

Research Article

The Antioxidant and Free Radical Scavenging Effects of Extracts of Seeds of Some Neglected Legumes of South-East Nigeria

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Abstract: The antioxidant and free radical scavenging capacity of five legumes namely- *Mucuna pruriens*, *Mucuna slonei*, *Monodora myristica*, *Mucuna rostrata*, *Curcubita pepo* and *Legioneria spherica* were assayed. The extracts of the samples were analyzed for chemical compositions, antioxidant vitamins C and E, phytochemicals. These were carried out by different extraction processes. The following assays were equally carried using current methods: free radical scavenging activity using hydroxyl and hydrogen peroxide radicals and peroxidation inhibiting activity through linolenic acid extracting system. Results revealed the presence of bioactive constituents, which include: alkaloids (2.0±0.05 mg/100g); saponins 1.2-5.9 mg/100g; oxalates (0.013 -0.390); cyanogenic glycosides (11.34-21.06 mg/100 g). These legumes contain various levels of phytochemicals, antioxidant vitamins (vit C. from 30.0mg-125.0mg/100g), vitamin E (0.092-2.665mg/100g; in all assays they exhibited high levels of antioxidant, reducing power and lipid peroxidation inhibition activities. They are equally sources of good quality antioxidants that have gainful uses as food additives, drugs, antimicrobials, colorants, preservative, some of which have been used prophylactically and therapeutically in the management of many human diseases and syndromes.

Keywords: Antioxidants, legumes, free radical scavenging functions

INTRODUCTION

Antioxidants are widely used as food additives to provide protection against oxidative degradation of foods by free radicals. Since ancient times, spices added to different types of food to improve flavors are also well known for their antioxidant capacities [1]. In recent times, there has been great interest in screening essential oils and various plant extracts for their good natural antioxidants properties. In order to prolong the storage of foods, several synthetic antioxidants such as butylated hydroxyanisole (BHA) are used currently, but these substances may be inappropriate for chronic human consumption as recent publications have indicated their possible toxic properties for human health and the environment [2, 3]. Hence, the development of alternative antioxidants of natural antioxidant origin has attracted considerable attention and is generally thought to be a desirable development. In plant cells, antioxidants help keep reactive oxygen species (ROS) at low concentrations, avoiding oxidative damage while allowing themselves play crucial functions in signal transduction [4]. However, little is known about the role of antioxidants during fruit maturation, especially in legumes. Leguminous plants such as pea (*Pisum sativa*), bean (*Phaseolus vulgaris*) or alfalfa (*Medicago sativa*) are crops of many economic values, such as protein source for human and animal consumption [5]. Antioxidants modulate the steady state concentrations of ROS, avoiding their potential cytotoxicity, while allowing them to function as signal molecules [6]. The roles of antioxidant enzymes have been documented.

Antioxidant enzymes which include, superoxide dismutase(SOD),Catalases, Peroxiredoxins(PRXs), Glutathione peroxidases (GPXs) and the four enzymes of Ascorbate-glutathione pathway [7]. In this pathway ascorbate peroxidase(APX) catalyzes the reduction of H₂O₂ to water by ascorbate, producing monodehydroascorbate and dehydroascorbate respectively. The antioxidants of leguminous leaves and nodules have been examined considerably [8, 9], but similar information on leguminous fruits/seeds is lacking. The study of antioxidants in seeds and fruits is very important for many reasons. Firstly, antioxidants may protect fruits/seeds from potentially toxic ROS and thereby contribute to stress tolerance of crops [6]. Secondly, fruits/seeds may possess nutritional values for animal and human consumption. Thirdly, in many cases, fruits/seeds have relatively short shelf- life following harvest, during which period, they undergo changes in texture, color and flavor, which may be accompanied by decrease in antioxidant levels. However, plants often contain substantial amounts of antioxidants including tocopherols (Vitamin E), carotenoids, ascorbic acid, flavonoids, phenolics and tannins [10].

Many people from developing countries have resorted to medicinal plants in order to treat large number of ailments or disease conditions. Three plant species with their antioxidant properties of anthocyanins, have been found to inhibit sickle cell polymerization of deoxyHbS and also reverse sickled erythrocytes. For example, three species of *Justicia*

namely, *J. tenella*, *J. gendarusa* and *J. insularis*, were found to possess profound antisickling potency as a result of high levels of anthocyanins [11-13]. Antioxidants have been found to possess anticancer, anticardiovascular, anti-inflammatory and other activities. Most antioxidants have been employed in the management and therapy of some malignant syndromes. For example, erythropoietin (Epo), a hormone released upon hypoxia mainly in the kidneys, enhances red cell production (RBC) erythropoiesis by stimulating the proliferation of erythroid progenitors and precursors in the bone marrow. Recombinant human Epo is widely used for the treatment of anemia. Although the main effect of Epo is related to the stimulation of erythropoiesis, it was suggested that in patients with chronic renal failure on dialysis, its anti-anemic effect may be associated with increasing the survival of mature red blood cells. Sickle cell disease is characterized by a pro-oxidant environment due to high production of reactive oxygen species (ROS), related to increased levels of free pathological iron and heme groups associated with reduction in antioxidant systems such as GSH. Studies in vitro on sickle cell disease (SCD) red cells have shown that iron chelation by deferiprone, reduce the sickle red cell membrane susceptibility to iron mediated oxidative damage. Antioxidants are widely used as food additives to provide protection against oxidative degradation of foods by free radicals [14]. Since ancient times, spices are added to different types of food to improve flavors and are, also well known for their antioxidant capacities [1]. In recent times, there has been great interest in screening essential oils and various plant extracts for natural antioxidants because of their good antioxidant properties. In order to prolong the shelf-life of foods, several synthetic antioxidants are employed by food and beverage industries [2, 3].

