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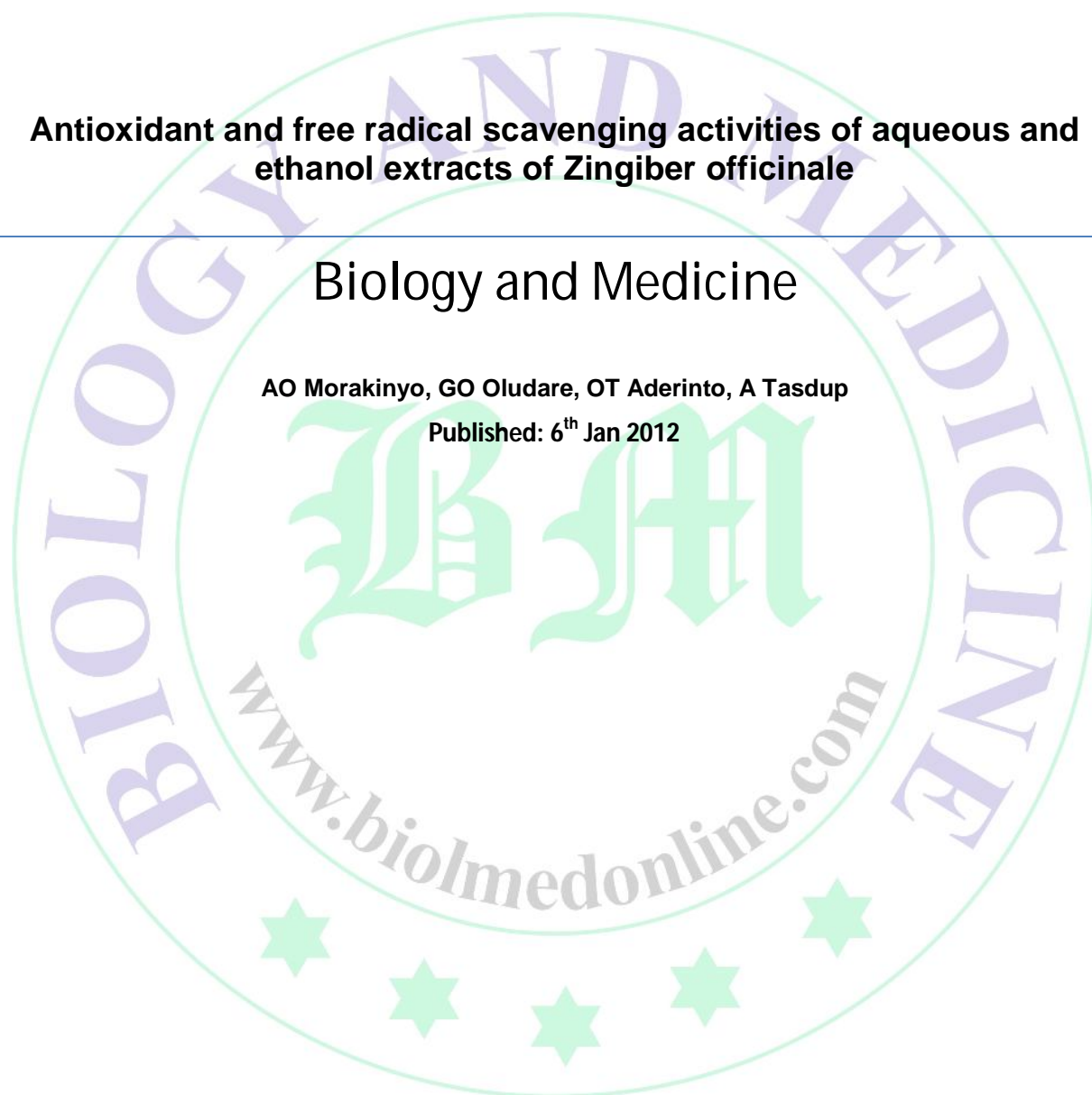
**Antioxidant and free radical scavenging activities of aqueous and ethanol extracts of *Zingiber officinale***

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## Antioxidant and free radical scavenging activities of aqueous and ethanol extracts of *Zingiber officinale*

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### Abstract

Oxidative stress contributes to the pathogenesis and progression of many diseases. The use of natural antioxidant as a therapeutic option is desirable and increasingly being practiced. The aim of the present study is to evaluate the in-vitro antioxidant property of *Zingiber officinale*, a known food additive. Aqueous and ethanol extracts of *Zingiber officinale* were evaluated for antioxidant activities using DPPH, ABTS and SOD scavenging assay; and lipid peroxidation assay. The results obtained indicated that both extracts possess potent antioxidant property as shown by significant scavenging of ABTS and SOD radicals. Similarly, MDA level (lipid peroxidation) was significantly reduced by the both extracts. However, there was no significant scavenging activity using the DPPH assay. The present study indicates that both aqueous and ethanol extracts of ginger are significant sources of natural antioxidant. Therefore, consumption of the plant material might be helpful in combating the progression of various diseases with oxidative stress components.

