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Original Article

Assessment of the *in vitro* antilipid peroxidative activity of *Costus afer* stem extract

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ABSTRACT

Objective: *Costus afer* is a perennial monocot consumed as a remedy for various ailments. The present study investigates the effect of *C. afer* stem extract on ferrous/ascorbate-induced lipid peroxidation in rat brain, liver, and kidney homogenates. The inherent antioxidant and free radical scavenging potentials of the extract were also assessed using various *in vitro* models. **Methods:** Homogenates of isolated tissues were prepared, and lipid peroxidation was induced using the ferrous/ascorbate system and subsequently incubated with various concentrations of the extract. The ability of the methanolic extract to scavenge hydroxyl radicals, hydrogen peroxide and its ability to reduce ferric ions were investigated. The total antioxidant potential through the phosphomolybdate method was also measured. **Results:** The results revealed that *C. afer* stem extract reduced tissue lipid peroxidation induced by ferrous/ascorbate. The extract also possessed significant antioxidant/free radical scavenging activity through the other *in vitro* models. In all cases, the effects were concentration dependent. **Conclusion:** The methanolic extract of *C. afer* stem possessed significant antioxidant potential ascribable to important phytochemicals and could protect cells/tissues from reactive oxygen species-induced damage. Thus, the plant has high pharmacological and ethnobotanical prospects.

KEY WORDS: *Costus afer*, hydroxyl radical, lipid peroxidation, phytochemicals

INTRODUCTION

Nature has endowed humankind with a floral diversity which can be exploited in various ways most importantly to the benefit of man. Preceding the advent of conventional medicine, human has relied on this natural endowment for the prevention and cure of various ailments. Up till now, many regions of the world still rely heavily on botanicals as the first line of treatment against many diseases. In addition to being relatively cheap and available, plants/plant-derived products seem to be better because of the reported less frequent side effects following their use when compared to chemotherapy.

Costus afer Ker Gawl (*Costaceae*) is a perennial rhizomatous monocot distributed in the moist and shady forest belts of Africa [1,2]. Among various ethnic groups in Nigeria, the plant is used as a remedy for measles, malaria, venereal diseases, and hypertension [3]. The plant has also been used to stop bleeding from wounds, toothaches, diabetes, epileptic attacks, and the management of tuberculosis [1,4,5]. Phytochemical screening of the plant has revealed the presence of alkaloids, tannins, saponins, flavonoids, phenols, cardiac glycosides, and terpenoids [2,5,6] and is thought that the beneficial role of the plant in agriculture and folk medicine is based on the presence of these phytochemicals [1].

This current work is aimed at assessing the effect of *C. afer* extract on lipid peroxidation on liver, brain, and kidney homogenates. Biological membranes are highly susceptible to oxidative attack due to the preponderance of polyunsaturated fatty acids. These attacks result in the production of lipid peroxides which can be quantified. The potential antioxidant and free radical scavenging potential of the plant were also assessed using various *in vitro* models.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents were of the highest grade and commercially available. All solutions and buffers were prepared in distilled water from a Milli-Q source.

Plant Material

The stems of *C. afer* were harvested from the bush in Amassoma, Bayelsa state. The plant was identified by Dr. I. Ogidi of the Department of Crop Production, Niger Delta University. A voucher specimen (16/280/Costus) of the plant is deposited at the herbarium of the Department of Pharmaceutical Chemistry, Niger Delta University. The stems were chopped into smaller bits and sun dried. They were ground to fine powder and soaked in absolute methanol for 3 days at room temperature and filtered. The extract was concentrated using a rotary evaporator at 40°C. Various concentrations of the extract (1, 5, and 10 mg/ml) were prepared in distilled water.

Preparation of Tissue Homogenates

Male albino rats were subjected to light anesthesia in urethane and sacrificed. Brain, liver, and kidney were excised, blotted, and homogenized in 0.02 M phosphate-buffered saline (pH 7.4) to give a 10% tissue homogenate. Each homogenate was centrifuged at 750 g for 10 min at 4°C. The supernatant (otherwise referred to as the homogenate in later sections) was used for analysis.

