

Preliminary Phytochemical Screening and in Vitro Antioxidant Potential of Aqueous, Hydro-Methanolic And Hydro-Ethanollic Extracts of Ginger (*Zingiber Officinale*): A Comparative Analysis Between Nigerian And Indian Variety.

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ABSTRACT: Ginger (*Zingiber officinale* Roscoe) is a herb that is well known and widely used, especially in Asia and Africa, which contains several interesting bioactive constituents and possesses health promoting properties. In this study, Phytochemical content and antioxidant potential of Nigerian and Indian ginger were assessed in an effort to compare and validate the medicinal potential of the plant. Aqueous, hydromethanolic and hydroethanolic extracts of Indian and Nigerian varieties were prepared by extraction process. Both varieties were found to contain high amount of secondary metabolites with exception of phlobotannins which happened to be in low quantity and tannins which is entirely absent in all the extracts. DPPH scavenging activity, Hydrogen peroxide and reducing power were assayed. The comparison carried out between different extracts of the two varieties using t-test at $p=0.05$ revealed a significant difference in terms of antioxidant activity. Indian variety was generally found to have more antioxidant activity than the Nigerian one with the exception of aqueous extract of Nigerian variety. This is probably due to high level of phenolics and flavonoids in the Indian variety. This study validated the medicinal potential of *Zingiber officinale* and established some relationship between the two varieties in terms of their antioxidant properties.

KEYWORDS: Ginger, *Zingiber officinale*, Phytochemical content, Antioxidant properties.

I. INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Medicinal plants are of great importance to the health of individuals and communities. Herbal medicines serve the health needs of about 80% of the world's population (Gosh *et al.*, 2011). From 250 to 500 thousand plant species are estimated to exist on the planet, and only between 1 and 10% are used as food by humans and other animals (Cowan, 1999). According to World Health Organization (WHO), traditional medicine is estimated to be used by 80% of the population of most developing countries (WHO Bulletin, 2002). The medicinal value of plants lies in some chemical substances or group of compounds that produce a definite physiological action in the human body. These chemical substances are called secondary metabolites. The most important of these bioactive groups of compounds are alkaloids, terpenoids, steroids, flavonoids, tannins and phenolic compounds (Hill, 1952).

Ginger (*Zingiber officinale*) is one of the most popular spices worldwide and is widely used in food, medicines, drinks and toiletries around the globe (Kokate 1999; Ali, 2009; Shah and Seth, 2010). The important medicinal part of the ginger plant is its rhizome which contain active compounds that inhibits colon cancer and suppression of the transformation, hyperproliferation of cells, and inflammation that initiate and promote carcinogenesis, angiogenesis and metastasis (Lee *et al.*, 2008).

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The aim of this study is to compare and determine the differences, in terms of total phytochemical contents and antioxidant potential, between Indian ginger and Nigerian ginger. It is planned to be achieved by carrying out the following processes;

- ❖ To prepare aqueous, hydroethanolic and hydromethanolic extracts of the plant of both varieties.
- ❖ To carry out both qualitative and quantitative phytochemical screening on the two varieties.
- ❖ To determine the antioxidant potential of both varieties.
- ❖ To carry out statistical analysis on the generated result so as to come up with useful information about the two varieties.

Figure 1: Ginger



II. MATERIALS AND METHODS

SAMPLE COLLECTION

The Indian ginger was collected from Spencer store in Ansal Plaza, Greater Noida, India. It was Utterkand variety from Utterkand State of northern India as gathered from the store's staff. It was dried fine-powder that is well packaged and stored at good environmental conditions with temperature of about 25°C. The fresh Nigerian ginger was collected from Rimi Market in Kano State of Nigeria. It was brought, as explained by the trader, from Kachia town in Kaduna state of northern Nigeria. Both varieties were identified at the department of Biotechnology, Sharda University. The fresh Nigerian ginger was air-dried and ground to fine-powder and both varieties were stored at laboratory standard conditions.

Chemical Reagents

All chemicals used in this study are of analytical grade and were obtained from laboratories of departments of Biotechnology and Chemistry, Sharda University, Greater Noida, India.