The plants involved in this study include: *Monodora myristica*. Investigations reveal that almost every part of this plant has economic importance [15]. The most economically important part is the seed, which is embedded in a white sweet smelling pulp of the fruit. The kernel is the popular condiment used as a scouring agent. When ground into powder, it is used or administered to women after childbirth to control passive uterine hemorrhage [15]. It has also been implicated with antisickling effectiveness [16].

Mucuna pruriens (Akurugba), the plant is an annual climbing shrub. When young, it is almost completely covered with fuzzy hairs; and when old, it is almost completely free from hairs. Its seeds are borne in pods which are about 10 cm long and covered with an array of hairs that cause severe itch when in contact with skin. The chemical compounds responsible are proteins and serotonin. In many parts of the world, *Mucuna pruriens* is used as forage and as green manure. The roots possess nodules containing nitrogen fixing bacteria. It is a widespread fodder plant. The silage

contains 11-23 % crude protein, 35-40 % crude fiber, and the dried beans 20-35 % protein. The seed in powdered form is used as coffee substitute called Nescafe. Unprocessed *Mucuna pruriens* seeds are toxic to some-ruminant animals including humans because of the anti-nutrients, such L-Dihydroxyphenylalanine (L-DOPA). A preparation from the leaves contain L-DOPA, used as an aphrodisiac and to increase libido in men and women respectively. *Mucuna pruriens* contain Serotonin, 5-HT, 5-HTP, Nicotine, bufotenine [17].

Most of the natural antioxidants include: Vitamin C (Ascorbic acid) and vitamin E. These antioxidants are vital for growth and maintenance of healthy bones, teeth, gums, ligaments and blood vessels [18-20]. Vitamin C is present in many fruits, legumes and vegetables, particularly in tomatoes, citrus fruits, spinach, green peppers, cabbage and potatoes. Vitamin C assists the immune system in two of its primary functions- (a) to rid the body of foreign invaders. It can accomplish these functions by stimulating the production of white blood cells, primarily neutrophils, which attack and neutralize foreign pathogens and antigens such as bacteria, high molecular weight polysaccharides and viral coat proteins. It also boosts the body's production of antibodies and interferon. Vitamin E is a generic name for the tocopherols and tocotrienols. It is a fat-soluble vitamin that acts in the body as antioxidant and important in the formation of erythrocytes. It is an antioxidant that inhibits the production of free radicals or reactive oxygen species (ROS) under lipid peroxidation [21-23]. Vitamin E is a family of α , β , and γ tocopherols. Tocotrienols have specialized roles in protecting neurons from damage, cancer prevention and cholesterol reduction by inhibiting the enzyme HMG-CoA reductase. Oral consumption of tocotrienols is also proven to protect against stroke associated brain damage [24].

Phytochemicals are plant derived chemical compounds that possess potential health promoting properties. Evidence suggests that, a diet high in fruits, vegetables and legumes may decrease the risk of chronic diseases such as cardiovascular diseases and colon cancer. Phytochemicals like phenolic compounds and derivatives, flavonoids, tannins, alkaloids, oxalates, saponins, cyanogenic glycosides, carotenoids and others, have been indicated in a wide array of applications. These may play key roles in reducing chronic disease risks. Diets high in fruits, vegetables and legumes have significant protective effect against a variety of disease states like coronary heart disease, cataract, diabetes, Alzheimer disease, cancer and even asthma. These protective effects are attributed to abundant phytochemicals resident in the plants [25-28]. Tannins are polyphenolics, obtained from various parts of plants. They are abundant on the bark of trees, fruits, fruit pods, leaves, legumes (seeds and leaves), vegetables and roots. They protect plants against bacteria and viruses. They are found in use in

photography, dyeing, beer and wine industry and employed as astringents in medicine.

Flavonoids are compounds found in fruits, vegetables, legumes and certain beverages and possess diverse biochemical and antioxidant effects. Their dietary intake is quite high compared to other dietary antioxidants like vitamins C and E respectively. Flavonoids are polyphenolic compounds that are ubiquitous in nature. They are characterized into flavonoids, flavones, flavonones, catechins, anthocyanidins and chalcones. Over 4000 flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. Flavonoids possess antiviral, anti-allergic, anti-platelet, anti-inflammatory, anti-tumor and antioxidant activities [29]. Recently, phytochemical compounds present in legumes have generated a lot of interest because they are considered to be possible chemoprotective agents. Extracts of 10 polyphenolic compounds from leguminous plants in Greece which contain mixtures of two compounds and five pure flavonoids extracted from aerial plant parts of *Vicia faba* and *Lotus edulis* have been characterized (Leguminosae). These fractions were found to exhibit significant DPPH radical scavenging capacity. They also exert significant protective activity against free radical induced DNA damage [30]. This current research work focuses on the phytochemical content, the antioxidant activity, the free radical scavenging effects of five indigenous legume seeds highly consumed in the South-East geo-political zone of Nigeria. These would in no small measure assess the nutritional and medicinal potentials, vis-à-vis, the pharmacological and therapeutic roles of these leguminous plant parts.

