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Research Article

Antioxidant Properties of Some Nigerian Green Leafy Vegetables

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Abstract

The anti-oxidant properties of acetone extracts of bitter leaf (*Vernonia anygdalina*), lemon basil (*Ocimun citriodorum*, candle bush (*Senna alata*) "Afang" (*Gnetum africanum*) and rat ear (*Portulaca oleracea*) were investigated using 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. The results obtained from the analysis of 1 mg/ml of five vegetable extracts indicate that *Vernonia amygdalina* (VA), *Senna alata* (SA), *Gnetum africanum* (GA), *Ocimun citriodorum* (OC) and *Portulaca oleracea* (PO) contain(s) 89.30%, 86.70%, 92.00%, 75.60% and 95.00% inhibition of DPPH respectively. In comparison with the reference sample {ascorbic (AA) acid and BHT which had 100% inhibition at 1 mg/ml}, it was observed that all the green vegetables investigated had a remarkably high antioxidant inhibition properties in DPPH. *Portulaca oleracea* had the highest DPPH inhibition property (95.00%) compared with *Ocimum citrodorum* which exhibited the least inhibition property of 75.00%. The degree of the inhibition property follows the order: PO > GA > VA > SA > OC. The range of DPPH inhibition for all the vegetables from highest to lowest was known to be 95% - 75% with 20% fundamental interval. The low range implies that antioxidant properties of all the vegetable are remarkably close.

Keywords: Antioxidant; Green Vegetables; Scavenging Method; Acetone Extracts

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 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 cell [1]. Many studies have shown that oxidation activity of free radicals and reactive oxygen species are the causes of several diseases like neuro degenerative disease, cancer, aging and coronary heart disease [2]. 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 of antioxidants

Based on the uses and the system, antioxidants are grouped into two major types, namely: biological system antioxidants and non-biological system antioxidants.

Biological system antioxidants are antioxidants 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 (Silvia., et al. 2004).

Non-Biological System Antioxidants are antioxidants that are useful outside the living system. Consequently, they are mainly utilized 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.

Based on solubility (i.e. with respect to the solvent by which it can be dissolved), antioxidants can also be classified as 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 [3]. Conversely, Hydrophobic Antioxidants are soluble in lipids. 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 [3].

Uses of antioxidants

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 scurvy and for stimulation of co-enzyme in the body [1], 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, however, 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.

Overview of sample

Vernonia amygdalina called bitter leaf in English is a perennial plant that belongs to the family called *Asteracea*. Apart from being highly nutritive, bitter leaf possesses high antioxidant properties of about 77% of DPPH inhibition [4]. More so, studies revealed that the 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 Fe^{2+} ascobate in rat liver for microsomal preparation [5].

Accordind to Erasto., *et al*. [6] the leaves of *Vernonia amygdalina* can be used for treatment of diabetic mellitus, malaria, venereal diseases, wounds, hepatitis and cancer [7].

Portuiaca oleracea is sometimes referred to as pig weed, moss Ross and rat ear. It is an annual succulent plant which belongs to the family called *portulacacea*. According to Aguwa [8], "The whole plant is considered antiphogistic (a substance that eliminates heat), bactericider, anaphrodiac (opposite of aphrodisiac), emollient, calmative, diuretic and refreshing agent". In addition to that, it is also used locally for the treatment of snake poison, insect bite and in cooking of native soup.

Senna alata locally called candle bush in English belongs to the family called *Fabacea*, subfamily, *Caesalpinioideae* [9]. It is locally used for the treatment of fungal skin diseases like ringworm, eczema, stomach problems, etc.

Studies have shown that *Senna alata* possesses anti-carcinogenic and anti-proliferation, anti-diabetic, anti-malaria and hepatoprotective abilities [10]. Researchers also indicate that astragalin from *Senna alata* induced DNA adducts *in vitro* and repairable DNA damage in the yeast *Saccharomyceae cerevisiae* [11].

Gnetum africanum belongs to the family of *Gnetaceae* and a division called *Gnetaphyte*. Ekpo [12] said that the leaves are edible and may also be used in the treatment of enlarged spleen, sore throat, worm expeller, snake poisoning, diabetes mellitus and pain at child birth [13].

Ocimum citrodorum generally called lemon basil in English belongs to the family *Lamiacea*. Its characteristic sweet aroma makes the leaves useful for cooking of soups, stews, and stir-fried dishes. More so, lemon basil possesses medicinal properties such as antidiabetic, immune stimulant, antifungal and antioxidant activities. And it also used in ayurvedic remedies for common cold, headaches, inflammation stomach disorder and malaria. The camphor oil that is present is *Ocimum citrodorum* have antibacterial properties [14].

Aims and Objectives

The aim of the investigation is to determine the antioxidant properties of the following leafy vegetables: *Vernonia amygdalina, Gnetum africanum, Senna alata, Ocimum citriodorum* and *Portulaca oleracea.*

Literature Review

The effect of food storage, processing and preparation on antioxidant properties was investigated by Esienet., *et al*. [15]. They observed that the concentration of genistein and diadzein derivative in vegetable decline at ambient temperature and accelerated approximately 40 – times at high temperature (70 to 90°C). Ferreira., *et al*. [16] on the other hand revealed that the sun drying of pear may cause a 64 percent decrease in total phenolic content on dry pulp basis from 3.7 gram per kilogram at harvest to 1.5 kilogram. They noticed that Procyanidins accounted for about 96 percent of the total phenolic content and that storage of wheat flour for six month led to a 70 percent decline in phenolic acid concentration.

