Evaluation of Antioxidant and Chelating activities of Seeds extracts of Aframomum sceptrum

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Abstract

Aframomum sceptrum (zingiberaceae) is a native spice commonly used to enhance cooking flavour, aroma and palatability in Southern parts of Nigeria, particularly among the Urhobos,Itsekiris and Ijaws. The seeds of Aframomum sceptrum were studied with the aim of evaluating the antioxidant and chelating activities of the seeds extracts in three different solvent (n-Hexane, ethylacetate and ethanol). The antioxidant activities were evaluated using in vitro assay which utilized the DPPH scavenging method. Antioxidant activities of the extracts were obtained by their reducing power. The extracts produced concentration dependent increase in antioxidant activities in 2, 2-diphenylpicrylhydrazyl photometric assay when compared with the standard gallic acid. The chelating ability of the examined extracts was also assessed in vitro. The extracts showed a dose dependent increase in chelating ability. The values of chelating ability for graded dose (5-25mg/ml) were 77.60%, 81.56%, 86.60%, 86.22% and 94.67% in the n- Hexane extract which has the highest chelating ability. The chelating abilityof the extract is comparable with that of the standard curcumin. These results suggest that seeds extracts of Aframomum sceptrum may be a potential source of antioxidants protection against oxidative stress and iron overload damage.

Keywords Aframomum sceptrum, Oxidative stress, Antioxidant, Chelating, Iron overload

Introduction

Antioxidants act as a defense mechanism that protect against deleterious effects of oxidative reactors produced by reactive oxygen species (ROS) in a biological system [1], [2] reactive oxygen species not only are produced naturally in cell following stress or respiration, but have also been reported to be produced by radiation, bacterial and viral toxin, smoking, alcohol and psychological or emotional stress. Over production of ROS and inadequate antioxidants has been implicated in the pathogenesis and complications of some disease condition like diabetes, cancer, atherosclerosis, arthritis, neurodegenerative disease, and aging process[3], [4].

Antioxidants have been reported to prevent oxidative damage caused by ROS by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers [5], [6]. The antioxidants in biological system can be either enzymatic or nonenzymatic. The enzymatic antioxidants include catalase, superoxide dismutase and glutathione which catalyze neutralization of many types of free radicals [7], [8], while the nonenzymatic antioxidants include vitamin C, Selenium, vitamins E, Carotenoids, and Polyphenols. There is growing evidence that antioxidants play a pivotal role in the prevention of heart disease, cancer, DNA degeneration, pulmonary disease, and neurological disorder [9]. Numerous studies have shown the antioxidant potentials of aromatic, spicy and medicinal plants [4], [7]. The use of these plant materials as natural antioxidants for food, cosmetics and other application becomes necessary because of food safety issues. Natural antioxidants as food additives for inactivation of free radical receive a lot of attention nowadays, not only for their scavenging properties, but also because they are natural, non-synthetic products and are more readily acceptable to the consumers [10, 11]. Different degrees of antioxidant activities have been reported from extracts of spices and herbs [12, 5].

Chelation of metal ions and quenching of singlet oxygen are the major characteristics of antioxidant activity [13]. Studies have shown that specialized phenol storing cells occur in several plant species [6]. A major disorder associated with iron overload is thalassemia. Thalassemia

usually results in under production of normal globin proteins, often through mutations in regulatory genes [14], [15]. *Aframomum sceptrum* is a species in the ginger family, Zingiberaceae, is a native spice commonly use to enhance cooking flavor, aroma and palatability especially in the South- eastern part of Nigeria, in particular the Ijaws, Urohobos and Itsekiri's of Delta State [16]. The antioxidant activity of *Aframomum sceptrum* has been reported [15]. However, studies on the chelating and antioxidant activities of *Aframomum sceptrum* seed extracted using different solvents based on solvent polarities has not been extensively study. This dearth of information prompted this study in order to provide useful data, either for practical use or for basic research needs.

Materials and Methods

Preparation of plant extract: *Aframomum sceptrum* seeds were purchased at Bode market in Ibadan, Oyo State, Nigeria. The plant had earlier been authenticated in the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Samples were dried in shade for extractions. Extraction of the plant seed was performed by maceration and decoction methods using three solvents viz n-Hexane, ethylacetate and ethanol. From each extract different concentrations 5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and 25mg/ml were prepared

Preparation of DPPH solution (stock): 0.3mM of DPPH was prepared by disolving 0.03g of DPPH in 250ml of each of the solvent used.

Preparation of gallic acid solution (stock): 0.2g of garlic acid was dissolved in 20ml of deionized water. The solution was incubated in water bath at 38_0C for 5mins because gallic was not completely soluble in water. Different concentration (grade doses) (500-300Ug/ml) of garlic were prepared from the stock gallic acid (10mg/ml).

Preparation of curcumin solution stock: 0.2g of curcumin was disolved in 20 ml of deionized water. The solution was incubated in water bath at 38^0C for 5 min because curcumin was not completely soluble in water. Different concentrations (grade doses) (500 - 300 ug/ml) of curcumin were prepared from stock curcumin (10 mg/ml).