MATERIALS AND METHODS

Plant materials

The experimental plant materials include the seeds of various plant species such as: *Mucuna pruriens* (Velvet beans), *Lagenaria spherica*, collected from Eziofodo village in Owerri, Imo State of Nigeria. Others include: *Curcubita pepo*, *Monodora myristica* and *Mucuna rostrata*, bought at the Owerri relief market. These legume seeds were identified and authenticated by a crop scientist Mr. Francis Iwueze, a plant taxonomist at the department of crop science and technology of the university, as being of the best varieties.

Fifty grams (50 g) of each of the seed samples were weighed, air dried at room temperature of 27 °C for 4 days to obtain constant weight. The samples were then oven dried at 60 °C for 3 days, for initial moisture analysis and at 105°C for final moisture content. The seeds were then milled into powder using a manual grinder, sieved and the flour stored in air tight bottles, properly labeled in a dessicator.

Determination of Antioxidants and Phytochemicals

Determination Of Antioxidants

Five grams (5 g) of the powdered samples were weighed into an extraction tube and 100 ml of EDTA/TCA (2:1) extraction solution mixed, the mixture shaken for 30 minutes. These were transferred to a centrifuge, centrifuged at 1500 X g for 20 minutes. The extracts (filtrates) were transferred into 100 ml volumetric flasks and volume made up to the 100 ml mark with the extracting solution. Twenty milliliters (20 ml) of each of the extracts were pipetted into a volumetric flask and 1% solution of starch (indicator) added and titrated with 20 % CuSO₄ solution, to get a dark end point [31]

Determination of Vitamin E

Two and a half milliliters (2.5 ml) of each of the samples were placed in two test tubes; 0.5 ml Nitric acid added to each tube and placed in boiling water for 3 minutes. Test tubes were cooled and allowed to stand in the dark for 15 minutes. The volume of solution in each test tube was brought to 5 ml with ethanol. It was then mixed and absorbance measured at 470 nm. The concentration of vitamin E in the test solution was determined using a calibration curve of standard vitamin E concentration [32]. A blank preparation containing 2.5 ml of distilled water was placed in a test tube and 0.5 ml nitric acid added, placed in a boiling water bath for 3 minutes. The tube was cooled and kept in the dark. The standard was prepared using a capsule of 1000 mg vitamin E purchased from Eva Pharmacy Ltd, Elele, PortHarcourt, Nigeria.

Determination of Phytochemicals

The phytochemicals -alkaloids, flavonoids, tannins, cyanogenic glycosides and oxalates were determined using standard methods of the Association of Official Analytical Chemists [33].

Determination of Antioxidant Activity

Rapid screening for free radical scavenging activity. Thin layer chromatographic screening for antioxidant activity was carried out by spotting a concentrated methanol solution of each extract on silica gel plates. The plates were sprayed with 0.2% w/v DPPH in methanol. The spots were visualized for the presence of yellowish spots.

DPPH's Radical Scavenging Activity

The radical scavenging activity of the plant extracts against 1,1-diphenyl-1-picryl-hydrazyl-(Sigma-Aldrich) radical was determined by measuring UV absorbance at 517 nm. Radical scavenging was measured by a slightly modified method [34, 35]. The following concentrations of the extracts were prepared: 0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml. Ascorbic acid and α -tocopherol were used as standards and the same concentrations were prepared as test solutions. All of the solutions were prepared with methanol. Two milliliters (2 ml) of each prepared concentration were placed into test tubes and 0.5 ml of 1mM DPPH solution in methanol was added

thereafter. The experiments were carried out in triplicates. The test tubes were incubated for 15 minutes at room temperature and the absorbance read at 517 nm. A blank solution was prepared and measured containing the same amount of methanol and DPPH. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The radical scavenging activity was calculated using the following formula:

$$\text{DPPH scavenging effect} = \left[\frac{AB - AA}{AB} \right] \times 100$$

Where, AB is the absorbance of the blank sample and AA is the absorbance of test extract solution.

Determination of total antioxidant Activity (FRAP Assay)

Total antioxidant activity was carried out by a modified method [35] which was adopted for the ferric reducing antioxidant power (FRAP) assay. It depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (111)-TPTZ) complex to ferrous tripyridyltriazine (Fe(11)-TPTZ) at low pH. Fe(11)-TPTZ has an intensive blue color which can be read at 593 nm. One and a half milliliter (1.5 ml) of freshly prepared FRAP solution, containing 25 ml of 300 mM acetate buffer, pH 3.6; 2.5 ml of 10 mM 2,4,6-tripyridyl-s-triazine(TPTZ) in 40 mM HCl, and 2.5 ml of 20 mM ferric chloride [FeCl₃ · 6H₂O] solution, was mixed with 1 ml of the extracts and the absorbance read at 593 nm. The standard curve was linear between 100 and 500 μM FeSO₄ · 7H₂O. Results are expressed in μM Fe (11)/g dry plant material compared with that of Ascorbic acid.