Preparations of Extracts

Aqueous Extract

50g of both Indian and Nigerian ginger powder were dissolved in 200ml of distilled water then placed on hot plate at 50-55°C for 2hrs. The mixtures were regularly shaken at intervals for 2-3 days after which they were filtered using filter paper. The filtrates were dried in a hot oven at 50°C. After drying, the extracts were stored in an air-tight container at 4°C for further use.

Hydroethanolic and Hydromethanolic Extracts

The hydroethanolic and hydromethanolic extracts of the samples (both Indian and Nigerian ginger) were prepared in a ratio of 20:80 respectively (i.e water:ethanol/methanol is 20:80). 50g of the sample was weighed using electric balance, dissolved and made 200ml solution. The mixtures were regularly shaken at intervals for 2-3 days after which

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they were filtered using filter paper. The filtrates were then dried in a hot oven at 50°C. After drying, the extracts were stored in air tight container at 4°C for further use.

PHYTOCHEMICAL SCREENING

Primary Metabolites

Test for Carbohydrates and Glycosides

Fehling's Test: 1ml each of the six extracts and Fehling's solutions A and B were boiled on water bath (Amin *et al.*, 2013).

Borfoed's Test: To 1ml of the six extracts, 1ml of Borfoed's reagent was added and heated on a boiling water bath for 2mins (Amin *et al.*, 2013).

Test for Proteins and Amino Acids

Ninhydrin Test: Two drops of ninhydrin solution were added to 1ml of the all the sample extracts (Amin *et al.*, 2013).

Test of Fixed Oils and Fats

Spot Test: A small quantity of each of the extracts was pressed between two filter papers to observe oil stain on the filter papers (Amin *et al.*, 2013).

Secondary Metabolites

Test for alkaloids

Mayer's Test: To a few ml of each of the extracts, a drop of Mayer's reagent was added by the side of the test tube (Evans, 1997).

Test for Flavonoids

To 5ml of dilute ammonia solution, a portion of each of the extracts was added, followed by addition of concentrated sulphuric acid (Sofowora, 1993; Khan *et al.*, 2011).

Test for Terpenoids (Salkowski Test)

5ml of each of the extracts were mixed with 2ml of chloroform and concentrated sulphuric acid to form a layer (Khan *et al.*, 2011).

Test for Ascorbic Acid

To each of the sample extracts, 2ml of water were added followed by addition of Sodium bicarbonate and ferrous sulphate and shaken well (Sharma and Paliwal, 2013).

Test for Saponins

Small portions of each of the solid extracts were diluted with distilled water and made up to 20ml. The suspensions were then shaken in a test tube for 15mins (Kokate, 1999).

Test for Phytosterols

Liebermann-Burchard's Test: Portions of each of the extracts were mixed in test tubes with 2ml of acetic anhydride. To this, 2 drops of concentrated sulphuric acid were added slowly along the sides of the test tubes (Finar, 1986).

Test for Phenolic Compounds

Ferric Chloride Test: To 5ml of each of the extracts, few drops of neutral 5% ferric chloride solution were added (Mace, 1963).

Test for Tannins

2ml of each of the sample extracts were diluted with distilled water and then followed by 2-3 drops of 5% ferric chloride (FeCl₂) solutions (Ciulci, 1994).

Test for Phlobatannins

10ml of each of the six different extracts were boiled with 1% aqueous HCl acid in a test tube (Krishnaiah *et al.*, 2009).

Test for Steroids

To 0.5 ml of each of the extracts, 2ml of glacial acetic acid and 2ml of Sulphuric acid were added (Venkatesan *et al.*, 2009).

Test for Cardiac Glycoside

To 2ml of each of the ginger extracts, 1ml of glacial acetic acid, 2ml ferric chloride and 2ml of concentrated sulphuric acid were added (Krishnaiah *et al.*, 2009).

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ESTIMATION OF ANTIOXIDANTS

Reducing Power Assay

The reducing power of each of the extracts was determined as reported by Oyaizu (1986). Different concentrations of the extracts [(0.1, 0.2, 0.4, 0.6, 0.8, 1.0) mg/ml] in 1ml of methanol were mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferrocyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20mins. A portion (2.5ml) of trichloroacetic acid (10%) was added to the mixture which was then centrifuged at 3000rpm for 10mins. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and FeCl₃ (0.5ml, 0.1%) and the absorbance was measured at 700nm and compared with standards. Increased absorbance of the reaction mixture indicates increased reducing power.

