EJEAFChe
Electronic Journal of Environmental, Agricultural and Food Chemistry

ISSN: 1579-4377

ANTIOXIDANT ACTIVITY OF NIGERIAN DIETARY SPICES

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KEYWORDS

Antioxidant activity, Nigerian spices, lipid peroxidation, reducing power, total phenolics.

ABSTRACT

Antioxidants are compounds that help to inhibit the many oxidation reactions caused by free radicals. The anti-oxidant activity of 20 extracts from 12 Nigerian spices Aframomum danielli K. Schum (Zingiberaceae), Allium cepa L. (Amarylliadaceae), Allium sativa L. (Amaryllidaceae), Capsicum frutescens L. (Solanaceae), Citrus sinensis (L.) Osbeck (Rutaceae), Curcuma longa L. (Zingiberaceae), Justicia flava (Forssk) Vahl. (Acanthaceae), Ocimum gratissimum L. (Lamiaceae), Piper guineense Schum. et Thonn.(Piperceae), Syzygium aromaticum (L.) Merr. et Perry (Myrtaceae), Xylopia aethiopica (Dun.) A. Rich. (Annonaceae) and Zingiber officinale Rosc. (Zingiberaceae) was evaluated by using the ferric thiocyanate method and reducing power. The total phenolics of the extracts was determined spectrophotometrically as Tannic Acid Equivalent (TAE) method of relative astringency of the plant extracts as a direct measurement of total soluble tannin. The anti-oxidant activity (expressed as per percent inhibition of oxidation) ranged from as high as 82.5% in turmeric extracts to as low as 8.6% in sweet orange peel. Anti-oxidant activity correlated significantly and positively with total phenolics ((R2 = 0.83), $P < 0.05$) while there was no linear correlation between total antioxidant activity and reducing power (R2 = -0.53) neither between reducing power and total phenolic content (R2 = -0.20). The results indicate that reducing power does not fully characterize the antioxidant activity, spices containing high phenolics provide a source of dietary anti-oxidants in addition to imparting flavor to the food. They possess potential health benefits by inhibiting lipid peroxidation, justifies their traditional use in pepper soup as a cure all medicine for the sick and potential use as a value-added ingredient for stabilizing food matrixes against lipid peroxidation reactions

INTRODUCTION

Free radicals are unstable molecules that include the hydrogen atom, nitric oxide (NO) and molecular oxygen (O_2). These naturally occur in the body as a result of chemical reactions during normal cellular processes. Reactive oxygen species (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions (O2-) and hydroxyl radicals (OH), as well as non free-radical species such as hydrogen peroxide (H2O2) [1,2]. In living organisms various ROSs can form in different ways, including normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes. These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals include tobacco smoke, ionising

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radiation, certain pollutants, organic solvents, and pesticides [3-5]. In an attempt for free radicals to stabilise, they attack other molecules in the body potentially leading to cell damage and triggering the formation of another free radical resulting in a chain reaction. These reactive oxygen species have been implicated in certain chronic and ageing diseases, including malaria, rheumatoid arthritis, cataracts acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, cancer and neurodegenerative diseases (Parkinson's and Alzheimer's diseases) [6-11]. Free radicals can also cause lipid peroxidation in foods, which leads to their deterioration. Oxidized polyunsaturated fatty acids may induce aging and carcinogenesis [12-14].

When produced in excess, ROSs can cause tissue injury. However, tissue injury can itself cause ROS generation [15]. Nevertheless, all aerobic organisms, including human beings, have antioxidant defences that protect against oxidative damages and repair enzymes to remove or repair damaged molecules [16, 17]. However, this natural antioxidant mechanism can be inefficient, and hence dietary intake of antioxidant compounds is important. Recent reports indicated that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human diseases [18].

Antioxidants are compounds that help to inhibit the many oxidation reactions caused by free radicals such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite thereby preventing or delaying damage to the cells and tissues. Their mechanisms of action include scavenging reactive oxygen and nitrogen free radical species, decreasing the localised oxygen concentration thereby reducing molecular oxygen's oxidation potential, metabolising lipid peroxides to non-radical products and chelating metal ions to prevent the generation of free radicals. In this way antioxidants limit the free radical damage from Oxidizing Low Density Lipoprotein (LDL) cholesterol, which may increase the risk of atherosclerosis, promoting platelet adhesion, which can lead to thrombosis thereby increasing the risk of heart disease or stroke, damaging the cell's DNA, which may lead to cancer, blocking the normal endothelial cell function and vasodilatation in response to nitric oxide, a potential mechanism for heart disease and cancer, triggering inflammation and Impairing immune function[19].

