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Original Article

Phytochemical Profile and Comparative Anti-radical Scavenging Activities of n-Hexane Extracts of Indigenous *Zingiber officinale* and *Curcuma longa*

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ABSTRACT

Objectives: The therapeutic and nutritional values of plants and plant produce for aliment management and as food supplements are evolving. Free radical scavenging activities of n-haxane extracts of indigenous *Curcuma longa* and *Zingiber officinale* was investigated and compared to CellGevity®; a nutraceutical antioxidant supplement. In addition, their phytochemical profile was qualitatively and quantitatively determined using standard procedure for phytochemical analysis. **Methods:** The antioxidant activities of the extracts were determined spectrophotometrically. We used 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, 2,2′-Azino-bis3-ethylbenzothialine-6-sulfonic acid (ABTS) radical cations and hydrogen peroxide (H_2O_2) to determine free radical scavenging activities of the extracts. The ability of the extracts to scavenge free radicals was determined following the discoloration of the solution mixtures, measured spectrophotometrically. The reducing power efficacy of the extracts was determined by their ability to reduce Fe^{3+} to Fe^{2+} ions. **Results:** *Curcuma longa* and *Zingiber officinale* have more free radical scavenging power compared to CellGevity®. *Curcuma longa* has more scavenging power against DPPH and H_2O_2 system, while *Zingiber officinale* has more scavenging power against ABTS cations. CellGevity® had the least scavenging activity against the free radicals, as observed in this study. The highest phenolic, flavonoid, Vitamin C, Vitamin B_1 and Vitamin B_2 quantified were found in *Curcuma longa* and *Zingiber officinale* compared to CellGevity®. **Conclusion:** The indigenous *Curcuma longa* and *Zingiber officinale* are natural sources of effective antioxidants with more scavenging power compared to a standard CellGevity® nutraceutical.

Key words: Oxygen-free radicals, Oxidative stress, Antioxidant, *Curcuma longa*, *Zingiber officinale*, Nutraceutical.

Key message: Dietary antioxidants would be obtained by incorporating the antiradical constituents from *Curcuma longa* and *Zingiber officinale* in the diet.

INTRODUCTION

The therapeutic and nutritional values of plants and plant produce for both the management and prevention of different ailments and food supplements have evolved. This is because oxidative stress leads to many pathological conditions and molecules with antioxidant properties would attenuate oxidative stress, thereby reducing the risks of health problems. This indicates that there is a link between nutrition and chemotherapy. In the African diet formula, plants and plant produce are essential component because they serve prophylactic purposes against ailments due to their large pool of antioxidants. *Curcuma longa* (Figure 1) and *Zingiber officinale* (Figure 2) are examples of the plant with prophylactic activity. *Curcuma longa* is widely grown and consumed in Nigeria. It is an herbaceous perennial plant of the ginger family *Zingiberaceae*,[1] popularly known as turmeric. *Curcuma longa* has served many purposes; in Nigeria cookeries, it is used as food ingredient to improve food texture because of the good flavor, it is also used to improve food appearance because of its yellowish color. It has been reported that in India, Asaia and China, it is used as spice and food preservative.[2] *Curcuma longa* exhibits numerous therapeutic actions including antioxidant activity,[3-5] anti-inflammatory activity,[3,6] anticarcinogenic and antimutagenic activity,[7,8] hypotensive and hypocholesteremic activities[9] among other therapeutic uses. *Zingiber officinale* is a sister plant of the *Zingiberaceae* family as *Curcuma longa*. It is commonly known as ginger and has been extensively consumed as food spice as well as medicinal agent in Indian, Asian and Arabic traditional medicine in the form of a fresh paste, dried powder, candy (crystallized ginger) or slices preserved in syrup.[10,11] It has been used tradomedically for the treatment of different ailments including neurological diseases, diabetes and diabetic complications,[12,13] analgesic and anti-inflammation.[11,12]

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Figure 1: *Curcuma longa* (Tumeric).