Hydrogen Peroxide Scavenging Activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the methods [36, 37]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230nm using a spectrophotometer. Extracts (0.1mg-1.0 mg) in distilled water were added to hydrogen peroxide solution (0.6 ml, 40mM). The absorbance of H₂O₂ at 230 nm was determined after 10

min against a blank solution containing phosphate buffer without hydrogen peroxide. Scavenging by the extract and standard compounds was calculated as follows: % scavenged [H₂O₂] = [A₀-A₁/A₀] X100, where A₀ is the absorbance of the control and A₁ is the absorbance in the presence of the extract and standard [36, 38].

Reducing Power Determination

The reducing power of the extracts was determined according to the method [39]. Different concentrations of each extract (25-80 μg /ml) in water were mixed with phosphate buffer 2.5ml, 0.2M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5ml, 1%) .The mixture was incubated at 50⁰ C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, the reaction mixture was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%) and the absorbance measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as positive control.

Statistical Analysis

All data were analyzed using on-way ANOVA, and results presented as Mean ±SD from triplicate determinations. The level of significance was placed at p≤0.05

RESULTS

Results are presented in tables 1-7 and Fig.1 below. Table 1 shows the moisture content of the samples expressed in g/100 g of sample. Table 2 shows the concentrations of two antioxidant vitamins C and E. Values are expressed in mg/ 100g of sample. Table 3 shows the quantitative phytochemical composition of samples. Table 4 shows the H₂O₂ (Hydrogen peroxide) scavenging activity of the samples. Table 5 shows the reducing power of the samples. Table 6 shows the results from FRAP and DPPH assays. Table 7 shows the total antioxidant activity of samples with time in days. Fig 1 shows a plot of total antioxidant activity against time in days.

Table 1: Moisture content of samples expressed in g/100 g of sample

Sample	Concentration g/100 g
<i>Mucuna pruriens</i>	5.313±0.01
<i>Lagenaria spherica</i>	11.305±0.10
<i>Curcubita pepo</i>	11.358±0.00
<i>Monodora myristica</i>	6.405±0.00
<i>Mucuna rostrata</i>	10.343±0.10

Values in the table are the Mean ± SD from triplicate determinations.

Table 2: Concentrations of Antioxidant Vitamins C and E of Samples

Sample	Vitamin C (mg/100g)	Vitamin E (mg/100g)
<i>Mucuna pruriens</i>	125.0	0.914
<i>Lagenaria spherica</i>	50.0	0.092
<i>Curcubita pepo</i>	35.0	0.943
<i>Monodora myristica</i>	30.0	0.665
<i>Mucuna rostrata</i>	95.0	1.840

Table 3. The Phytochemical Compositions of the Samples

Sample	Alkaloids (%/5g)	Saponins (%/5g)	Tannins (mg/100g)	Flavonoids (%/5g)	Oxalate (mg/100g)	Cyanogenic glycosides (mg/100g)
<i>Mucuna pruriens</i>	1.40±0.0	2.40±0.1	7.99±0.2	6.40±0.0	3.57±0.2	11.31±0.1
<i>Lagenaria spherica</i>	1.00±0.0	6.20±0.0	8.26±0.1	17.00±0.0	2.61±0.1	21.06±0.1
<i>Curcubita pepo</i>	0.80±0.0	3.80±0.2	9.18±0.0	18.40±0.0	1.10±0.0	12.96±0.0
<i>Monodora myristica</i>	6.60±0.0	11.80±0.0	7.75±0.1	6.80±0.0	2.61±0.1	14.58±0.2
<i>Mucuna rostrata</i>	1.20±0.0	5.00±0.0	8.83±0.1	16.20±0.1	3.16±0.1	17.82±0.2

The values in the table are the Mean ± SD from triplicate determinations

Table 4: Hydrogen peroxide (H₂O₂) and Hydroxyl radical (OH) Scavenging Activities of samples

Sample	H ₂ O ₂ (%)	OH (%)
<i>Mucuna pruriens</i>	4.62	7.42
<i>Lagenaria spherica</i>	4.72	6.21
<i>Curcubita pepo</i>	4.88	4.64
<i>Monodora myristica</i>	4.62	1.84
<i>Mucuna rostrata</i>	50.0	3.73
Vitamin C	62.0	7.92

Table 5: Antioxidant Reducing Power of the Samples

Sample	% Reducing power
<i>Mucuna pruriens</i>	7.12
<i>Lagenaria spherica</i>	6.22
<i>Curcubita pepo</i>	5.47
<i>Monodora myristica</i>	2.76
<i>Mucuna rostrata</i>	4.25
Vitamin C	10.22

Table 4: Results of DPPH and FRAP assays showing IC₅₀ values for the DPPH

Extracts/Standard	FRAP	DPPH(IC ₅₀ µg/ml)
<i>Mucuna pruriens</i>	225.0±0.01	303.21±14.05
<i>Lagenaria spherica</i>	200.0±0.0	108.30±8.60
<i>Curcubita pepo</i>	270.0±0.1	317.36±11.21
<i>Monodora myristica</i>	210.0±0.0	229.60±10.56
<i>Mucuna rostrata</i>	281.0±0.0	231.40±12.21
Vitamin C	300.0±0.1	561.10±20.10

Values I the table are the Mean± SD from triplicate determinations

Table 7: Antioxidant activity of samples expressed in percentage (%) FTC method at different time intervals using 0.2mg/ml of sample and 0.1 mg/ ml of Vitamin C

Sample/ Time(hr)	0	24	48	72	96
<i>Mucuna pruriens</i>	90	80	70	60	50
<i>Lagenaria spherical</i>	90	60	50	45	40
<i>Curcubita pepo</i>	90	65	60	50	40
<i>Monodora myristica</i>	90	65	50	40	35
<i>Mucuna rostrata</i>	90	50	45	40	35
Vitamin C	90	80	75	65	50

Values in the table are the percent antioxidant activity of samples determined by the FTC method.