Antioxidant assay

To get free radical scavenging activity of the extracts, the free-radical scavenging activity of each of the extracts were measured by decrease in the absorbance of methanol solution of 1-1-diphenyl-2-picryl-hydrazyl (DPPH) (Umamaheshwari *et al.*, 2012). A stock solution of DPPH (33mg in 1L) was prepared in methanol, which gave an initial absorbance of 0.493, and 5ml of this stock solution were then added to 1ml of each of the extracts different concentrations (100, 200, 400, 600, 800, 1000 µg/ml). After 30mins, absorbance was measured at 517nm and compared with standards (10-50 mg/ml). Scavenging activity is expressed as the percentage inhibition calculated using the following formula:

$$\% \text{ Anti - radical activity} = \frac{\text{Control Abs} - \text{Sample Abs} \times 100}{\text{Control Abs}}$$

Scavenging of Hydrogen Peroxide

A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (100, 200, 400, 600, 800, 1000 µg/ml) of each of the extracts were added to a hydrogen peroxide solution (0.6ml, 40mM). Absorbance of hydrogen peroxide at 230nm was read after 10mins against a blank solution containing phosphate buffer without hydrogen peroxide.

The percentage scavenging activity of hydrogen peroxide of each of the extracts and standard compounds were calculated using the following formula: % scavenged [H₂O₂] = [(A₀-A₁)/A₀] x 100; where A₀ is the absorbance of the control and A₁ is the absorbance in the presence of each of the samples and standards (Kumar *et al.*, 2007).

III. RESULTS AND DISCUSSION

Percentage yield and phyto-profile of the extracts from successive extraction of *Zingiber officinale* of Nigeria and India

The yield of successive extracts of *Zingiber officinale* (in grams) and their corresponding percentage yields are shown on table 1 and phyto-profile on table 2. Total weight of ginger powder taken is 50g.

Table 1: Weights (in grams) and percentage yield of different extracts of *Zingiber Officinale* (50g)

S.No	Extract	Weight (gram)	% Yield (w/w)
1	Aqueous (Nigeria)	3.045	6.09%
2	Hydromethanolic (Nigeria)	5.070	10.14%
3	Hydroethanolic (Nigeria)	5.402	10.80%
4	Aqueous (India)	2.142	4.28%
5	Hydromethanolic (India)	4.120	8.24%
6	Hydroethanolic (India)	3.605	7.21%

Table 1 presents the values of extracts (in percentage) of different solvents from highly-polar to less-polar i.e. Aqueous, hydro-ethanolic and hydro-methanolic. The values were high in hydro-ethanolic and hydro-methanolic extracts of Nigerian ginger; 10.14% and 10.80% respectively. But aqueous extract of Indian variety 4.28% was the minimum value obtained.

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This indicates that most of the phytochemicals present in ginger are less-polar in nature and could best be extracted using less-polar solvents.

Figure 2: Graphical representation of percentage yield of different extracts of *Zingiber Officinale* (50g)