**Keywords:** *Zingiber officinale*; antioxidant; lipid peroxidation; superoxide dismutase; DPPH; ABTS.

### Introduction

Increased oxidative stress has been implicated in the incidence of chronic diseases (Dhalla, 2000; Valko *et al.*, 2004; Dalle-Donne *et al.*, 2006). Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation (Drodge, 2002). This contributes to the pathogenesis of many disorders including coronary heart disease, neurodegenerative disorders, diabetes, arthritis, inflammation, lung damage and cancer (Dhalla, 2000; Valko *et al.*, 2004; Dalle-Donne *et al.*, 2006). Since numerous physiological and biochemical processes in the human body may produce oxygen-centred free radicals and other reactive oxygen species as by-products, the body has developed an elaborate and effective protective mechanism against excessive accumulation of these free radicals (Sika, 2004; Masella *et al.*, 2005; Landis and Tower, 2005). However, these protective mechanisms are sometimes disrupted by various pathological processes, and antioxidant supplements are vital to combat oxidative damage. Since, antioxidants are capable of preventing oxidative damage, the wide use of natural antioxidants as a replacement of conventional synthetic antioxidants in food and food supplements has been employed owing to the fact that natural

products are considered to be promising and safe source.

Ginger (*Zingiber officinale*, Zingiberaceae) is widely used around the world as a spice. It is also widely used in traditional alternative medicine in the treatment and management of various disorders including catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes (Wang and Wang, 2005; Tapsell *et al.*, 2006). Evidence abounds in literature that shows that ginger is endowed with strong *in-vitro* and *in-vivo* anti-oxidant properties. The antioxidant property of ginger has been proposed as one of the major possible mechanisms for the protective effects of the plant against toxicity and lethality of radiation (Jagetia *et al.*, 2003; Haksar *et al.*, 2006) and a number of toxic agents such as carbon tetrachloride, arsenic and cisplatin (Amin and Hamza, 2006; Yemitan and Izegebu, 2006; Morakinyo *et al.*, 2010). Furthermore, the antioxidant efficacy has also been implicated in its use as an anti-ulcer drug (Siddaraju and Dharmesh, 2007). In spite of the numerous studies dedicated to this area, comprehensive investigations of the *in-vitro* antioxidant properties of aqueous and ethanol extracts of ginger (Nigerian variety) are very limited in the literature. Since the constituents of ginger are numerous and vary depending on the place of origin and whether the rhizomes are fresh or dry (Badreldin *et al.*, 2008), it is

therefore imperative to evaluate the biopharmacological activities of a Nigerian variety of ginger vis-a-vis its antioxidant activities. The objective of the present study is to evaluate the antioxidant potential and free radical scavenging activity of both aqueous and ethanol extracts from a dry Nigerian variety of *Zingiber officinale* rhizome.

## Materials and Methods

### Drug and extract preparation

The *Zingiber officinale* rhizome (ginger) was purchased from local commercial sources, identified and authenticated in the Department of Cell Genetics and Botany, University of Lagos. The plant material was shade dried at room temperature before being pulverized with an electric grinder. The powdered ginger was extracted with distilled water and ethanol using Soxhlet apparatus for 48 hr. The extract was concentrated under reduced pressure to yield a semi-solid mass of aqueous ginger extract (AGE) and ethanol ginger extract (EGE). These extracts were used as the test material or sample throughout this study.

### Reagents / chemicals used

DPPH (2,2-diphenyl-1-picryl hydrazyl), Methanol (HPLC grade), Positive control (Ascorbic acid), Reduced nicotinamide adenine dinucleotide sodium salt (NADH), Phenazinemethosulphate (PMS), Nitrobluetetrazolium (NBT), Ammonium persulphate (APS), ABTS (2,2'-azinobis-3-ethylbenzothiozoline-6-sulphonic acid).