Lipid peroxidation is a major cause of food deterioration, leading to a loss of functional properties and nutritional value [20]. Oxidized polyunsaturated fatty acids may induce aging and carcinogenesis. The major pathway of lipid peroxidation contains a self-catalytic free radical chain reaction. However, lipid peroxidation can be catalyzed by environmental factors, such as light, oxygen, free radicals and metal ions [21]. The discovery of the inhibition of lipid peroxidation by some phenolic compounds during the late 1940s, contributed to the application of synthetic antioxidants in the food industry [22]. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertbutylhydroquinone(TBHQ) have been dominant since their introduction. They have been used as an antioxidant in foods for years. However, some physical properties of BHT and BHA such as their high volatility and instability at elevated temperatures, side effects and strict legislation (even recently by the country's food and drug regulating body NAFDAC) on the use of synthetic food additives. Consumers are increasingly avoiding foods prepared with preservatives of chemical origin, and natural alternatives are therefore needed to achieve sufficiently a long shelf life of foods and a high degree of safety. Therefore, the commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest. Many spices possess potent antioxidant activity and examples of them are rosemary and sage. Herbs are also found to be potent sources of natural antioxidants as well as retard lipid oxidative rancidity in foods.

Medicinal plants possessing natural antioxidants polyphenolics such as anthraquinones, flavonoids, aromatic acids, and tannins have been shown to have ROS scavenging and lipid peroxidation prevention effects [23, 24]. The commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest [25]. Epidemiological studies have suggested positive associations between the consumption of phenolic-rich foods or beverages and the prevention of diseases [26]. These effects have been attributed to antioxidant components such as plant phenolics, including flavonoids and phenylpropanoids among others [27].

Evidence is mounting for the role of these dietary phytochemicals, including flavonoids, ascorbic acid, ∞-tocopherol, and carotenoids, in the maintenance of health and protection from disease [28, 29]. As plants produce antioxidants to control the oxidative stress caused by sunlight and oxygen, they became a source of useful new compounds with antioxidant activity.

The aim of the present study was to evaluate the in vitro antioxidant activity of aqueous extracts of popular plants spices widely used in Nigeria, using lipid peroxidation as standard classical assay.

MATERIALS AND METHODS

All the plant materials used (both fresh and dry as appropriate) were purchased on separate occasions from different open markets in Lagos Nigeria and authenticated by Mr. Gabriel at the Herbarium of the Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria by comparing with herbarium specimens. Odukoya keeps museum samples of materials.

Preparation of Extracts

The fruits were picked to remove debris, while Onions, ginger, turmeric and garlic having dead and dry skins had their skins removed, washed to show only edible portions and cut into small pieces. The materials were weighed (20g) and homogenized with (100mL) of distilled water using a Moulinex blender. The homogenate was boiled for 3min and allowed to stand at room temperature for for 24h before filteration. The filterates were diluted to produce a 200mg/L of extract needed for the antioxidant/reduction assays.

Antioxidant activity

The antioxidant activity was determined using the ferric thiocyanate method as described by [30]. Two milliliters of 200mg/L extract, 2mL of 2.5%(w/v) linolenic acid in ethanol 95%(v/v) 4mL of 0.05M of phosphate buffer(Ph7.0) and 2mL of distilled water were mixed in a 10mL test tubes covered with aluminum foil and fastened with rubber band. A blank sample was prepared using 4mL of distilled water, 2mL of 2.5 %(w/v) linolenic acid in ethanol (95%) 4mL of 0.05M of phosphate buffer (Ph7.0).

The test tubes were placed in a water bath at 37°C and kept in the dark cupboard to accelerate oxidation.

0.1mL of mixture above was added to 9.7mL of 75% ethanol and 0.1mL of 30 %(w/v) ammonium thiocyanate. After 5minutes, 0.1mL of 0.02M ferrous chloride solution in 3.5 %(v/v) HCl was added to the mixture and stirred. The amount of peroxide formed was determined by reading absorbance at 500 nm at intervals for 24h during incubation. ∞ -Tocopherol (Sigma) was used as standard antioxidant while a blank of distilled water was ran with each assay. All determinations were carried out in triplicate. The inhibition of lipid peroxidation as a percentage was calculated by following equation:

$$\%\,Inhibition = \frac{(A1 - A2)}{A1} \times 100\%$$

where A1 was the absorbance of the control reaction and A2 was the absorbance in the presence of the extract sample.