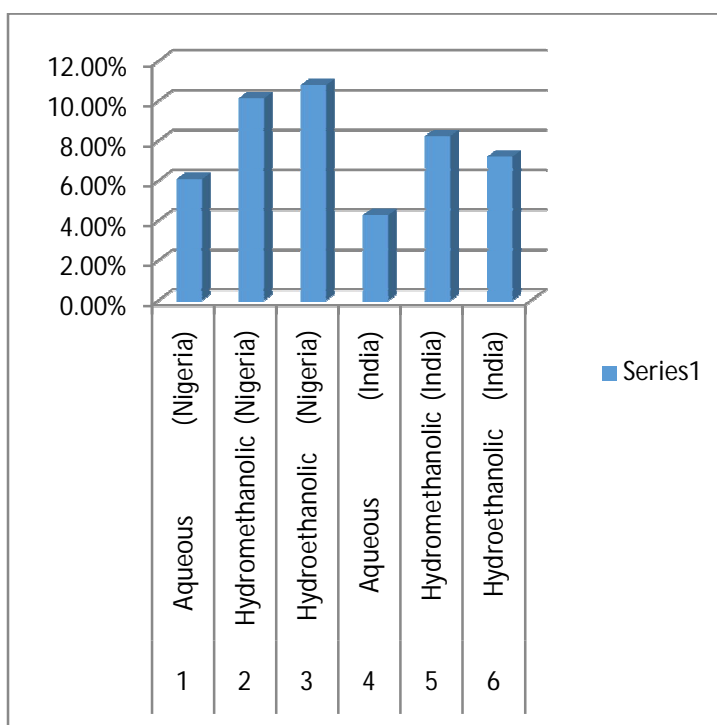


Table 2: Phyto-profile and percentage yield of different extracts of *Zingiber officinale*

S.No	Extract	Nature	Consistency	Colour	Odour	% Yield (w/w)
1	Aqueous (Nigeria)	Solid	Dry	Light brown	Pungent	6.09%
2	Hydromethanolic (Nigeria)	Solid	Sticky	Blackish brown	Pungent	10.14%
3	Hydroethanolic (Nigeria)	Solid	Sticky	Yellow brown	Pungent	10.80%
4	Aqueous (India)	Crispy solid	Dry	Dark brown	Pungent	4.28%
5	Hydromethanolic (India)	Solid	Sticky	Orange brown	Pungent	8.24%
6	Hydroethanolic (India)	Solid	Sticky	Yellow brown	Pungent	7.21%

Fluorescence analysis of *Zingiber officinale*

Fluorescence is an important phenomena exhibited by various chemical constituents present in plant material. Many phytochemicals fluorescence when suitably illuminated. The Fluorescence characteristics of different extracts of *Zingiber officinale* is shown on table 3. The Fluorescence was observed under UV light at 280nm.

Table 3: Fluorescence characteristics of different extracts of *Zingiber officinale*

S.No	Extract	Under ordinary light	Under UV light (280nm)
1	Aqueous (Nigeria)	Light brown	Colourless
2	Hydromethanolic (Nigeria)	Blackish brown	Brownish yellow
3	Hydroethanolic (Nigeria)	Yellow brown	Pale yellow
4	Aqueous (India)	Dark brown	Pale brown
5	Hydromethanolic (India)	Orange brown	Pale yellow
6	Hydroethanolic (India)	Yellow brown	Yellow

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The fluorescence colour observed is specific for each compound. A non-fluorescence compound may fluorescence if mixed with impurities that are fluorescent. Some constituents show fluorescence in the visible range in a day light. The UV produces fluorescence in many natural products. If the substance themselves are not fluorescent, they may often be converted into fluorescence derivatives after reacting with different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmaceutical evaluation (Paliwal, 2015).

Phytochemical screening of different extracts of *Zingiber officinale*

Phytochemical components are responsible for both pharmacological and toxic activities in plants. These metabolites are said to be useful to a plant itself but can be toxic to animal, including man. The presence of these chemical compounds in this plant is an indication that the plant, if properly screened, could yield drugs of pharmaceutical importance (Paliwal, 2015). Phytochemical constituents of *Zingiber officinale* are shown on table 4.

Table 4: Qualitative phytochemical screening of different extracts of *Zingiber officinale*

S.No	Plant Constituents	Test Performed	Nigeria			India		
			Aq	HM	HE	Aq	HM	HE
1	Carbohydrates	Fehling's Test Barfoed's test	-	-	-	-	-	-
2	Proteins and amino acids	Ninhydrin	-	-	-	-	-	-
3	Fats and Oil	Stain test	-	++	++	-	++	+
4	Alkaloids	Mayer's test	-	-	-	-	-	-
5	Flavonoids	Ammonia test	++	++	+	+	+++	+++
6	Terpenoids	Yellow brown	++	+++	+	+++	+	++
7	Steroids	Liebermann- Burchard's Test	-	+	+	-	+	+
8	Saponins	Frothing test	++	++	++	++	+++	+++
9	Tannins	Ferric chloride test	-	-	-	-	-	-
10	Phenolics	Ferric chloride test	++	+++	+++	++	+	++
11	Phytosterol	Acid test	+	+	+	+	+	+
12	Ascorbic acid	Ferrous sulphate test	+++	+++	+++	+++	+++	+++
13	Phlobotannins	Hydrochloric acid test	+	-	-	+	-	-
14	Cardiac glycosides	Killer killani test	++	+	+++	+	++	++

WHERE (-): absent, (+): Weak, (++) : Moderate: (+++): Strong

Aq: Aqueous, **HM:** Hydro-methanolic, **HE:** Hydro-ethanolic

The results showed the presence of flavonoids, phenolics, saponins, terpenoids, phytosterol, steroids, fats and oil, and cardiac glycosides and high amount of ascorbic acid in different extracts of *Zingiber officinale* of both Nigeria and India.