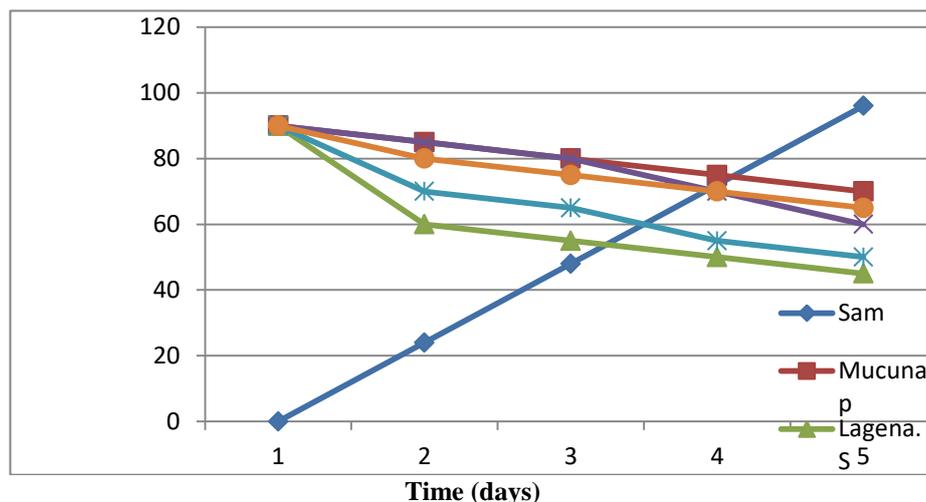


Fig. 1 Antioxidant activity of samples in FTC method at different time intervals using 0.2 mg/ml of samples

DISCUSSION

The study aims at evaluating the antioxidant content as well as, the free radical scavenging abilities of crude aqueous extracts of the following indigenous leguminous seeds of *Mucuna pruriens*, *Lageneria spherica*, *Monodora myristica* (Ehuru) or African nutmeg, *Curcubita pepo*, and *Mucuna rostrata*. The plant *Monodora myristica*, is more prevalent in the Southern part of Nigeria. The seed/kernel is a popular condiment used as a scouring agent for hemorrhage in post partum women [15]. It has been reported for *Mucuna pruriens* to be endowed with abundant phytochemicals and bioactive substances that have found therapeutic values. Its crude protein content which within 11-23 % and crude fiber (31-40%). It is an aphrodisiac, other common compounds identified include: 5-HT, nicotine, N,N-DMT bufotenine. It is the world richest source of dopamine for the treatment of Parkinsonian epilepsy. From our study, the phytochemical compositions of the seeds remain outstanding. Table 3 shows the content of alkaloids, saponins, flavonoids, tannins and cyanogenic glycosides from these leguminous seeds. It has been discovered that flavonoids show strong antioxidant activity and their effects on human nutrition and health are considerable. The mechanism of action of the flavonoids are through scavenging or chelating processes [41,42]. The flavonoid content of the samples range from 6.4mg/5g of sample for *Mucuna pruriens* to 18.4mg/5g for *Curcubita pepo*. Apart from flavonoids, saponins are abundantly present in the samples and play outstanding role as antiseptics. Their concentrations range from 1.0%/ 5g for *Lageneria spherica* to 6.6%/5g for *Monodora myristica*. Phytochemicals are determined along with the antioxidant vitamins C and E, because of their similar role in scavenging free radicals from cells undergoing apoptosis. Flavonoids are potent water- soluble antioxidants and free radical scavengers, which possess anticancer activity [43]. Flavonoids in the intestinal tract lower the risk of colon

cancer and also provide anti-inflammatory activity [43]. As antioxidants oxidants, flavonoids from these legumes such as *C. pepo*, *L. spherica* and *Mucuna rostrata*, have been used for the treatment of wounds, burns and ulcers in herbal medical practice. Tannins have astringent properties hasten the healing of wounds and reduction of inflamed mucous membranes. The high saponin content of *M. myristica*, *L. spherica* and *M. rostrata* compare to *M. pruriens* and *C. pepo*, justifies the use of the extracts to stop bleeding. Saponins have the property of precipitating and coagulating red blood cells. Alkaloids and their synthetic derivatives are used as basic medicinal agents such as analgesics, antispasmodics and antibacterial agents [44]. They exhibit marked physiological activity when administered to animals. Cyanogenic glycosides were detected in all samples but at low concentrations. Some of these legumes seem to be toxic and need to be processed before consumption. Two samples may be rich sources of cyanogenic glycosides namely-*Mucuna pruriens* and *Mucuna rostrata* respectively.