A detailed study by Gahler., *et al.* 2003 assessed the impact of various tomato processing methods on total phenolic, vitamin C content and antioxidant capacity. They observed that 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 [17]. Overall, the team judged that tomato processing leads to a general improvement of the level of individual antioxidants [18].

An exception was documented in a study assessing the impact of tomato processing on carotenoid levels, where modest reduction was found [19]. A third detailed study focused on change in vitamin C, lycopene and total antioxidant capacity in raw tomatoes and after thermal processing. This showed a significant reduction in vitamin C while lycopene levels increased more than 2.5-fold after 30 minutes of processing. The total antioxidant capacity rose to 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" [20].

Experimental procedure

Collection of samples

Gnetum africanum, Ocimum citrodorum, Vernonia amygdalina were bought in Watt market located at Calabar metropolis while *Senna alata* and *Portulaca oleracea* was obtained from botanical garden located at Malabor, University of Calabar, Calabar, Nigeria.

Analysis of sample

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 each of *Venonia amgygdalina, Ocimum citrodorum, Senna alata, Gnetum africanum, Portulacfa oleracea* were weighed and then placed into five different labeled test-tubes each for the five different extract samples, the 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 and then placed in respective labeled sample tubes and covered properly. Furthermore, 1 ml of DPPH was carefully added to all the various concentrated extract solutions and the reference followed by shaking 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 analysis. The spectroscopic analysis was carried out using optima SP-300 spectrophotometer at the wavelength of 517 nm. During the analysis, the cuvette was rinsed with acetone followed by the sample to be analyzed after each analysis [21-30].

Result and Discussion

Figure 1: Variation of Absorbance with concentration of samples and control.

Note: Where

$$\text{Inhibition \%} = \frac{\text{absorbance of control x absorbance of sample}}{\text{Absorbance of control x 100}}$$

Sample	Concentration (in g/m)	Absorbance	Inhibition (%)
Vernonia amygdalina	0.2	0.623	62.23
	0.4	0.687	86.70
	0.6	0.791	79.10
	0.8	0.855	85.50
	1.0	0.893	89.30
Senna alata	0.2	0.652	65.20
	0.4	0.683	68.30
	0.6	0.776	77.60
	0.8	0.833	83.30
	1.0	0.867	86.70
Gentum africanum	0.2	0.528	52.80
	0.4	0.743	74.30
	0.6	0.823	82.30
	0.8	0.871	87.10
	1.0	0.920	92.00
Portulaca oleracea	0.2	0.520	52.00
	0.4	0.756	75.60
	0.6	0.899	89.90
	0.8	0.921	92.10
	1.0	0.950	95.00
Ocunum citriodorum	0.2	0.435	43.50
	0.4	0.491	49.10
	0.6	0.587	58.70
	0.8	0.651	65.10
	1.0	0.756	75.60
BHT	0.2	0.842	84.20
	0.4	0.967	96.70
	0.6	1.000	100
	0.8	1.000	100
	1.0	1.000	100
Ascorbic	0.2	0.8087	80.87
	0.4	0.838	83.80
	0.6	0.952	95.20
	0.8	1.000	100
	1.0	1.000	100
Control sample DPPH + acetone		0.227	
Acetone		0.000	

Table 1

Table 1 shows the antioxidant properties of *Venonia amygdalina, Ocimum citrodorum, Senna alata, Gnetum africanum, Portulacfa oleracea*. The results obtained from the analysis of the sample extracts indicate that *Vernonia amygdalina, Senna alata, Gnetum africanum, Ocimun citriodorum* and *Portulaca oleracea* contain(s)

89.30%, 86.70%, 92.00%, 75.60% and 95.00% inhibition of DPPH respectively. The antioxidant property of *Portulaca oleracea* (95%), *Vernonia amygdalina* (89.30%), *Senna alata* (92.00%) and *Gnetum africanum* (92.00%) are higher than the value of 77% obtained by Imaga and Bamigbetan, 2013 for bitter leaf; however, *Ocimun citriodorum* (75.60%) is slightly lower though comparable to that of bitter leaf in their analysis.

Figure 2: Variation of absorbance and concentration for the samples.

Summary

Antioxidant scavenging properties of *Vernonia amydalina, Ocimum citrodorum, Gnetum africanum, Senna alata, Portulacfa oleracea* were studied using analytical methods. The results obtained using 2,2 – diphenyl 1 – picrahydrazyl (DPPH) radical showed that *Portulacfa oleracea* had the highest of antioxidant scavenging properties among the five vegetables whereas *Ocimum citrodorum* exhibited the lowest property.

Conclusion

In conclusion, all the five leafy vegetable studied are rich in antioxidants. An diet consisting of *Vernonia amydalina, Ocimum citrodorum, Gnetum africanum, Senna alata* and *Portulacfa oleracea* could be supplemented with other foods, which are rich in protein and the nutrients.

Recommendation

I hereby recommend that the vegetables should be used as food supplement and more work should be carried out on vivo assessment of antioxidants of the samples. Further studies should also be centered on toxicant composition of the samples as well as the effects of storage and processing on nutrient and antioxidant properties on the sample.

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