Antilipid Peroxidation Assay

The ability of *C. afer* to inhibit tissue lipid peroxidation was performed according to the method of Kimuya *et al.* [7] with some modifications. Initial assay mixture was made up of 0.2 ml of 0.02 M phosphate buffer (0.02 M, pH 7.4), 0.04 M ferrous chloride (0.1 ml), 0.1 ml of ascorbic acid (0.1 mM), 0.5 ml of homogenate, and 0.5 ml of either extract or control (quercetin monohydrate). This was incubated for 30 min in a boiling water bath after adding 1.8 ml of distilled water and 2 ml of 2% thiobarbituric acid. After cooling, n-butanol (5 ml) was added and the mixture shaking vigorously. The n-butanol layer was collected through centrifugation and the amount of thiobarbituric acid reactive substances assessed by measuring absorbance at 532 nm.

Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity was assessed based on the Fenton reaction according to the method of Yu *et al.* [8]. Briefly, 1 ml of either extract or control was incubated with 20 μl of 0.02 M ferrous chloride, 0.5 ml of 40 mM 1, 10 phenanthroline and 1 ml of phosphate buffer (0.02 M, pH 7.4). Hydrogen peroxide (7 mM, 0.05 ml) was added to initiate the reaction and the absorbance was measured at 560 nm after incubation at room temperature for 5 min. Scavenging activity was expressed as relative activity and calculated as follows:

$$\text{Scavenging activity (\%)} = (A_{blank} - A_{sample})/A_{blank} \times 100$$

Total Antioxidant Activity

Determination of the antioxidant activity through the phosphomolybdate method was done according to Jayaprakasha *et al.* [9] as modified by Okoko and Ere [10]. Briefly, 0.2 ml of extract or control was mixed with 1 ml of a reagent stock made up of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixture was incubated in a boiling water bath for 90 min. The contents were cooled and the absorbance was measured at 695 nm. Final reading was expressed as relative antioxidant activity.

Hydrogen peroxide Scavenging Activity

The Hydrogen peroxide scavenging activity was determined according to the method of Ruch *et al.* [11] with some modifications. Either extract or control (1 ml) was incubated with 0.6 ml of 40 mM hydrogen peroxide (prepared in phosphate buffer, 0.02 M, pH 7.4) for 10 min at room temperature. The absorbance was measured at 230 nm against a reagent blank. The hydrogen peroxide scavenging activity was also expressed as relative activity.

Reducing Ability

The ability of the extract to reduce ferric ions was determined according to the method of Oyaizu [12] with some modifications. Either the extract or control (5 ml) was mixed with 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 5 ml of 1% potassium ferricyanide. After incubation for 20 min at 50°C, 0.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer (0.5 ml) was mixed with distilled water (0.5 ml) and 0.1% ferric chloride. The absorbance was measured at 700 nm after incubation at room temperature for 10 min. Increasing absorbance indicates increasing reducing ability.

Statistical Analysis

Results were expressed as mean ± standard error mean from five replicate experiments. Quercetin monohydrate (10 μg/ml) was used as the control for the antilipid peroxidation assays while vitamin C (5 mg/ml) was used for the other assays. Data were analyzed using two-tailed Students *t*-test or analysis of variance. Confidence was set at $P < 0.05$. Where appropriate, the concentration of the extract to reduce lipid peroxidation or to scavenge reactive oxygen species (ROS) (via the *in vitro*) by 50% was estimated graphically using a linear regression model.

RESULTS

The effect of *C. afer* extract on ferrous/ascorbate-induced lipid peroxidation is shown in Figures 1-3. The results revealed that the extract reduced chemically induced lipid peroxidation in all the tissue homogenates in a concentration-dependent manner and the variations were significant ($P < 0.05$). The extract also exhibited significant ability to scavenge hydroxyl radicals [Figure 4], hydrogen peroxide [Figure 5] and also possess significant ability to reduce ferric ions [Figure 6]. *C. afer* stem extract also possess significant antioxidant activity assessed through the phosphomolybdate

method [Figure 7]. In all cases, the observed potentials were concentration dependent and the differences were statistically significant ($P < 0.05$). The inhibitory concentration 50 values of the extract at inhibiting lipid peroxidation and other antioxidant effects are shown in Table 1. It revealed that the values were moderate when compared to the reference antioxidants.