Table 2 shows the antioxidant compositions of vitamins C and E of the samples respectively; the values are well pronounced. The vitamin E content ranged from 0.914 mg/100g for *Mucuna pruriens* to 2.665 mg/100 g for *Monodora myristica*. It can be seen that the antioxidant status in terms of vitamin E is remarkable, but differs from that of vitamin C. It could be that many plant seeds have low vitamin C content, which nonetheless is present more in leafy and juicy parts of vegetables and succulent fruits than in the seeds. In table 2, the highest vitamin C content is shown by *Mucuna pruriens* (125 mg/100g of sample, while *Monodora myristica* shows the least (30 mg/100g of substance). Free radicals are involved in many disorders like neurodegenerative syndromes or diseases such as cancer, HIV/AIDS, Parkinsonian epilepsy, diabetes mellitus, sickle cell disease and ageing. Antioxidants through their radical scavenging ability

have proved relevant for the effective management of these diseases and the protection of various cells from mutagens. Antioxidants are used as medications to treat various forms of brain injury. Superoxide dismutase mimetics [45], Sodium thiopental and Propofol are used to treat reperfusion and traumatic brain injuries [46], while some other drugs like Disufenton sodium^R and EbseleN^R [46, 47] are used for treatment of stroke. These compounds appear to prevent oxidative stress in neurons and prevent apoptosis and neurological damage. Antioxidants are also being investigated as possible treatment for neurodegenerative disorders such as Alzheimer's disease and Parkinsonian disease and any atrophic lateral sclerosis [49,50]. This review is in line with the literature on the roles of *Mucuna pruriens* and *Mucuna rostrata* that have been used in the treatment of Parkinsonian epilepsy. It has been stated that some targeted antioxidants may lead to better medical effects. For example, mitochondria-targeted ubiquinone, may prevent damage to the liver caused by excessive alcohol [51]. It has equally been stated that the antioxidant effects of flavonoid-rich foods seem to be due to fructose induced increases in the synthesis of the antioxidant uric acid and not to dietary antioxidants per se [52].

Reducing power assayed by Fe (111) reduction, is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action [37]. In the reducing power assay, the presence of antioxidants in the sample results in the reduction of Fe³⁺ to Fe²⁺ by the donation of an electron. This mechanism becomes very relevant for almost all antisickling agents. Most, if not all antisickling agents increase the Fe²⁺/Fe³⁺ ratio, this in other words, measures the increase in oxygen affinity of sickle erythrocytes and the reversion of the sickling syndrome [16]. The amount of Fe²⁺ complex can be monitored by measuring the formation of Perl's Prussian blue at 700nm. Increasing absorbance at 700nm indicates an increase in reductive ability. In table 5, the results of the reducing power assay were displayed; the result showed that *Mucuna pruriens* had the highest level of reducing power among the samples almost comparable to antioxidant vitamin C. All extracts showed good reducing power, although only one concentration of samples was used. Results of the FTC antioxidant assay focused on the effect of peroxidation on membrane lipids. Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Specifically, two types of lipids are susceptible namely, linoleic acid and arachidonic acid [37]. The seed extracts possess notable vitamin E concentration. Studies indicated that oxidation of low density lipoproteins in the blood contributes to heart disease and initial observational studies found that people taking vitamin E supplements have a lower risk of developing heart disease [52, 53]. Fig 1 shows the time course plots for the antioxidant activity of the plants extracts using the FTC method. All extracts exhibited

moderate concentration dependent antioxidant activity. There are significant differences ($p \leq 0.05$) among plant extracts and vitamin C at different incubation times. The research work has investigated the antioxidant and free radical scavenging effects of extracts of some underutilized legumes in our region. Some of these legumes have been consumed for years by the residents of the sub- ethnic group without health hazards. Some of these legumes are consumed after processing. *Mucuna rostrata* is used as soup thickener in food preparation. The incidence of degenerative disorders in this region is very low and of low occurrence. The nutritional and medicinal roles of some antioxidant vitamins have already been reviewed [54-56]. We are of the opinion that these under-utilized legumes can be consumed with caution and only when well processed because of their cyanogenic glycoside content. They are rich sources of antioxidant vitamins C and E; very potent antioxidant vitamins. From the preponderance of phytochemicals, antioxidant vitamins, minerals, drugs, nutrients and other medicinal components; these under-utilized legumes can readily provide the pharmaceutical and other industries, unlimited source of necessary raw materials for technological revolution in the Nigerian economy.

REFERENCES

1. Madson HI and Bertelson G; Spices as antioxidants. Trends Food Science Tech.,1995; 6:271-277.
2. Ito N, Hirose M, Fukunshima H, Tsuada I, Shirai T, Intenntsu M; Studies on antioxidants, their carcinogenic and modifying effects on chemical carcinogens. Food Chemistry and Toxicology, 1980; 24:1071-1092.
3. Stich HF; The beneficial and hazardous effects of simple phenolic compounds. Mutation Research, 1991, 239: 307-324.
4. Manuelli AM, Jerge L, Karl-Josef Dietz, Pedro M, Aparicio T, Maniust Becana; Functions of antioxidant enzymes and metabolites during maturation of Pea fruits. Journal of Experimental Botany, 2010; 6(1): 87-97.
5. Graham PH, Vance CP; Legumes, importance and constraints to greater use. Plant Physiology, 2003; 131:872-879
6. Mittler R, Vaderaawera S, Gollery M, Van Breusegen F; Reactive oxygen gene network of plants. Trends in Plant Science, 2004; 9: 490-498.
7. Dietz K J; Plant peroxiredoxins. Annual Review of Plant Biology, 2003; 54: 93-107.
8. Amarowicz R, Pegg RR, Meghaddam PR, Bart B, Weil JA; Free radical scavenging capacity and antioxidant activity of selected plant species from Canadian prairies. Food Chem., 2004; 84: 551-562.
9. Mohammed A, Ebrahim Z, Seyed M, Nehavi S, Fazel N, Fatemeh B, Aihmad PB; Antioxidant and free radical scavenging