### DPPH radical scavenging assay

DPPH [1,1-diphenyl-2-picryl hydrazyl] is a stable free radical with purple colour, the intensity of which is measured at 510 nm spectrophotometrically. DPPH is reduced to a colourless compound, 1,1-diphenyl-2-picryl hydrazine, by antioxidants. Briefly, 1 ml of 0.3 mM alcohol solution of DPPH was added to 2.5 ml from the samples with different concentrations of ginger extract. The samples were kept at room temperature in the dark and after 30 min the optical density was measured at 510 nm. A control reaction was carried out without the test sample. The % inhibition was calculated according to the following equation; % Inhibition =  $(A_0 - A_t) / A_0 \times 100$ ; where  $A_0$  was the absorbance of the control (blank, without extract) and  $A_t$  was the absorbance in the presence of the extract.

### ABTS radical scavenging assay

ABTS (2, 2'-azinobis-3-ethylbenzothiozoline-6-sulphonic acid) assay is based on the scavenging of light by ABTS radicals. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical, a process which is associated with a change in absorption which can be followed spectrophotometrically. The relatively stable ABTS radical has a green colour and is quantified spectrophotometrically at 734nm. The  $ABTS^+$  radical cation was pre-generated by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and incubating for 12–16h in the dark at room temperature until the reaction was complete and the absorbance was stable. The absorbance of the  $ABTS^+$  solution was equilibrated to 0.70 ( $\pm$  0.02) by diluting with water at room temperature, then 1 ml was mixed with 10  $\mu$ l of the test sample (0.05–10 mg/ml) and the absorbance was measured at 734 nm after 15 min. All experiments were repeated four times. A control reaction was carried out without the test sample.

### Superoxide radical scavenging activity (PMS-NADH System)

Superoxide anions were generated using PMS / NADH system. The superoxide anions were subsequently made to reduce nitrobluetetrazolium (NBT) to a purple formazan. The test solution (0.1ml) in 0.1M phosphate buffer pH 7.4, 62.5  $\mu$ l of 468  $\mu$ M NADH solution, 62.5  $\mu$ l of 150  $\mu$ M NBT solution and 62.5  $\mu$ l of 60  $\mu$ M PMS solution were added to a microwell plate. After incubation for 5 min at ambient temperature, the absorbance at 562 nm was measured against an appropriate blank to determine the quantity of formazan generated. All tests were performed four times.

### Lipid peroxidation assay

The quantification of the end products of lipid peroxidation, specifically malondialdehyde (MDA), is the most commonly used test to evaluate lipid peroxidation. This assay is used for putative antioxidants which diffuse freely in lipids. Lipid peroxidation was induced in the liver homogenate with hydrogen peroxide and used for the evaluation of MDA.

### Statistical analysis

All values were expressed as Mean  $\pm$  SEM (standard error of mean) and analysed using the GraphPad InStat Version 3.05 (GraphPad Software, San Diego California, USA) using the one-way ANOVA followed by Student's



Newman-Keuls post-hoc test. Differences were considered significant when  $P < 0.05$ .

**Results**

*DPPH radical scavenging assay*

The antiradical activity assay is based on the reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The ability of the extracts and standard ascorbic acid to scavenge free radicals and pair off the odd electron was shown in this assay. It was observed that

aqueous and ethanol extracts of *Zingiber officinale* is a poor scavenger of DPPH radical compared with the standard ascorbic acid. The highest inhibition effect of aqueous and ethanol extracts of *Zingiber officinale* were 2.5% and 2.7% respectively compared with ascorbic acid with 92.9% at concentration of 25  $\mu\text{g/ml}$ . However, further increase in the concentration of the *Zingiber officinale* extracts showed decreasing inhibition of DPPH radical (Table 1).

**Table 1: Scavenging effects of *Zingiber officinale* extracts (aqueous and ethanol) and standard ascorbic acid on DPPH radical. The data represents percentage inhibition of DPPH radical.**

