle bush (Senna alata) Afang (Gnetum africanum) and Rat ear (Portulaca oleracea).
II. AN OVERVIEW

2.1 Effect of Food Storage, Processing and Preparation on Antioxidant Properties

Because of the growing importance of antioxidants in diet, the stability of isoflavones and other antioxidants have been extensively studied. The concentration of genistein and diadzein derivative vegetable decline at ambient temperature (Esien et al., 2003) and accelerated approximately 40 – times at high temperature (70 to 90 °C). It was revealed that the sun drying of pear may cause a 64 percent decrease in total phenolic content on dry pulp basic dropping from 3.7 gram per kilogram at harvest to 1.5 kilogram (Ferreira et al, 2002). Procyanidins accounted for about 96 percent of the total phenolic content. Storage of wheat flour for six month led to a 70 percent decline in phenolic acid concentration.

A detailed study assessed the impact of various tomato processing methods on total phenolic, vitamin C content and antioxidant capacity (Gahler et al., 2003). Tomato juice, baked tomatoes sauce and tomato product decreased during thermal processing, while total phenolic concentration and water soluble antioxidant capacity increased. Another team assessed three of the same processing techniques and found the largest reduction in naringenin concentrations, whereas chlorogenic acid levels were elevated (Re et al., 2002). Overall, the team judged that tomato processing leads to a general improvement of the level of individual antioxidants (Benbrook, 2005).

An exception was documented in a study assessing the impact of tomato processing on carotenoid levels, where modest reduction was found (Tateoka et al 2001). A third detailed study focused on change in vitamin C, lycopene and total antioxidant capacity in raw tomatoes and after thermal processing showed a significant reduction in vitamin C while lycopene levels increased more than 2.5-fold after 30 minutes of processing and total antioxidant capacity rose about 50 percent. The authors of this third study concluded that: “these findings indicate that thermal processing enhanced the nutritive value of tomatoes by increasing the bio-accessible lycopene content and antioxidant activity and are against the notion that processed fruits and vegetable have lower nutritional value than fresh produce”. (Dewanto et al, 2002).

2.2 Vernonia amygdalina (Bitter Leaf)

Vernonia amygdalina is a perennial plant that belongs to the family called Asteraceae. It is generally called bitter leaf in English because of the characteristic bitter taste of the leaf. It is known as “Ewuro” in Yoruba, “Etiodi” in Ibibio, “Onugbu” in Igbo, “Ituna” in TIV, “Ilo” in “Oriwa” in Edo and “Chusar-doki” in Hausa. The leaves are about 2mm-6mm in diameter depending on the variety. The leaves are used for the preparation of soup either in the washed or unwashed form (Nwanjo, 2005).

Nevertheless, bitter leaf, apart from being highly nutritive also possesses high antioxidant properties of about 77 % of DPPH inhibition (Imaga and Bamigbetan, 2013). Studies have revealed that aqueous and ethanolic extracts of Vernonia amygdalina have further antioxidant properties like ability to inhibit the bleaching of B-carotene, oxidation of linoleic acid and lipid peroxidation induced by Fe²⁺ ascorbate in rat liver microsomal preparation (Ayola et al., 2008).

Apart from cooking of soup, its leaves are employed in the treatment of diabetic mellitus, malaria, venereal diseases, wounds, hepatitis and cancer (Hamzah et al., 2013 and Erasto et al, 2007).

2.3 Portulaca oleracea (Rat Ear)

Portulaca oleracea is sometimes referred to as pig weed, moss ross and rat ear “Utung Ekpu”. It is an annual succulent plant which belongs to the family called portulacaceae. It possesses smooth, reddish, prostrate stem and alternate leaves that looks like rat ear which are clustered at the stem and joint.

According to Agwu (2006), “The whole plant is considered antiphlogistic (take the heat out), bactericider, anaphrodisiac (opposite of aphrodisiac), emollient, calmative, diuretic and refreshing agent”. In addition to that, it is also used locally in “Ibibu” for the treatment of snake poison, insect bite and in cooking of local soup called “Efere Ibaba”.

2.4 Senna alata (Awolowo)

Senna alata is locally called “Awolowo” in South-eastern Nigeria and also called candle bush in English. It belongs to the family called Fabacea, subfamily, Caesalpinioideae. It is a perennial shrub that stands 3-4 cm tall, with leaves 50-80 cm long.

Its inflorescent flowers resemble yellow candle (Chattergee et al., 2013). It is locally used for the treatment of fungal skin diseases like ringworm, eczema, stomach problems, etc.