- activity of *H. officinales* L.var, *Angustifolios* V, *Odorota* B, *Hyrcana* and *C. speciosum*. *Pakistan Journal Pharm Sci.*, 2010; 23(1): 29-34.
10. Larson RA; The antioxidants of higher plants. *Phytochemistry*, 1988; 27: 969- 978.
 11. Mpiana PT, Bokota MT, Ndjete MBZ, Mudago V, Ishibange DST, Ngbolua KN *et al.*; Antisickling activity of three species of *Justicia* from Kissangari (DR Congo). *J. tenella*, *J. genderassa* and *J. insularia*. *International J Biol Chem.*, 2010b; 4 (6): 1953-1961.
 12. Mpiana PT, Mudago V, Tshibangu DST, Ngbolua KN, Shetorde OM, Mbala MB; *In vitro* antisickling activity of Anthocyanin extract from *Occimum basilicum* (L). *International Journal of Pharmacology*, 2007a; 3(4):371-374.
 13. Mpiana PT, Mudago V, Tshibangu DST, Kitwa EK, Kanangila AB, Lambu JBS *et al.*; Antisickling activity of Anthocyanidins from *Bombax pentadrum*, *Ficus capensis*, *Ziziphus mucronata*; photodegradation effect. *Journal Ethnopharmacology*, 2008; 120: 413-418.
 14. Gulcin I, Okta M, Kufrevioghu OI; Aslun A .Determination of antioxidant activity of Lichen (*Cetraria islandica*(L), *Arch J Ethnopharmacology*, 2002; 79: 325-329.
 15. Okafor JC; Edible indigenous woody plants in the rural economy of the Nigerian forest zone. *Forest Ecology and Management*, 1981; 3: 48-55.
 16. Uwakwe AA, Nwaoguikpe RN; *In vitro* antisickling effects of *Xylopiya aethiopica* and *Monodora myristica* on sickle cell blood. *Journal of Medicinal Plant Research*, 2008; 2 (6):119-124.
 17. Nwaoguikpe R, Braide W, Ujowundu CO; The effect of processing on the Proximate and Phytochemical Compositions of *Mucuna pruriens* seeds (Velvet Beans). *Pakistan Journal of Nutrition*, 2011; 10(10): 947-951.
 18. Duru M, Amadi B, Agomuo E, Eze A; Chemical profile of a antimalarial concoction ‘UdU’ used in Umunchi Autonomous Community in Isiala Mbano LGA of Imo State, Nigeria. *Journal of Emerging Trends in Engineering and Applied Sciences*, 2012; 3 (3): 444-447.
 19. Nadeem AA, Atio A, AzharA IA; The effect of anti-browning agents on quality changes of Loquat fruit after harvest. *Pakistan Journal of Botany*, 2013; 45(4):1391-1396.
 20. Dawey MW, Montagu MV, Inze D, Sanmartia A, Kanellis N, Smimoff IF *et al.*; Plant L-Ascorbic acid chemistry, functions, metabolism, bioavailability and effects of processing. *Journal of Food Science and Agriculture*, 2000; 89: 825-860.
 21. Uttara AV, Singh PZ, Mohaja RT; Oxidative stress and neurodegenerative disease: a review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacology*, 2009; 7(1):65-74.
 22. Smith MA, Perry P, Richey L; Oxidative damage in Alzheimer’s. *Nature*, 1996; 382(6587):120-121.
 23. Ames B; Dietary carcinogens and anti-carcinogens. Oxygen radicals and degenerative diseases, *Science*, 1983; 221(4617): 1256-1263.
 24. Aruoma OI; Free radicals, oxidative stress and antioxidants in human health and disease. *Journal of the American Oil Chemists Society*, 1998; 75(2): 199-212.
 25. Shi J, Arunasalem K, Yeung D, Kakada Y, Mittal G, Jiang Y; Saponins from edible legumes, chemistry, processing and health benefits. *Journal Medicinal Food*, 2004; 7(1): 67-78.
 26. Phan-Huy LA, He H, Pham-Huye C; Free radicals antioxidants, in disease and health. *International Journal of Biomedical Sciences*, 2008; 4(2): 89-96.
 27. Terao J, Piskula MK; Flavonoids as inhibitors of lipid peroxidation in membranes. In Rice-Evans CA, Packer L editors; *Flavonoids in health and disease*, Marcel Dektar, NewYork, 1997: 277-295.
 28. Cook NR, Albert CM, Gaziano JM; A randomized factorial trial of vitamins C and E, and beta carotene in the secondary prevention of cardiovascular events in women; results from Women’s cardiovascular antioxidant study. *Arch. Intern Medicine*, 2007; 167(15): 1610-1618.
 29. Cook NC, Samman S; Flavonoids, Chemistry, Metabolism, Cardio-protective effects and Dietary sources. *Nutritional Biochemistry*, 1996; 7: 66-76.
 30. Miller AL; Antioxidant flavonoids, structure, function and usage. *Alternative Medicine Review*, 1996; 1(2): 103-111.
 31. Barakat MZ, Shelab SK, Darwish N, Zaheameyv EL; Determination of Ascorbic acid from plants. *Analytical Biochemistry*, 1973; 53: 225-245.
 32. Okwu DE; Phytochemicals and vitamins content of Indigenous spices of South-Eastern Nigeria. *Journal Sustainable Agric Environ.*, 2004; 6: 30-34.
 33. AOAC; Official Methods of Analysis. International 17th edition. Association of Official Analytical Chemists, Washington, DC, USA, 2000.
 34. Brand-Williams W, Cuvelier ME, Berset C; Use of free radical method to evaluate antioxidant Iron chelating activity screening, phenol and flavonoid contents of some