DISCUSSION

In healthy individuals, there is always a balance between the production of ROS and the innate antioxidant defenses which could be enzymatic or non-enzymatic. Any aberration to that imbalance causes oxidative stress which may be implicated in many disorders. Among the important ROS is hydrogen peroxide which is kept in check by the activities of catalase. Overproduction of hydrogen peroxide or probably if catalase activity is impaired, lesions like hyperpolarization of cell membranes [13], cell damage could occur which are implicated in a number of diseases such as diabetes, neurodegenerative, cardiovascular disorders, cancer, and aging [14]. Hydrogen peroxide reacts with partially reduced transition metal to generate the hydroxyl radical which is considered to be the most potent among the biologically active ROS [15,16]. Production of hydroxyl radicals is also induced by the Fenton reaction through the reduction of ferric ion, tricarboxylic acid cycle, and the electron transport chain [17].

ROS react with membrane polyunsaturated fatty acids to cause lipid peroxidation and many diseases have been attributed to this. For example, in the erythrocytes, ROS cause functional impairment due to the disruption of cellular components [18]. In the present experiment, lipid peroxidation in brain, liver, and kidney tissue was induced by the ferrous/ascorbate system. This system has been shown to induce lipid peroxidation in rat liver microsomes and mitochondria [19]. The results revealed that the extract reduced *in vitro* lipid peroxidation in all tissues and was concentration dependent ($P < 0.05$); thus, *C. afer*

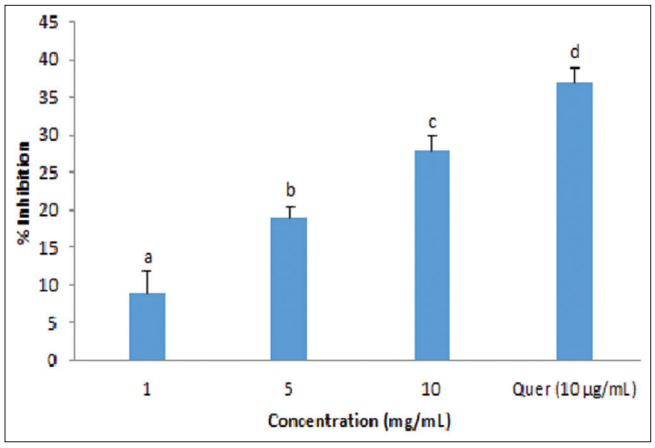

Figure 1: Inhibitory effect of *Costus afer* extract on $FeCl_2$-ascorbic acid-induced lipid peroxidation in rat brain homogenate. Each bar represents mean ± standard error mean from five replicate experiments. Values having different superscript letters differ significantly ($P < 0.05$)

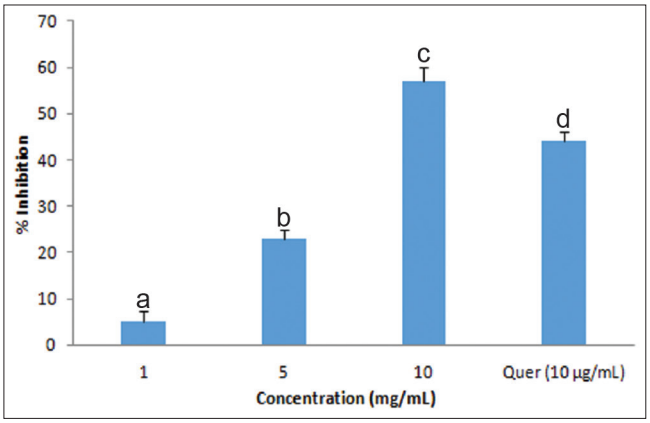

Figure 2: Inhibitory effect of *Costus afer* extract on $FeCl_2$-ascorbic acid-induced lipid peroxidation in rat liver homogenate. Each bar represents mean ± standard error mean from five replicate experiments. Values having different superscript letters differ significantly ($P < 0.05$)