Studies have shown that Senna alata possesses anticarcinogenic and anti-proliferation, anti-diabetic, anti-malaria and hepatoprotective abilities (Mortada et al., 2011). Researchers also indicate that astragalin from Senna alata induced DNA adducts in vitro and repairable DNA damage in the yeast saccharomyceae Cerevisiae (Saito et al., 2012).

2.5 Gnetum africanum

Gnetum africanum is usually called “eru” in Yoruba “Okazi” in Igbo and “Afang” in Efik and Ibibio. It belongs to the family of Gnetaceae and a division called Gnetaphyte. It is a perennial plant that grows approximately 10 meters long with thick papery-like leaves growing in groups. The leaves may grow approximately 8cm long and at maturity it produces a small flower (Ali et al., 2011). The leaves are edible and are used in the cooking of soups and stew. The leaves may also be used in the treatment of enlarged spleen, sore throat, worm expeller, snake poisoning, diabetes mellitus and pain at child birth (Ekpo, 2007).

2.5 Ocimum citriodorum

Ocimum citriodorum is generally called lemon basil in English and “Ikoh” in Efik and Ibibio. It belongs to the family Lamiacae, Lemon basil is a small shrub of about 20-40 cm tall and small leaves which resemble Ocimum basilicum leaves that tend to be broad.

Secondly, its characteristic sweet aroma makes the leaves useful for cooking of soups, stews, and stir-fried dishes. It is also used in salads.

Moreover, lemon basil also possesses medicinal properties such as anti-diabetic, immune stimulant, antifungal and antioxidant activities. And it also used in ayurvedic remedies for common cold, headaches, inflammation stomach disorder and malaria. And camphor oil that is present is Ocimum citriodorum have antibacterial properties (Janathanam and Sumathi, 2012).
III. MATERIALS AND METHODS

3.1 Collection of Samples

Gnetum africanum (Afang), Ocimum citrodium (Ikoh), Vernonia amygdalina (bitter leaf) were bought in Watt market located at Calabar metropolis. While Senna alata and Portulaca oleracea was obtained from botanical garden located at Malabar, University of Calabar, Calabar, Cross River State. The purchased samples were taken to the Department of botany, University of Calabar for Identification. The identification was carried out by Mr. Henry Ephraim, the Chief Herbarium Officer of the Department of Botany, University of Calabar. 2,2-diphenylpicryl-1-hydrazyl(DPPH), butylated hydroxytoluene (BHT) and ascorbic acid were bought in Victam chemical store at U. J. Esuene stadium, 134 Murtala Mohammed Highway, Calabar, Cross River State. Acetone and distilled water were obtained in the Department of Chemistry, University of Calabar- Nigeria.

3.2 Preparation of Samples, Extracts and Extractants, Reference and DPPH Solutions.

The chemical analysis for antioxidant properties of the sample were carried out using the method of the association of the Official Analytical Chemists (A. O. A. C, 2000). The samples were taken to the laboratory in the Department of Pure and Applied Chemistry, University of Calabar. All the samples were washed thoroughly with distilled water before being dried in an electric oven at the temperature of 60 °C for 2 days. Griffin and George oven was used for the drying process. After the samples were well dried, they were ground with electric blender to give a fine powder, stored in air-tight specimen bottles and then placed in a refrigerator. (Jimoh et al., 2012).

3.3 Preparation of Extracts and Extract Solutions

20 g each of the dry powdered samples were added to 200 ml of acetone in a conical flask and stirred. The flask was covered with stoppers and the mixtures were soaked for 24 hours followed by filtering using Whatman no. 1 filter paper each before the extracts were dried in an electric oven at 40 °C for 2 days.

The acetone was evaporated from the extract solutions and 0.2 mg, 0.4 mg, 0.6 mg, 0.8 mg and 1.0 mg of Vernonia amygaldalina (bitter leaf), Ocimum citrodium (Ikoh), Senna alata (Awolowo), Gnetum africanum (Afang), Portulaca oleracea were weighed with an electric balance using a clean spatula, and were placed into five different labeled test-tubes each for the five different extract samples, they, burrete was filled with acetone and exactly 1ml acetone was added to each 0.2 mg, 0.4 mg, 0.6 mg, 0.8 mg and 1.0 mg labeled test-tubes for each of the five extracts and all the test-tubes were shaken as the acetone was being added until the solid extract dissolved totally. Thereafter, all the extract solutions in all the test tubes were placed into respective labeled sample tubes and covered properly.

3.4 Preparation of Reference Solutions

Exactly, 0.2 mg, 0.4 mg, 0.6 mg, 0.8 mg and 1.0 mg of ascorbic acid and butylatedhydroxytoluene (BHT) were weighed with electric balance and placed in five different labeled test-tube for each of the samples. (1 ml) of acetone was added to each of the test tubes and mixture in each tube was shaken until the content dissolved, thereafter, all the solution in the test tubes were placed into each of their respective labeled samples tubes, and they were well covered.