Crude flavonoids and saponins were present in high amount while phenolics were relatively low. While Indian ginger was found to contain relatively high amount of flavonoids and saponins than Nigerian one, it also contain more phenolics in comparison with Nigerian variety. This could be attributed due to different geographical location and soil constituents.

Flavonoids shows a wide range of biological activities such as inhibition of cell-proliferation, induction of apoptosis, inhibition of enzymes and other antibacterial and antioxidant effects (Paliwal, 2015). Saponins helps in boosting the immune system in lowering the cholesterol levels in the blood and reducing risk of getting intestinal cancer. Various reports have shown that phenolics compounds contribute to the quality and nutritional value of ginger in terms of modifying colour, taste, aroma and flavor and also in providing beneficial health effect. Phenolics also

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provide plants with defense mechanism to neutralize reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganism, insects and herbivores (Paliwal, 2015).

All these phytochemicals possesses good antioxidant properties and has been reported to exhibit multiple biological effects including anti-inflammatory and antitumor activities.

Antioxidant test on reducing power of successive extracts of *Zingiber officinale*

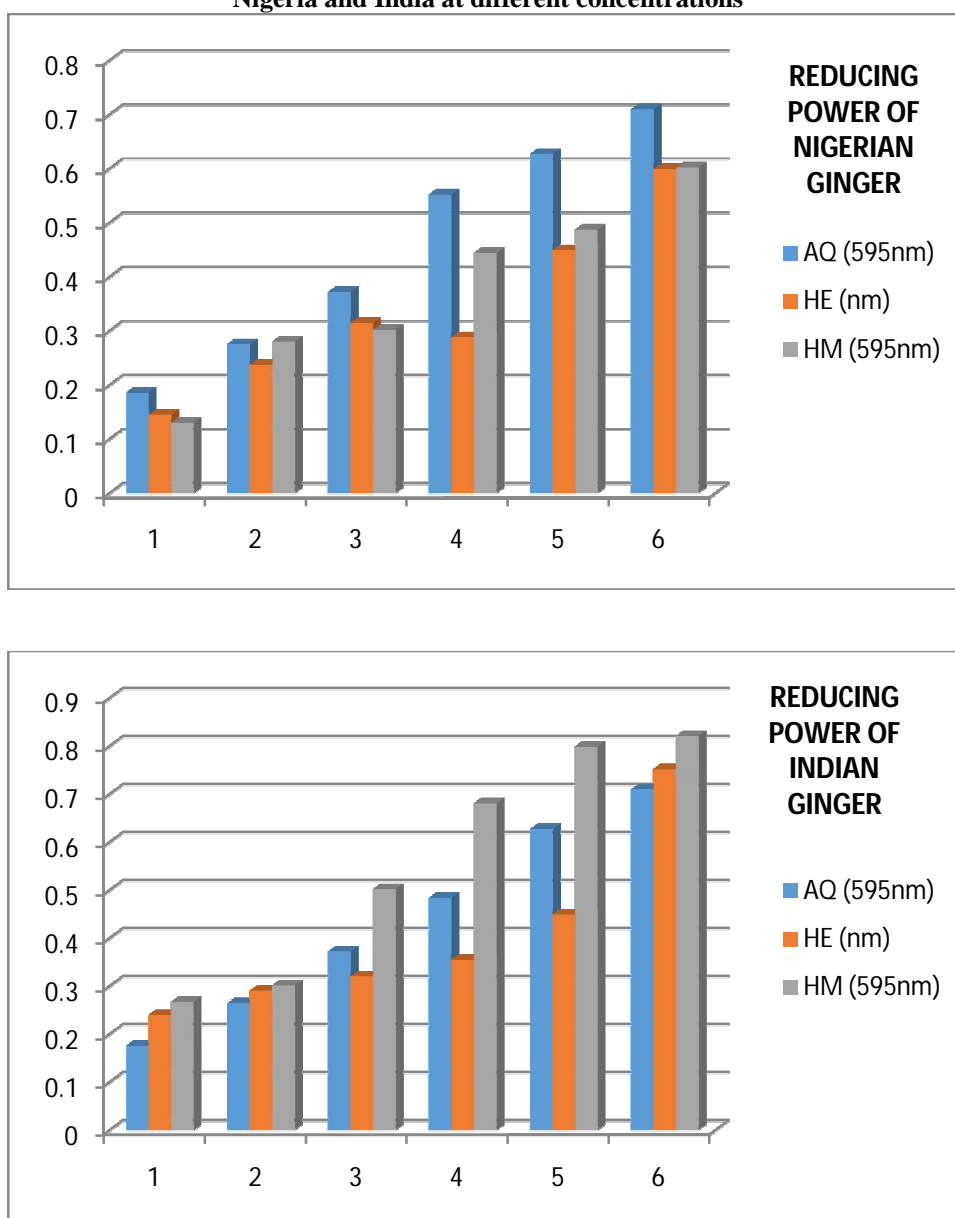
The extracts of the two samples' reducing power were tested and found to have strong antioxidant activity successively at different concentrations table 5. The reducing power of each of the extracts was measured by decrease in the absorbance of the standard used i.e tocopherol.