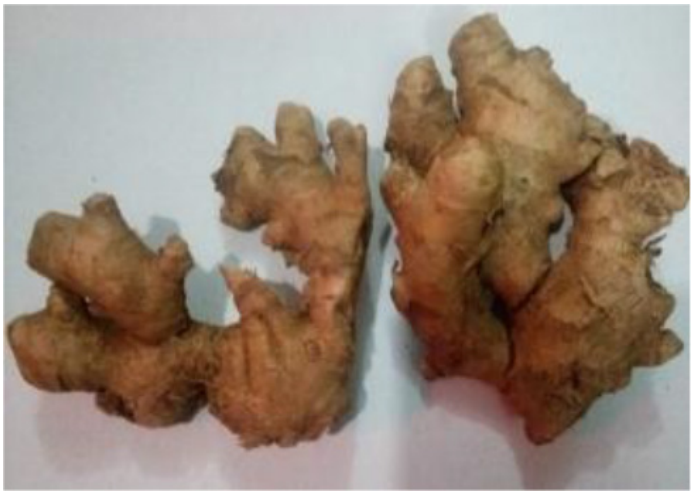

Figure 2: ***Zingiber officinale*** (Ginger).

The tendency of oxygen molecules to create free radicals is the crucial problem associated with the metabolic process, such as oxidation.[14] Oxidation, which is the biological process for energy production by the body,[15] increased exposure to environmental toxicants and dietary xenobiotics[16] are the complex biochemical reactions that result in the generation of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). These free radical by-products lead to oxidative stress by attacking vital organs; causing the cells to malfunction[15] and ultimately result in the development abnormal physiological conditions including mutagenesis, cellular ageing, coronary heart disease, diabetes.[17] Antioxidants are known to act protectively through one-electron reactions with free radicals,[16,18] thereby reducing the oxidative damage caused in the body, inhibiting the peroxidation of lipids and by retarding the progress of many diseases.[19] This highlights the need for a balance between free radical and antioxidant concentrations for proper physiological functions.[18]

Consideration the evolution of the application of these plants as effective therapeutic agents and as food supplement, there is need to reveal the phytochemical profile and antioxidant properties of Nigerian *Curcuma longa* and *Zingiber officinale* varieties. Available antioxidant studies on *Curcuma longa* and *Zingiber officinale* are majorly varieties from Asia, India and China.[2] Studies on Nigeria variety are virtually few.[20] It is important to comparatively study the phytochemical profile of *Curcuma longa* and *Zingiber officinale* from Nigeria since there is variation in the phytochemical constituents among the same plant cultivated in different geographical locations.[21] Therefore, we present the first report on the phytochemical profile and comparative free radical scavenging activities of n-hexane extracts of indigenous *Zingiber officinale* and *Curcuma longa*.

MATERIALS AND METHODS

Chemicals

The 2,2-diphyenyl-1-picrylhydrazyl (DPPH), 2,2'-Azinobis3-ethylbenzothialine-6-sulfonic acid (ABTS) and hydrogen peroxide (H_2O_2) were purchased from Sigma Aldrich USA. Nutraceutical (CellGevity®) was purchased from a distributing company; Max International® Nigeria. All other chemical and reagents used were of analytical grade and purchased from reputable chemical companies.

Preparation of Nutraceutical

Exactly 1 g of Cellgevity was weighed and dissolved in 100 mls of distilled water. This was placed on bench top shaker (MaxQ 4000 orbital shaker) for 1 h to obtain thorough mixture. Afterwards, the solution was kept in a refrigerator until needed.

Plant material and extraction

The plant materials were extracted following slight modification of the procedure described by Azmin.[22] The indigenous *Zingiber officinale* (ginger) and *Curcuma longa* (tumeric) were freshly harvested in January 2018 from Kachia and Jaba village farmlands respectively, both in Southern Kaduna, Kaduna State, Nigeria. They were identified at the Herbarium by Taxonomist in the Department of Botany, Ahmadu Bello University Zaria with voucher numbers deposited. They were washed with neat tap water and dried at room temperature 38°C for 21 days. The dried samples were pulverized using mortar and pestle and stored air-tight container until need. Using a weighing balance (Contech® Instruments Ltd., India. Model CAC-224), 100g each of the grinded *Zingiber officinale* and *Curcuma longa* powder were independently extracted by cool maceration with 250 ml n-hexane for 48 h using soxhlet extractor. About 4 drops of chloroform was added to ensure there was no fungi growth during the extraction duration of 48 h. The extracts were filtered using Whatman filter paper No.1 and concentrated by freeze drying using bench top freeze dryer (Labconco™) at 4°C for about 3 h. They were stored in the refrigerator until used. This served as the crude extracts.