Theoretical calculation for the groups of DPPH that was used for the preparation of 0.135 mM.

Number of mole of DPPH = 0.135 mM = 1.35 10^{-4} M

Molecular weight of DPPH = 394.32 g/mol

… The number of gram of DpH that will give 1.35 x 10^{-4} Mole = \frac{\text{gram}}{\text{molecular weight}}

=\frac{\text{gram} =\text{mole} \times \text{molecular weight}}{1.35 \times 10^{-2} \text{ mol} \times 294.32/\text{mol}}

=0.053 g

0.135 mM of DPPH was prepared when 0.053 g of DPPH was weighed using electric balance and added to 100 ml of acetone in volumetric flask until DPPH purple pellet was dissolved.

3.5 Determination of Antioxidant Properties

1 ml of DPPH was carefully added to all the various concentrated extract solutions and the reference before they were shaken thoroughly until the DPPH purple color changed to yellow. 1 ml of DPPH was placed in an empty sample tube and was used as a control sample. Thereafter all the mixtures of extracts, reference and control sample were placed in dark cupboard for 30 mins before all the samples were analyzed. The spectroscopic analysis was carried out using optima SP-300 spectrophotometer at the wavelength of 517 nm. During spectroscopic analysis, the cuvette was rinsed with acetone followed by the sample to be analyzed after each analysis.

IV. DISCUSSION

Table 1: Concentration, Absorbance and Inhibition Properties of Sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (mg/ml)</th>
<th>Absorbance</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bitterleaf</td>
<td>0.2</td>
<td>0.623</td>
<td>62.23</td>
</tr>
<tr>
<td>(Vernonia Amygdalina)</td>
<td>0.4</td>
<td>0.687</td>
<td>863.70</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.791</td>
<td>79.10</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.855</td>
<td>85.50</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.893</td>
<td>89.30</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Plant Type</th>
<th>Concentration (mg/ml)</th>
<th>DPPH Scavenging (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candle bush (Senna alata)</td>
<td>0.2</td>
<td>0.652</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.683</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.776</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.833</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.867</td>
</tr>
<tr>
<td>Afang (Gentum Africanum)</td>
<td>0.2</td>
<td>0.528</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.743</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.823</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.871</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.920</td>
</tr>
<tr>
<td>Rat ear (Portulaca Oleracea)</td>
<td>0.2</td>
<td>0.520</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.756</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.899</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.921</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.950</td>
</tr>
<tr>
<td>Lemon basil (Ocimum citriodorum)</td>
<td>0.2</td>
<td>0.435</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.491</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.587</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.651</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.756</td>
</tr>
<tr>
<td>BHT</td>
<td>0.2</td>
<td>0.842</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.967</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.000</td>
</tr>
<tr>
<td>Ascorbic</td>
<td>0.2</td>
<td>0.8087</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.838</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.952</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.000</td>
</tr>
<tr>
<td>Control sample DPPH + acetone</td>
<td></td>
<td>0.227</td>
</tr>
</tbody>
</table>
Acetone | 0.000

Note
Where inhibition % = absorbance of control x absorbance of sample

| Absorbance of control x 100

4.1 DISCUSSION
The result obtained from the analysis of 1 mg/ml of five vegetable extracts indicate that Vernonia amygdalina, S. alata, G. africanum, O. citriodorum and P. Oleracea contain(s) 89.30 %, 86.70 %, 92.00 %, 75.60 % and 95.00 % inhibition of DPPH respectively. In comparison with the reference sample (ascorbic acid and BHT which had 100% inhibition at 1 mg/ml) all the vegetables had some remarkably high antioxidant inhibition properties in DPPH.

It was also shown that Portulacea oleracea had the highest DPPH inhibition property compared with Ocimum citriodorum that exhibited 75% inhibition properties.

The range of DPPH inhibition for all the vegetables from highest – lowest
=95 % - 75 %
= 20 %
The low range showed that antioxidant properties of all the vegetable are remarkably close.

V. CONCLUSION
In conclusion, all the five leafy vegetable studied are rich in antioxidants. The results obtained using 2,2 – diphenyl 1 – picrylhydrazyl (DPPH) radical showed that Portulaca oleracea had the highest of antioxidant scavenging properties among the five vegetables whereas Ocimum citriodorum exhibited the lowest property. A diet consisting of Vernonia amygdalina, Ocimum Citridorum, Gnetum africanum, Senna Alata and Portulaca oleracea could be supplemented with other foods, which are rich in protein and the nutrients.

REFERENCES
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