Reducing Power

The reducing power of extracts was determined using a modified method of [18, 31].

Two mL of each sample was added to 2 mL of 0.2 M phosphate buffer (pH 6.6) and 2 mL of 1% potassium ferricyanide. The mixtures were incubated at 50°C for 25 minutes, 2mL of 10% tricholoroacetic acid was added to each reaction mixture and centrifuged for 10 min. 2 mL of upper layer solution was mixed with 2 mL distilled water and 0.5mL FeCl3 (0.1%), in the tubes. After 10 minutes the solutions were measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Distill water, was used as the blank sample.

Determination of total phenolic content

Extracts were screened for presence of phenolics with ferric chloride solution before commencement of assay. Total phenolics were determined using the Tannic Acid Equivalent (TAE) method of relative astringency of the plant extracts as a direct measurement of total soluble tannin as earlier described by [32].

STATISTICS ANALYSIS

The data were presented as mean ± standard deviation of 3 determinations. Statistical analysis was performed electronically using Java script E – Labs learning probability and statistics resources to determine correlation coefficient at 95% confidence level [33].

RESULTS AND DISCUSSIONS

Antioxidant activity was determined by the thiocyanate method by determining amount of peroxides formed in emulsion during incubation spectrophotometrically by measuring absorbance at 500 nm. High absorbance is an indication of high concentrations of formed peroxides. Description of spices used is shown in Table 1.

The relative antioxidant activity of fresh plant extracts was higher than dry ones (Table 2). Fresh ones decreased in the order of tumeric, ginger, garlic, basil, red bird's pepper, green bird's pepper, bastered melegueta pepper, onions and sweet orange peel. While dry ones decreased in the order of tumeric, Ashanti pepper, Ethiopian pepper, cloves, ginger, bastered melegueta pepper, basil, red bird's pepper, yellow justicia and sweet orange peel but with inhibition values lower than the fresh ones (Table 2).

Different anti-oxidant substances occur in plant tissues especially fruits and vegetables. This makes it relatively difficult to measure each anti-oxidant component separately. Therefore several methods including oxygen radical absorption capacity method, ferric reducing antioxidant capacity method, liposome assay, lipid peroxidation and total oxyradical scavenging capacity assay have been developed in recent years to calculate the total anti-oxidant activity of biological samples [34-43]. Antioxidation activity represents the capability of scavenging free radical and offering hydrogen atom. Higher antioxidation activity indicates stronger capability [44].

The vehicle used ensures maximum extraction of the available anti-oxidants from the sample. In the present study, water was used in extracting the anti-oxidants present in the spices because that is the way it is used traditionally as pepper soup in Nigeria as a combination of at least any of the five plant materials combined with fish or meat as an appetizer or as a cure for all medicine for the sick.

Dry spice mix namely turmeric, Ashanti pepper, Ethiopian pepper, cloves, ginger, bastered melegueta pepper, basil, red bird's pepper, yellow justicia and sweet orange peel showed significant cumulative inhibition of lipid peroxidation thus exhibiting their synergistic antioxidant activity Table 2. This highlighted the importance of exploring the changes in antioxidant activity during drying for optimizing processing technologies since the spices are used in the dried form traditionally. The statistical analysis showed a positive and strong linear relationship between the total phenolic content and the antioxidant

activity (R^2 = 0.83, P=0.000 for control and extracts) suggesting that the antioxidant activity in these spices is largely due to the presence of phenolic components. The same relationship was also observed between phenolics and antioxidant activity in rosehip extracts and some other vegetables [18, 36, 40, 45]. While there was no linear correlation between total antioxidant activity and reducing power (R^2 = -0.53) neither between reducing power and total phenolic content (R^2 = -0.20).