Qualitative Screening of phytochemicals

The qualitative phytochemical screening for alkaloids, glycosides, steroids, flavonoids, tannins, saponin, terpenoid, phenol, were done following the standard analytical procedures for preliminary phytochemicals determinations described by Brain and Turner 1975[23] and Evans 1996.[24] For the qualitative determinations, all filtrations were done using 12.5cm Whatman filter paper. Determinations were in triplicates.

Test for Alkaloids

Extracts (0.2g) were individually mixed with 10ml 2% HCl and was heated for 5 min then filtered. 1 ml of filtrate was pipetted into a test tube and 1 ml of Wagner's reagent was added. Formation of brown or reddish-brown precipitate indicates the presence of alkaloids. The test was confirmed by treating the filtrate with Mayer's reagent (Mayer's test). Formation of a yellowish cream precipitate infers the presence of alkaloids.

Test for Glycosides

Glycoside presence was determined following the method by Hikino 1984.[25] Distilled water (20 ml) was added to 2g of each extract and then heated for 5 min on a water bath at 100°C and filtered using 12.5 cm Whatman filter paper. 0.2 mls Fehling's solutions A and B were thoroughly mixed with 5 ml of the filtrate until became alkaline as confirmed with litmus paper test. Brick-red color on heating indicated presence of glycoside. This was confirmed via a re-test using 15 ml of 1.0 M sulphuric acid. The quantity of precipitate obtained was the indices for inference when compared to the water test above. High precipitate content indicated the presence of glycoside while low precipitate content indicates absence of glycoside.

Test for Steroids

To 0.2g of each of the extracts, 2 ml acetic anhydride was added, followed by 2 ml of concentrated H_2SO_4. Color change from violet to either blue or green indicated presence of steroid.

Test for Flavonoids

Few drops lead acetate solution was added to 0.2g of each of the extracts. Yellow precipitate indicated the presence of flavonoid. Also, the extracts were treated with few drops of H_2SO_4, orange color formation indicated presence of flavonoid.

Test for Tannins (Ferric chloride method)

A 0.2g of each of the extracts reconstituted with distilled water was heated with 10ml of 45% ethanol on the water bath. The solution was filtered and the filtrate treated with 200µl of ferric chloride. An observation of brownish green precipitate indicates the presence of tannins.

Test for Saponins

To 0.2g of each of the extracts, 10ml distilled water was added, warmed for a minute on a water bath and then filtered. Then, 1ml of each filtrate was added 4 ml of distilled water, shaken thoroughly for 5 min and allowed to stand for 1 min. Formation of frothing of creamy bubbles indicated the presence of saponin.

Test for Terpenoids

To 0.2g of each of the extracts, 2 ml of chloroform and 3 ml of concentrated H_2SO_4 were mixed together. A change in colour from pink to violet showed the presence of terpenoids.

Test for Phenols

The 0.2g of each of extracts, few drops of ferric chloride solution was added; appearance of bluish-black indicated the presence of phenol. Also drops of lead acetate solution were added to the each of the extracts, appearance of yellowish color indicated the presence of phenol.