Table 5: Absorbance of antioxidant activity: reducing power of *Zingiber officinale* extracts of both Nigeria and India at different concentrations.

Nigeria	Absorbance	Absorbance	Absorbance
Concentration (mg/ml)	AQ (595nm)	HE (595nm)	HM (595nm)
0.1	0.186	0.145	0.130
0.2	0.275	0.237	0.280
0.4	0.372	0.315	0.302
0.6	0.552	0.288	0.444
0.8	0.627	0.449	0.487
1.0	0.710	0.599	0.602

India	Absorbance	Absorbance	Absorbance
Concentration (mg/ml)	AQ (595nm)	HE (595nm)	HM (595nm)
0.1	0.176	0.240	0.267
0.2	0.264	0.290	0.301
0.4	0.372	0.320	0.502
0.6	0.484	0.355	0.681
0.8	0.627	0.449	0.799
1.0	0.710	0.752	0.821

Figure 3: Graphical representation of antioxidant activity: reducing power of *Zingiber officinale* extracts of both Nigeria and India at different concentrations



For the measurements of the reducing ability, “Fe³⁺ to Fe²⁺ transformation” in the presence of ginger extract was found. The antioxidant activities of the Indian ginger were slightly higher than that of Nigeria with hydro-methanolic extract of Indian ginger having the highest activity. But, the aqueous extract of Nigerian ginger has higher activity than that of India. Based on the results obtained, it is possible that several compounds present at different concentrations might have contributed to the antioxidant properties of the plant to vary remarkably.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reductive capabilities of the extracts were compared with alpha-tocopherol. The reducing power of ginger was found to increase with rising concentrations (Fig. 3). Kikuzaki (1993) reported that hydromethanolic extracts may contain phenolic and hydroxy-phenolic compounds with acid, alcohol, sugar or glycoside.

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DPPH scavenging activities of successive extracts of *Zingiber officinale*

Table 6. DPPH scavenging activities of the extracts of two samples of *Zingiber officinale*
BHT was used as positive control. tocopherol at 50 µg/ml is 67.54±0.8%

Nigeria		DPPH radical Scavenging activity (% Inhibition)		
Concentration (µg/ml)	AQ	H.M	H.E	
100	78.27 ± 3.20	48.31 ± 3.20	48.68 ± 2.06	
200	76.16 ± 2.34	35.02 ± 2.34	42.86± 2.77	
400	67.52 ± 1.12	40.09 ± 2.13	59.70 ± 2.89	
600	68.54 ± 3.11	35.59 ± 2.14	67.80 ± 3.01	
800	76.51 ± 2.11	52.48 ± 2.34	67.60 ± 2.99	
1000	81.64 ± 3.94	76.90 ± 1.67	76.60 ± 3.11	