Table1. Description of spices used

S/N	COMMON NAME	BOTANICAL NAME	PART USED	FORM	CODE
1	Bastered Melegueta	Aframomum danielli K. Schum (Zingiberaceae)	Seed	Fresh	ADF
				Dry	ADD
2	Onions	Allium cepa L.(Amarylliadaceae)	Bulb	Fresh	ACF
3	Garlic	Allium sativa L.(Amaryllidaceae)	Bulb	Fresh	ASF
4	Bird's Pepper	Capsicum frutescens L. (Solanaceae)	Fruit	Fresh (Green)	CFG
				Fresh (Red)	CFF
				Dry (Red)	CFD
5	Sweet Orange	Citrus sinensis (L.) Osbeck (Rutaceae)	Peel	Fresh(Ripe)	CSF
				Dry(Ripe)	CSD
6	Tumeric	Curcuma longa L. (Zingiberaceae)	Rhizome	Fresh	CLF
				Dry	CLD
7	Yellow Justicia	Justicia flava(Forssk) Vahl.(Acanthaceae)	Seed	Dry	JFD
8	Basil	Ocimum gratissimum L. (Lamiaceae)	Leaf	Fresh	OGF
				Dry	OGD
9	Ashanti/Black Pepper	Piper guineense Schum. et Thonn. (Piperceae)	Seed	Dry	PGD
10	Cloves	Syzygium aromaticum(L.) Merr. et Perry (Myrtaceae)	Flower	Dry	SAD
11	African/Ethiopian Pepper	Xylopia aethiopica (Dun.) A. Rich.(Annonaceae)	Fruit	Dry	XAD
12	Ginger	Zingiber officinale Rosc. (Zingiberaceae)	Rhizome	Fresh	ZOF
				Dry	ZOD
13	Mixture	All plant materials above		Dry	MPM

Natural extracts with proven antioxidant activity usually contain compounds with phenolic moiety, for example coumarins, flavonoids, tocopherols and catechins. Organic acids, carotenoids, proteins, hydrolysates and tannins can also be present and act as antioxidants or have a synergistic effect with phenolic compounds. Recent studies have demonstrated that the antioxidant activity is correlated with the number of hydroxyl groups [46]. This study provided some essential further evidence on this point based on the reported data and mechanisms underlying the antioxidant functions as well as the anodic oxidation of phenolic antioxidants, indicating that further consideration and investigation should be made before reducing power are used as the absolute measure of antioxidant activity.

These results indicate that spices containing high phenolics provide a source of dietary anti-oxidants and in addition to imparting flavor to the food, they possess potential health benefits by inhibiting lipid peroxidation and justifies their traditional use in pepper soup as a cure for all medicine for the sick and potential use as a value-added ingredient for stabilizing food matrixes against lipid peroxidation reactions.

Table2. Antioxidant activity, reducing power and total phenolics of extracts

S/N	CODE	% ANTIOXIDANT ACTIVITY	% REDUCING POWER	TOTAL PHENOLICS (mg/100g)
1	ADF	46.20 ± 0.41	3.26 ± 0.72	93.78 ± 2.61
2	ADD	56.98 ± 1.82	8.84 ± 0.35	116.52 ± 1.32
3	ACF	44.23 ± 1.12	1.80 ± 0.51	58.30 ± 1.46
4	ASF	60.06 ± 2.59	1.35 ± 0.27	136.48 ± 4.72
5	CFG	47.19 ± 2.30	5.36 ± 0.56	63.29 ± 1.81
6	CFF	52.96 ± 3.32	10.99 ± 0.86	89.36 ± 1.01
7	CFD	49.65 ± 2.74	6.65 ± 0.47	72.85 ± 5.64
8	CSF	21.37 ± 0.21	51.34 ± 0.21	38.41 ± 4.21
9	CSD	12.94 ± 0.13	67.81 ± 0.16	30.03 ± 1.48
10	CLF	82.51 ± 5.13	19.65 ± 0.41	176.25 ± 8.13
11	CLD	78.29 ± 4.70	17.48 ± 1.26	146.54 ± 5.47
12	JFD	49.62 ± 1.72	9.77 ± 0.41	107.88 ± 3.40
13	OGF	56.41 ± 0.49	9.12 ± 0.28	138.21 ± 0.96
14	OGD	51.79 ± 1.05	7.56 ± 0.59	121.79 ± 1.47
15	PGD	76.94 ± 2.64	15.34 ± 0.38	169.52 ± 0.19
16	SAD	68.65 ± 3.07	11.37 ± 1.13	216.38 ± 7.94
17	XAD	72.47 ± 2.65	13.89 ± 0.20	152.24 ± 6.01
18	ZOF	64.33 ± 1.82	13.92 ± 0.49	229.83 ± 10.17
19	ZOD	58.74 ± 0.98	9.64 ± 0.07	182.69 ± 9.25
20	MPM	91.23 ± 7.01	23.97 ± 0.27	331.10 ± 14.62
21	TOCOPHEROL	65.21 ± 1.42		

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