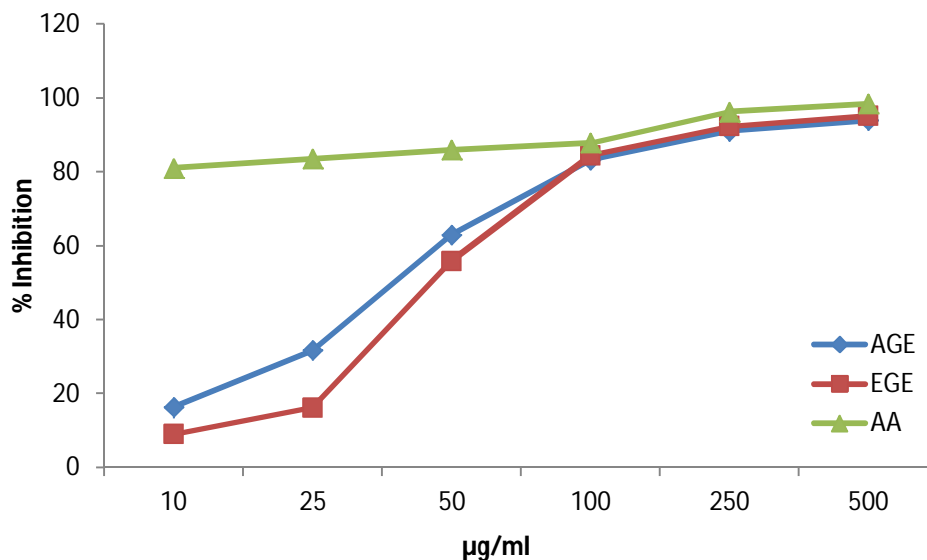
Conc ( $\mu\text{g/ml}$ )	AGE	EGE	AA
10	2.079	3.611	86.214
25	2.516	2.735	92.888
50	0.547	-1.422	93.217
100	-19.753	-16.204	95.799
250	-30.093	-20.833	97.068
500	-67.284	-41.512	98.104

AGE, aqueous ginger extract; EGE, ethanol ginger extract; Ascorbic acid (AA) was used as the positive control.

*ABTS radical scavenging assay*

The scavenging activity for ABTS radical by *Zingiber officinale* extracts was shown in Figure 1. The data indicated that both aqueous and ethanol extracts of *Zingiber officinale* were effective scavenger of ABTS radical and this

activity was comparable with ascorbic acid. At 500 $\mu\text{g/ml}$ , the percentage scavenging activities of the both aqueous and ethanol extracts of *Zingiber officinale* were 93.9% and 95.1% respectively whereas that of lipoic acid was 98.4%.



**Figure 1: Scavenging effects of *Zingiber officinale* extracts (aqueous and ethanol) and standard ascorbic acid on ABTS radical. The data represents percentage inhibition of ABTS radical. AGE, aqueous ginger extract; EGE, ethanol ginger extract; Ascorbic acid (AA) was used as the positive control.**

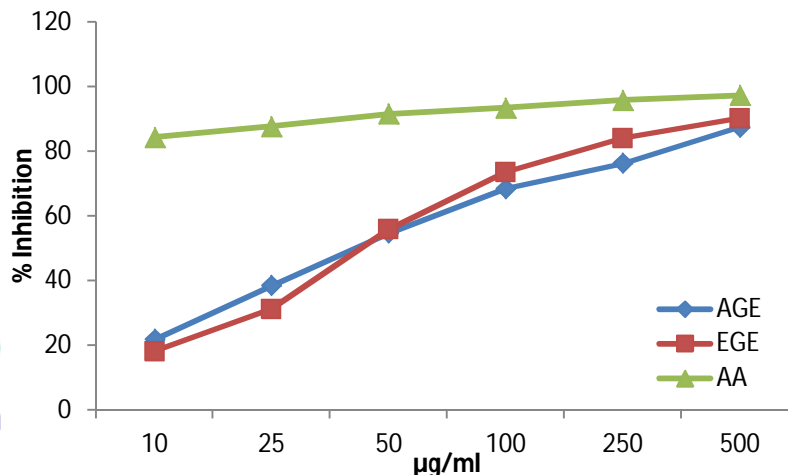
*Superoxide radical scavenging activity*

PMS-NADH coupling yields superoxide radicals that can be measured by their ability

to reduce NBT. The ability of the extracts and standard ascorbic acid to quench superoxide radicals from reaction mixture is reflected in

the decrease of the absorbance at 560 nm as shown in Figure 2. At 500mg/ml, the scavenging activities of both aqueous and

ethanol extracts of *Zingiber officinale* were 87.4% and 90.2% respectively compared with the standard ascorbic acid with 98%.