Biochemical Assays: Antioxidant activity determination: The antioxidant activities of n-hexane, ethylacetate and ethanolic extracts were determined according to the method of Blois [17]. 1ml of 0.3min of DPPH solution was added to 1ml each of the test solution and this was allowed to react at room temperature in the dark for 30min. the absorbance of the solution was read at 517nm in a UV/visible spectrophotometer against blank (distilled water). Gallic acid was used as standard antioxidant. The antioxidant activity of was extract expressed as percent of inhibition as follows:

$$\% \text{ inhibition} = \left[\left(A_O - A1\right)/ A_O\right] x100$$

Ao was the absorbance of control and Ai was the absorbance of the extracts [18].

Metal chelating activity: The iron chelating activity of the spice extracts was determined by method of Minotti [19] with slight modification by Puntel [13]. To 0.2ml of each extracts was added 0.5ml of freshly prepared 500uM $FeSO_4$. 0.3ml of 0.1M Tris –HC l (Ph 7.4) and 0.4ml of saline were then added. The mixture was incubated for 5 min, followed by the addition of 3 drops of 0.25% 1, 10-Phenanthroline (w/v). The absorbance was measured at 510nm with UV/visible spectrophotometer. The iron chelating ability was subsequently calculated with respect to the control by using the formula:

Chelating activity (%) = $A_{control}$ - A_{sample} /$A_{control}$ * 100

Results

Table 1: shows the dose dependency of DPPH radical scavenging activity of the n-Hexane, ethylacetate and ethanolic extracts of *A. sceptrum* with standard antioxidant -

gallic acid. The n-hexane extract had the highest activity than the ethylacetate extract while ethanolic extract had the least activity.

At the highest concentration of 25mg/ml, the scavenging activity of n-hexane extract reached 66.99%, while at this concentration it was 63.10% and 28.20% respectively for ethylacetate and ethanolic extracts. Only at 20mg/ml that ethylacetate exhibited (45.6%) activity higher than the standard gallic acid.

From Table 2, the chelating activity showed a dose dependent relationship in all the tested extracts with n-hexane having the highest at 94.6% with ethyl acetate and ethanol extracts having 19.0% and 8.6% respectively. This result shows that the n- hexane extract has a higher metal chelating activity than gallic acid used as the control.

Table 1: Antioxidant activities of *Aframomum sceptrum* seed extract

Concentration mg/ml	n-Hexane extracts (%)	Ethylacetate extract (%)	Ethanol extract (%)	Gallic acid
5	2.9+2.0.4	13.6+3.11	6.79+2.14	19.54+4.26
10	8.74+2.23	16.5+2.91	9.70+8.74	48.54+4.98
15	31.1+1.55	28.2+2.72	10.6+2.33	49.78+4.94
20	31.1+2.91	45.6+2.72	14.60+1.65	39.56+3.21
25	66.99+1.65	63.1+2.91	28.20+8.74	79.94+1.12

Table 2: Chelating ability of *Aframomum sceptrum* seed extract

Concentration (mg/ml)	n-Hexaneextracts (%)	Ethylacetate extract (%)	Ethanol extract (%)	Gallic acid
5	77.60+7.89	10.00+9.89	0.62+4.99	24.97+4.88
10	81.56+8.00	11.56+1.25	3.07+2.50	29.72+4.92
15	86.06+9.52	11.91+6.36	8.04+5.37	62.178+5.74
20	86.22+8.11	16.89+1.35	8.40+7.60	77.84+5.68

25	94.67+1.80	19.07+2.60	8.62+4.67	83.26+4.88

Discussion

This study reports antioxidant activities of *Aframomum sceptrum*. Spices have been acknowledged not only to have properties that make food more pleasant but also important preservative and antioxidant properties [20].The relationship between total phenol contents and antioxidant activity has been widely studied in different food stuffs [20] . Antioxidant activity of food stuff significantly increases with the presence of high concentration of total phenol and flavonoid contents.

The observed scavenging ability of the extracts of *A. sceptrum* againt stable DPPH followed a dose-dependent pattern, with the highest activity observed at the highest concentration. The n- hexane extract has the highest antioxidant and chelating activities of the three extracts examined. This suggests that the extract possessed high content of polyphenol and flavonoid. This is an indication that *A.septrum* is a potential source of dietary antioxidant that can be used in the prevention and management of various ROS-related ailments such as Parkinson's and Alzheimer's diseases.

The maximum in vitro chelating ability of the n-hexane extract is 94.67% at 25% concentration, while, minimum in vitro chelating ability is 77.6% at 5% concentration, this shows that the extract having more chelating activity than gallic acid. Chelation property may afford protection against oxidative damage and iron - overload [13]. Chelating ability of the extract provide a strategy to avoid free - radical generation and iron - overload by chelation of the metal ion [19]. *A. sceptrum* extracts was a good chelator for iron removal at *in vitro* condition.

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