Quantitative phytochemical analysis

Determination of Total Flavonoid

Total flavonoid was estimated in triplicates following a standard method as described by Ejikeme 2014[26] and Boham and Kocipai 1994.[27] In a beaker of 250 mls, 2 g of each of the extracts were mixed separately with 50 mls of 80% aqueous methanol. The mixture was covered allowed to stand at room temperature, after 24 h, the supernatant was discarded and the residue re-extracted by dissolving in 50 mls of 80% aqueous methanol. Each of the sample extracts was filtered using Whatman filter paper number 42 (125 mm). The filtrate in a crucible was evaporated to dryness in a water bath, cooled in a desiccator and weighed. The percentage of flavonoid was estimated using a formula thus: Percentage (%) Flavonoid = Weight of flavonoid /Weight of sample × 100

Determination of Phenols

Initially, using a soxhlet apparatus, defatting was carried out by suspending 1 g of each of the extracts in 100 ml ether for 2 h before extraction of phenol. After defatting, the phenols was extracted weighing 0.50 g of the defatted sample into 50 ml of ether and boiled in a water bath at 100°C for 15 min. A mixture of 10 ml of distilled water, 2 ml of 0.1N ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added to 5 mls of the extract. The mixture was allowed to stand for 30 min until a change in colour was seen, before taking the optical density measurement at 505 nm. A phenol standard curve was prepared according to a standard method.[28] Exactly 0.2 g tannic acid was dissolved in distilled water and diluted to a volume of 200 mL to a final concentration of 1mg/mls. This served as the standard tannic acid solution. Different concentrations (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) of the tannic acid was prepared in a test tube by serial dilution, 2 mls of NH_3OH, 5 ml of amyl alcohol and 10 ml of distilled water were added to each of the test tubes and allowed for 30 min for colour development to be seen before taking the reading of the optical density at 505 nm.

Determination of Alkaloids

The total alkaloid was determined as described.[29] In a 250 ml flat bottom beaker, 200 mls of 10% acetic acid in ethanol and 2 g of each of the sample extracts were mixed together and allowed to stand at room temperature for 4 h. The mixture was partially concentrated in a water bath and filtered using Whatman filter paper number 42 (125 mm). Concentrated ammonium hydroxide was added in aliquots of about 20 drops to each extract to precipitate and allowed to sediment. The supernatant was discarded and the precipitates were washed with 20 mls of 0.1M of ammonium hydroxide and then filtered using Gem filter paper (12.5 cm). The residue was dried in an oven and weighed using a weighing balance (Contech® instruments Ltd India, model CAC-224). The alkaloid was estimated using a formula thus: Percentage (%) Alkaloid = Weight of alkaloid / Weight of sample × 100

Determination of Saponin

Saponin was quantitatively determined as described standardly.[26,27] In a 250 ml conical flask, 1 g of the extract and 100 ml of 20% aqueous ethanol were mixed together. The mixture was heated water bath at 55°C for 4 h with continuous stirring with a glass rod and filtered. The residue of the mixture was re-extracted with another 100 ml of 20% aqueous ethanol after filtration and heated in a water bath at 55°C for 4 h with constant stirring. The extract was concentrated to 30 mls in a water bath at 90°C. About 20 ml diethyl ether was added to the concentrate in a 250 mls separator funnel and vigorously agitated. The resulting aqueous layer was retained while the ether layer was discarded, then, 60 ml of n-butanol was added into the aqueous layer and extracted twice with 10 mls of 5% sodium chloride. After discarding the sodium chloride layer the remaining solution was heated in a water bath for 30 min. It was thereafter concentrated to dryness in a water bath and weighed. The total saponin was estimated thus:

$$\text{Percentage (\%) of Saponin} = \frac{\text{Weight of saponin}}{\text{Weight of sample}} \times 100$$

Determination of Tannin

Total tannin was determined according to the analytical method for quantitative determination of tannin as described.[30,31] In conical flasks containing 100 ml distilled water, 1 g of each of the extracts was added and heated gently for 1 h on an electric hot plate connected to power source. It was filtered into a 100 ml volumetric flask using Whatman filter paper number 42. For color development, 5.0 mls Folin-denis

reagent, 10 ml of saturated Na_2CO_3 solution, 50 ml of distilled water and 10 mls of diluted extract (aliquot volume) were mixed together in a 100 ml conical flask with thorough agitation and allowed to stand for 30 min in a water bath at a temperature of 25°C. The optical density was measured using UV-2450 spectrophotometer (Shimadzu, Japan) at 700 nm and compared on a standard tannic acid curve, prepared by dissolution of 0.20 g of tannic acid in distilled water and diluted to 200 ml. Varying concentrations (0.2–1.0mg/ml) of the standard tannic acid solution were pipetted into five different test tubes to which Folin-denis reagent (5 ml) and saturated Na_2CO_3 (10 ml) solution were added and made up to the 100 mls mark with distilled water. The solution was left to stand for 30 min in a water bath at 25°C. this served as standard tannic acid.[32] A graph of the absorbance (OD) and tannic concentration was obtained and tannic acid content determined thus:

$$\text{Tannic acid(mg)} = \frac{\text{C} \times \text{extract volume}}{(100\text{ g})\ \text{Aliquot volume} \times \text{weight of sample}} \times 100$$

C is concentration of tannic acid read off the graph.

Test for Ascorbic acid

Ascorbic acid (Vitamin C) was determined following standard method described.[33] About 0.5ml of each of the extracts was mixed with 1.5ml of 6% TCA and centrifuged for 10 min at 3000 rpm using Sorvall MTX 150 Bench Micro-Ultracentrifuge (Thermo Fisher Scientific®), after which 0.5 ml of the supernatant was mixed with 0.5 ml of Dinitrophenylhydrazine reagent (2% DNPH and 4% thiourea in 9N Sulphuric acid) and allowed to stand at room temperature for an additional 3 h then 2.5ml of 80% sulphuric acid was added and left undisturbed for 30 min. The absorbance was read using UV-spectrophotometer at 530nm. Optical densities of varying concentration (10-50 µg) of ascorbic acid standard were taken at 530 nm.

Test for Thiamine

Thiamine (Vitamin B_1) was determined following standard method as described.[34] Briefly 5g of the sample were homogenized with ethanolic sodium hydroxide (50ml). It was filtered into a 100ml flask and 10ml of the filtrate was pipetted. The colour developed by addition of 10ml of potassium dichromate and the absorbance was read using UV-spectrophotometer at 360nm.

Test for Riboflavin

Riboflavin (Vitamin B_2) was determined following standard method as described.[34] About 5g of the sample was extracted with 100ml of 50% ethanol solution and shaken for one h and filtered into a 100ml flask. Exactly 10ml of the extract was pipetted into 50ml volumetric flask followed by addition of 10ml of 5% potassium permanganate and 10ml 30% H_2O_2 and allowed to stand over a hot water bath for 30min. Thereafter, 2ml of 40% sodium sulphate was added. This was made up to 50ml mark. The absorbance was read using UV-spectrophotometer at 510nm.

Antioxidant assessment

The 2, 2-diphenyl-1-picryl hydrazil DPPH free radical scavenging assay

The free radical scavenging power of the extracts was ascertained using the DPPH assay, as described.[35] In a test tube, 1 ml of 0.3 Mm DPHH and 2.5 mL of the extract were mixed together and allowed to stand for 30 min at room temperature. The mixture was transferred to a cuvette and the optical density was read at 518 nm using UV-2450 spectrophotometer (Shimadzu, Japan). The control reaction was a mixture of all reagents except the test sample extract. The Antioxidant Activity (AA) was estimated thus:

$$\text{AA\%} = \frac{100 - (\text{Sample Absorbance} - \text{Absorbance empty Sample})}{\text{Control Absorbance}} \times 100$$

The result obtained was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in 1 g of dry sample thus:

$$\text{AEAC} = \frac{\text{IC}_{50\ \text{Ascorbic acid}}}{\text{IC}_{50\ \text{sample}}} \times 10^5$$

The 2,2′-Azinobis3-ethylbenzothialine-6-sulfonic acid (ABTS) radical cation decolorization Assay

The ABTS decolorization was assayed as described.[36] This assay was carried out in the dark room and at dark room temperature. In a test tube, 7 mM ABTS solution and 2.45 mM potassium persulfate was mixed and allowed to stand for 15 h. The solution was diluted with methanol to obtain absorbance of 0.7 ± 0.2 units at 734 nm. The extracts were separately dissolved in methanol to yield a concentration of 1 mg/mL. Exactly 200 µL of methanolic test solution of each sample was added to 2 mL of ABTS free radical cation solution. The solution was thoroughly mixed together by vortexing for 1 min. The absorbance of the solution was read using UV spectrophotometer at 734 nm. The result obtained was expressed as the ascorbic acid equivalent antioxidant capacity (AEAC) in 1 g of dry sample.