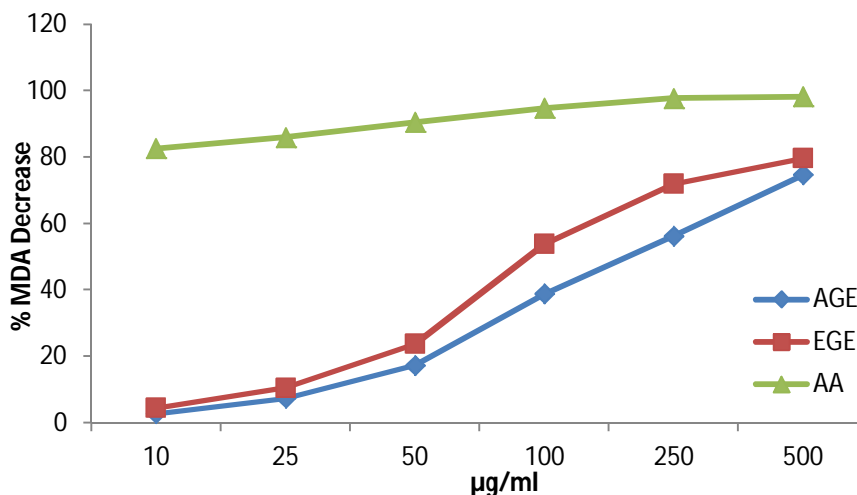


**Figure 2: Scavenging effects of *Zingiber officinale* extracts (aqueous and ethanol) and standard ascorbic acid on superoxide radical. The data represents percentage inhibition of superoxide radical. AGE, aqueous ginger extract; EGE, ethanol ginger extract; Ascorbic acid (AA) was used as the positive control.**

*Lipid peroxidation assay*

As illustrated in Figure 3, both aqueous and ethanol extracts of *Zingiber officinale* were shown to have strong antioxidant efficiency to inhibit lipid peroxidation. The reduction in peroxidation represented by the % MDA

inhibition values for aqueous (74.6%) and ethanol (79.6%) extracts *Zingiber officinale* were lower than that of ascorbic acid (98.2%) but nevertheless, a good antioxidant potential was established.



**Figure 3: Antioxidant effects of *Zingiber officinale* extracts (aqueous and ethanol) and standard ascorbic acid on TBARS in liver microsomes. AGE, aqueous ginger extract; EGE, ethanol ginger extract; Ascorbic acid (AA) was used as the positive control.**

**Discussion**

In living organisms, free radicals are constantly generated from numerous physiological and biochemical processes (Valko *et al.*, 2007; Jain and Agarwal, 2008). These radicals are responsible for damage to cellular bio-

molecules such as proteins, enzymes, nucleic acids, lipids and carbohydrates leading to many disease conditions (Sayre *et al.*, 2001; Jenner, 2003; Dalle-Donne *et al.*, 2006; Valko *et al.*, 2006). It is also well known that all living organisms possess protective antioxidant

systems against the oxidative damage of free radicals (Sika, 2004; Smith *et al.*, 2004; Schrauzer, 2006). However, these protective mechanisms are sometimes not adequate to prevent all possible damage. Thus, the use or intake of antioxidant to provide enhanced and greater protection against free radical generation and oxidative damages is increasingly practised.

Natural products with strong antioxidant properties have received great attention (Zheng and Wang, 2001) with their use as a replacement of synthetic antioxidants in food and food supplements (Yazdanparast *et al.*, 2008). Ginger as a widely used food additives has been shown in this study to have good antioxidant ROS scavenging activities. ABTS is a blue chromophore produced by the reaction of ABTS with potassium persulphate. This pre-formed radical was reduced to ABTS with the addition of both aqueous and ethanol extracts of ginger in a dose-dependent manner. These results indicate that ginger extracts possess strong antioxidant radical activities as evidenced by the ABTS assay. The hydroxyl radical is one of the radicals in living systems that causes DNA blockage leading to many debilitating diseases (Pastor, 2000; Leonard *et al.*, 2004). Superoxide anion is known to be harmful to cellular components in a biological system (Kovacic *et al.*, 2005; Valko *et al.*, 2004). It indirectly induces the peroxidation of lipid membranes by generating singlet oxygen. The extracts of ginger were shown in this study to dose-dependently scavenge the  $O_2^-$  radicals and thereby confer protection on the system. Furthermore, the results shows that ginger extracts prevent lipid peroxidation damage. Ginger extracts, therefore, could prevent and / or attenuate the free radical- induced peroxidation of cellular structures such as lipids, proteins and DNA as shown by the decrease in MDA level. Free radicals are known to be highly reactive causing severe damages to membranes, proteins and DNA (Stadman, 2004).

### Conclusion

On the basis of the results obtained in the present study, ginger has been shown to be a functional food material with medicinal benefits. The present study indicates that both aqueous and ethanol extracts from the Nigerian variety of ginger have significant natural antioxidant activity. Therefore, consumption of the plant material might be helpful in combating the progression of various diseases with oxidative stress components

such as atherosclerosis, diabetes mellitus among others.

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