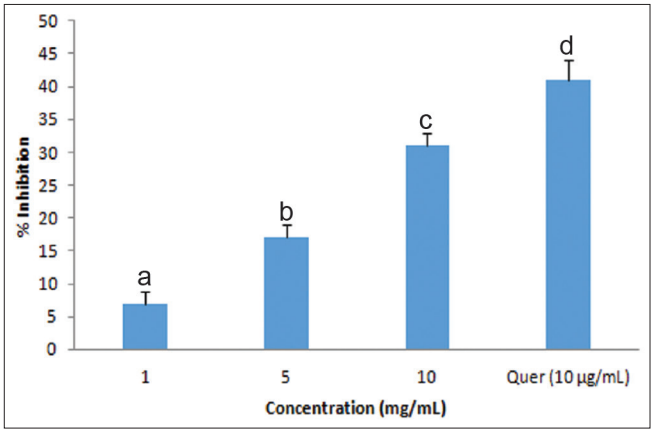

Figure 3: Inhibitory effect of *Costus afer* extract on $FeCl_2$-ascorbic acid-induced lipid peroxidation in rat kidney homogenate. Each bar represents mean ± standard error mean from five replicate experiments. Values having different superscript letters differ significantly ($P < 0.05$)

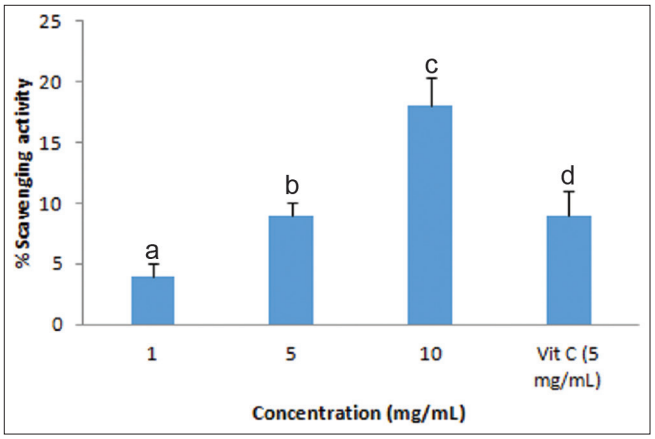

Figure 4: Hydroxyl radical scavenging activity of *Costus afer*. Each bar represents mean ± standard error mean from five replicate experiments. Values having different superscript letters differ significantly ($P < 0.05$)

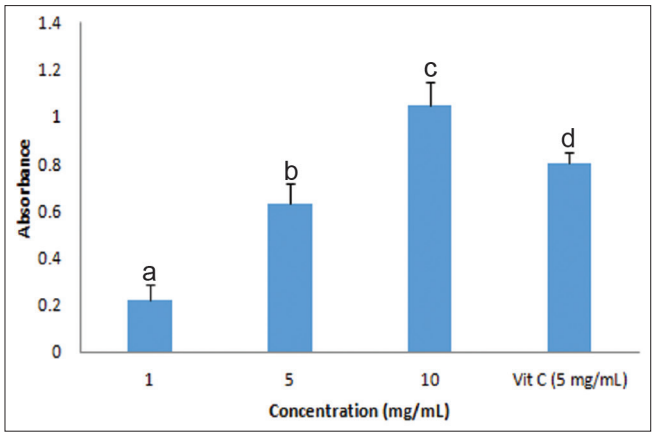

Figure 5: Hydrogen peroxide scavenging activity of *Costus afer*. Each bar represents mean ± standard error mean from five replicate experiments. Values having different superscript letters differ significantly ($P < 0.05$)

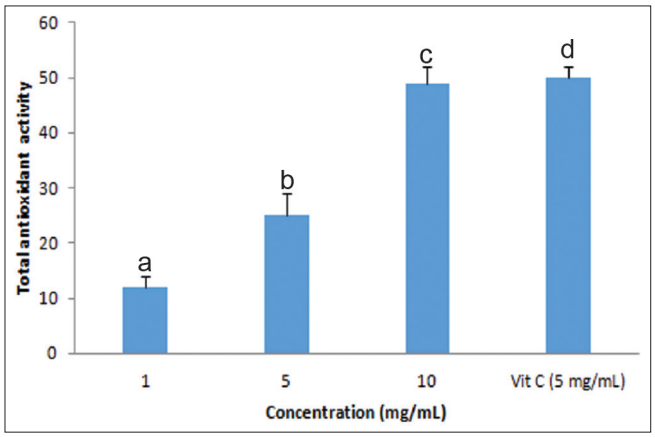

Figure 6: Ferrous ion reducing ability of *Costus afer* stem extract. Each bar represents mean ± standard error mean from five replicate experiments. Values having different superscript letters differ significantly ($P < 0.05$)