The Ferric Reducing Antioxidant Power Assay (FRAP)

The ability of the extracts to reduce Fe^{3+} to Fe^{2+} was determined using the Ferric Reducing Power Antioxidant (FRAP) assay by method of Benzie[37] as modified.[38] A working FRAP reagent was prepared by mixing 20 mL of 4 2,4,6-Tripyridyl-S-Triazine, 200 mL acetate buffer, 20 mL ferric chloride and 24 mL distilled water in a 10:1:1 ratio. The mixture was heated to 37°C in a water bath. Exactly 0.3 mL FRAP reagent was added to a cuvette and blank reading was then taken at 593 nm using UV-2450 spectrophotometer (Shimadzu, Japan). Then, a total of 100 µL of sample extract and 300 µL distilled water was then pipetted into a cuvette and measured at 593 nm using the spectrophotometer. The sample extract were then added to the prepared FRAP reagent and allowed to stand for 4 min before taking a sec reading at 593 nm. The change in absorbance after 4 min from the initial blank reading was then compared with the standard curve. Varying concentration (100 to 1000 µM) of known standard Fe^{2+} was prepared by serial dilutions. A standard curve was prepared by plotting the FRAP value of each standard against its concentration. The final result was expressed as the concentration of antioxidant having ferric reducing ability.

Hydrogen Peroxide Scavenging Activity

The ability of the extract to break down hydrogen peroxide to water and oxygen was determined according to the method described.[39] About 4mM of hydrogen peroxide was prepared in phosphate buffered saline of pH 7.4. Exactly, 4 ml of various concentrations (0.2-1.0mg/ml) of each extract was added to 0.6 ml of hydrogen peroxide. The absorbance was read after 10 min at 230nm using a UV-spectrophotometer against a blank solution containing sample without hydrogen peroxide. The percentage of hydrogen peroxide scavenging ability of the extracts was determined thus:

$$\%\ \text{hydrogen peroxide scavenged} = \frac{\text{A}_{\text{control}} - \text{A}_{\text{sample}}}{\text{A}_{\text{control}}} \times 100$$

Where, $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

Statistical analysis

The results are presented as mean ± standard deviation. The differences between the mean values of their anti-radical scavenging powers were compared with the mean of CellGevity nutraceutical antioxidant supplement using one-way ANOVA. A post hoc multiple test was used to compare the level of significance. *P*-value of > 0.05 was considered statistically not significant while *P*-value < 0.05 was considered statistically significant.

RESULTS

Result of phytochemical screening of n-hexane extracts of the indigenous *Zingiber officinale* and *Curcuma longa* is summarized in Table 1. *Zingiber officinale* showed positive for all the phytochemicals screened except steroid. Glycoside and steroids were the phytochemicals absent in *Curcuma longa.* For all the Tables, except Table 1, values are expressed as mean ± SD (n=3); values with different superscripted alphabets along a row are significantly different at P< 0.05.

Result of the quantitative determination of total flavonoids, phenols, alkaloids, saponins and tannins, as well as the determination of the Vitamin C, Vitamin B_1 (thiamine) and Vitamin B_2 (riboflavin) contents is presented in Table 2. Comparing the two plants to Cellgevity, it showed that there was significant difference (p<0.05) in the total flavonoids, phenol, alkaloids, saponins and tannins. There is also significant difference in the total antioxidant capacity (TAC), ascorbic acid and thiamine contents of *Zingiber officinale* and *Curcuma longa,* compared to CellGevity. There was no significant difference in the riboflavin content among *Zingiber officinale, Curcuma longa* and CellGevity.

From the results of the antioxidant activity of the extracts against DPPH radicals presented in Table 3, there was significant difference (p<0.05) in the scavenging power (inhibition) of the extracts against DPPH radicals, compared to Cellgevity. Overall, *Zingiber officinale* and *Curcuma longa* have more scavenging power that Cellgevity.