- medicinal plants from Iran. Afr Journal of Biotechnology, 2008a; 32: 43-49.
35. Benzie IEF, Strain JJ; The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power; the FRAP assay. Analytical Biochemistry, 1996, 239(1): 70-76.
 36. Nebavi SM, Ebrahimzadeh WA, Nebavi SF, Hanidinia A, Bekhrandnia AR; Determination of antioxidant activity, phenol and flavonoid contents of *Perrotia Persia*. Pharmacology online, 2008a; 2: 560-567.
 37. Nebavi SM, Ebrahimzadeh MA, Nebavi SF, Bahramcan F; *In vitro* antioxidant activity of *Phytolacca americana* berries. Pharmacologyonline, 2009b; 1:81-88.
 38. Yen GC, Chen HY; Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal Agricultural Food Chemistry, 1995; 43 (1): 27-32.
 39. Nebavi SM, Ebrahimzadeh MA, Nebavi SF, Jafari M; Free radical scavenging activity and antioxidant capacity of *Eryngium caucasicum* Trautz and *Froripia subpinata*. Pharmacologyonline, 2008b; 3:19-25.
 40. Shahidi F, Waasundara PK; Phenolic antioxidants. Critical Reviews in Food Science and Nutrition, 1992, 32: 67-103.
 41. Okwu DE, Okwu ME; Chemical composition of *Spondias mombia* plant parts. Journal of Sustainable Agric Environ., 2004; 6:140-147.
 42. Warner D, Sheng H, Batinin-Haberte I; Oxidants, Antioxidants and the Ischemic brain. Journal Experimental Biology, 2004; 207: 3221-3231.
 43. Wilson J, Gelb A; Free radicals, antioxidants and neurologic injury; possible relationship to cerebral protection by anaesthetics. Journal Neurosurgery Anaesthetics, 2002; 14(1): 66-79.
 44. Lees K, Davalos A, Davis S, Diener H, Grotta J, Lyden P *et al.*; Additional outcomes and subgroup analyses of NXY-059 for acute ischaemic stroke in the SAINT 1 trial. Stroke, 2006; 37(12): 2970-2978.
 45. Yamaguchi T, Aok K, Takakura K, Saito L, Shinohara Y, Asano T *et al.*; Ebselen in acute ischaemic stroke, a placebo controlled-double blind clinical trial. Ebselen study Group, Stroke, 1998; 29(1):12-70.
 46. Lees K, Zivin J, Ashwood T, Davalos A, Davis S, Diener H *et al.*; NXY-059 for acute ischemic stroke. New England Journal of Medicine, 2006b; 354(6):588-600.
 47. Di Matteo V, Esposito E; Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinsonian disease, amyotrophic lateral sclerosis. Current Drug Targets CNS Neurological Disorders, 2003; 2(2): 95-102.
 48. Rao A, Balachandran H; Role of oxidative stress and antioxidants in neurodegenerative diseases. Nutritional Neurosciences, 2002, 5(8): 291-309.
 49. Antioxidants may prevent alcohol-induced liver disease. e -Science News, 2 May, 2011.
 50. Litito SB, Frei B; Consumption of flavonoid rich foods and increased plasma antioxidant capacity in humans. Free Radical Biol Medi., 2006; 41(12): 1727-1746.
 51. Nebavi SM, Ebrahimzadeh MA, Nebavi SF, Fazelian M, Eslami B; *In vitro* antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus brossieriana* growing in Iran. Pharmacology Magazine, 2009a; 4(18):123-127.
 52. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willet WC; Vitamin E consumption and the risk of coronary heart disease in men. New Engl Journal Med., 1993; 328(20):1450-1456.
 53. Sesso HD, Buring JE, Christen WG; Vitamins E and C in the prevention of cardiovascular disease in men. The physician's Health Study II randomized controlled trial. JAMA, 2008; 300(18): 2123-2138.
 54. Burkill HN; The useful plants of West Tropical Africa, 2nd edition, volume 3, Families J-L, Royal Botanical Garden, Kew-Richmond, United Kingdom, 1995:857.
 55. Oboh G; Antioxidant properties of some commonly consumed and underutilized tropical legumes. European Food Research Technology, 2006; 224: 61-65.
 56. Wang X, Quin PJ; Vitamin E and its functions in Membranes. Progress in Lipid Research, 1999; 38 (4): 309-336.