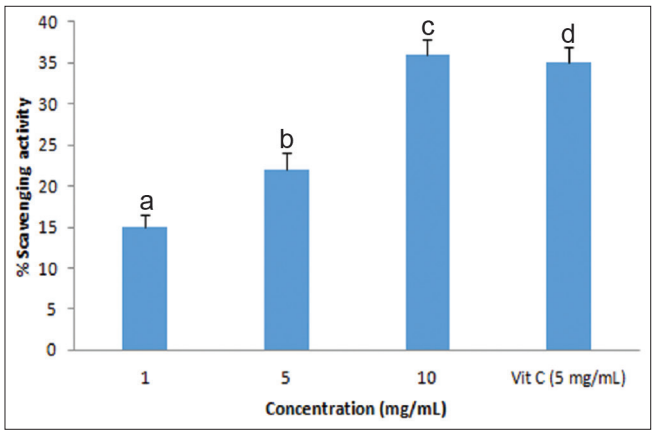

Figure 7: Total antioxidant activity of *Costus afer* extract via the phosphomolybdate method. Each bar represents mean ± standard error mean from five replicate experiments. Values having different superscript letters differ significantly ($P < 0.05$)

Table 1. IC_{50} values for antioxidant activities of *Costus afer* extract

Entity	Antilipid peroxidation			Hydroxyl	H_2O_2
	Brain	Liver	Kidney		
Extract (mg/ml)	20.27	9.06	17.18	30.67	16.24
Vitamin C (mg/ml)	NA	NA	NA	28.86	12.53
Quercetin monohydrate (μg/ml)	14.43	12.27	15.55	NA	NA

NA: Not applicable, IC_{50}: Inhibitory concentration 50

could reduce the incidences of diseases associated with lipid peroxidation. Malondialdehyde (product of lipid peroxidation) is very reactive and damages various cellular targets; thus, the reduction of its production could enhance health in no small measure.

To further assess the bioactivity of C. *afer*, various *in vitro* models were used to investigate the potential antioxidant ad free radical scavenging activity. The results revealed that the extract possessed significant hydroxyl radical scavenging ability, hydrogen peroxide scavenging ability, ferric ion reducing ability, and significant ability to reduce molybdate species. In all cases, the variations were concentration dependent. Hydrogen peroxide affects the blood–brain barrier by attacking the endothelial cells and neuron, thereby causing cell death and perhaps hemorrhages thus could contribute significantly to the progression of neurodegenerative disorders [20,21]. The findings imply that C. *afer* could contribute to the health of the nervous system.

Redox active metals such as iron and magnesium (especially in their high oxidation states) initiate radical-mediated oxidative chain reactions in biological systems [22]; thus, any condition that either shields or reduces these metals could contribute to health. The aforementioned biological effect of C. *afer* could be ascribed to the antioxidation of various inherent phytochemicals.

Studies have shown that phytochemicals especially polyphenols donate hydrogen to scavenge the so-called free radicals, but it has been observed that their cellular effects are mostly mediated by interaction of various signal transduction cascades [23-25]. Terpenoids are important phytochemicals that have significant antioxidant and anti-inflammatory potentials [26,27]. Alkaloids have also been demonstrated to scavenge diphenyl-2-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radicals, reduce cupric ions attributed to the ability of the pyrrole nitrogen to form an iminoquinone structure by donating either a hydrogen or electrons to the free radicals [28]. It has also been suggested that the alkaloids could also strengthen the antioxidation of other phytochemicals such as polyphenols [29]. Flavonoids, terpenoids, and alkaloids have all been detected in C. *afer* [2,6] and thus the observed bioactivities are attributed to these important phytochemicals which could probably act in concert.

In conclusion, the ability of the C. *afer* to significantly reduce lipid peroxidation and its *in vitro* antioxidation may be partly

responsible for its reported traditional uses; thus, the plant has high pharmacological and ethnobotanical prospects. Evaluation of the potential bioactivity of the plant in cell-based systems is currently in progress.

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