India		DPPH radical Scavenging activity (% Inhibition)		
Concentration (µg/ml)	AQ	HM	HE	
100	60.27 ± 3.20	48.31 ± 3.20	48.68 ± 2.06	
200	66.16 ± 2.34	35.02 ± 2.34	42.86± 2.77	
400	67.52 ± 1.12	40.09 ± 2.13	59.70 ± 2.89	
600	68.54 ± 3.11	35.59 ± 2.14	67.80 ± 3.01	
800	76.51 ± 2.11	52.48 ± 2.34	67.60 ± 2.99	
1000	80.64 ± 3.94	77.9 ± 1.67	78.60 ± 3.11	

Results were expressed as: mean ± standard deviation
n = number of sample (n=6).

The free radical scavenging activity was evaluated by various in vitro assays. DPPH radical was used as a substrate to evaluate free radical scavenging activities of different Ginger extracts. It involves reaction of specific antioxidant with a stable free radical DPPH. As a result of this reaction, there is reduction of DPPH concentration by antioxidant, which decreases the optical absorbance of DPPH; this is detected by spectrophotometer at 517nm. Tocopherol was used as standard. These results indicated that the extracts have noticeable effect on scavenging the free radicals. The test showed that ginger extracts from both samples have good free radical scavenging ability and can be used as a radical inhibitor or scavenger, acting possibly as a primary antioxidant.

Antioxidant activities of successive extracts of *Zingiber officinale* on Hydrogen peroxide

Table 7. Antioxidant activities of successive extracts of two samples of *Zingiber officinale* on Hydrogen peroxide.

Nigeria		Hydrogen peroxide radical Scavenging activity (% Inhibition)		
Concentration (µg/ml)	AQ	HM	HE	
100	50.27 ± 3.20	38.31 ± 0.20	40.68 ± 0.20	
200	60.16 ± 3.21	38.07 ± 1.10	42.86 ± 1.20	
400	67.52 ± 3.44	40.09 ± 2.20	59.76 ± 2.20	
600	68.54 ± 3.11	48.76 ± 1.20	67.89 ± 2.20	
800	76.61 ± 1.21	52.44 ± 3.00	70.62 ± 3.10	
1000	81.61 ± 2.23	66.96 ± 3.25	75.68 ± 2.67	

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India	Hydrogen peroxide radical Scavenging activity (% Inhibition)		
	Concentration ($\mu\text{g/ml}$)	AQ	HM
100	45.27 \pm 3.21	50.31 \pm 0.20	56.68 \pm 1.50
200	60.16 \pm 3.21	57.07 \pm 1.10	60.86 \pm 2.24
400	64.52 \pm 3.45	60.09 \pm 2.20	66.76 \pm 1.20
600	66.54 \pm 3.12	66.76 \pm 2.20	73.89 \pm 2.10
800	73.61 \pm 1.21	78.44 \pm 2.00	78.62 \pm 3.20
1000	76.61 \pm 2.22	88.96 \pm 3.23	87.68 \pm 2.07

H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Thus, removing of H_2O_2 is very important for protection of food systems. Table 7 shows the H_2O_2 scavenging activity by different concentrations of ginger extracts and comparison with $50\mu\text{m/ml}$ of BHA. The percentage of H_2O_2 scavenging activity of ginger extract and BHA was found to be 88.85 and 89.25%, respectively. This indicated that the extracts contain high amount of antioxidants.

The Hydromethanolic and Hydroethanolic extracts of Indian ginger were found to be more active than that of Nigeria. In addition, anti-oxidative activities observed in ginger varieties could be a synergistic effect of more than two compounds that might be present in the plant.

IV. CONCLUSION

The results of this study indicated that *Zingiber officinale* from both India and Nigeria is very rich in phytochemicals that are very active in serving as antioxidant agents. This could be attributed to the fact that high amount of phenolic and flavonoid compounds were recorded in both samples. There were some differences between the two samples in terms of their antioxidant activities. This might not be unconnected to the fact that the different geographical location of the two samples can play a vital role in determining the total content of their phytochemical constituents, hence the level of their antioxidant potential.

Further work is required to establish the components in phenolics and flavonoids that might have contributed to the high antioxidant activities so far observed.

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