Result of the antioxidant scavenging activity using ABTS radical is presented in Table 4. *Zingiber officinale* has more scavenging power against ABTS radicals. There was significant difference (p<0.05) in the free radical scavenging activity of *Zingiber officinale, Curcuma longa* extracts against ABTS and when compared to CellGevity, with Cellgevity having the lesser scavenging power.

Result of the antioxidant activity of the extracts against H_2O_2 is summarized in Table 5. *Curcuma longa* exhibited more activity against H_2O_2. At 0.2 0.4 and 1 mg/ml, there was significant difference (p<0.05) in the scavenging ability of *Zingiber officinale, Curcuma longa* and CellGevity. At 0.6 and 0.8 mg/ml, there was no significant difference (p>0.05) between *Zingiber officinale* and *Curcuma longa*.

Table 1: Phytochemical Composition of n-Hexane extracts of *Zingiber Officinale* and *Curcuma longa*.

Phytochemicals	*Zingiber officinale*	*Curcuma longa*
Phenols	+++	+++
Flavonoids	+++	+++
Saponins	++	+++
Steroids	-	-
Tannins	+++	+++
Alkaloids	+++	+++
Terpenoids	++	+
Glycosides	+++	+

Heavily present: +++; slightly present: ++; present: +; absent: –

Table 2: Quantitative Phytochemicals, Total Antioxidant Capacity and Vitamin contents of n-haxane extracts of *Zingiber officinale* and *Curcuma longa*.

Phytochemical/vitamins	CellGevity	*Zingiber officinale*	*Curcuma longa*
Total Flavonoids (mg/ml)	0.03^{b}±0.00	0.15^{a}±0.00	0.14^{a}±0.00
Total Phenols (mg/ml)	0.73^{c}±0.01	0.97^{a}±0.01	0.88^{b}±0.02
Total Alkaloids (mg/ml)	0.67^{b}±0.43	0.77^{a}±0.23	0.81^{a}±0.04
Total Saponins (mg/ml)	0.59^{b}±0.41	0.83^{a}±0.13	0.79^{a}±0.08
Total Tannins (mg/ml)	0.61^{b}±0.06	0.68^{a}±0.51	0.71^{a}±0.09
Total antioxidant capacity	0.76^{c}±0.61	0.92^{a}±0.02	0.84^{b}±0.42
Vitamin C (mg/ml)	118.81^{b}±8.04	158.47^{a}±2.26	159.52^{a}±4.76
Vitamin B_1 (mg/dL)	28.15^{b}± 0.33	34.62^{a}±1.93	37.39^{a}±0.65
Vitamin B_2 (mg/dL)	0.16^{a}±0.00	0.17^{a}±0.00	0.18^{a}±0.00

Table 3: DPPH radicals scavenging activity of the n-hexane extracts of *Zingiber officinale* and *Curcuma longa*.

Sample Conc. (mg/ml)	CellGevity	*Zingiber officinale*	*Curcuma longa*
0.2	37.46^{c}±0.21	57.23^{b}±0.01	68.77^{a}±0.41
0.4	41.90^{c}±0.11	65.47^{b}±0.12	68.64^{a}±0.21
0.6	45.20^{b}±0.01	69.65^{a}±0.01	73.25^{a}±0.14
0.8	51.62^{b}±0.06	73.86^{a}±0.51	74.82^{a}±0.51
1.0	55.15^{b}±0.32	73.94^{a}±0.02	74.39^{a}±0.09

Table 4: The 2,2′-Azinobis3-ethylbenzothiazoline-6-Sulfonic Acid (ABTS) radical Scavenging activity.

Sample conc.(mg/ml)	CellGevity	*Zingiber officinale*	*Curcuma longa*
0.2	74.17^{c}±0.41	89.23^{b}±0.53	80.05^{a}±0.16
0.4	74.36^{c}±0.22	92.39^{b}±0.21	87.33^{a}±0.07
0.6	75.46^{c}±0.31	90.76^{b}±0.17	85.09^{a}±0.40
0.8	74.28^{b}±0.11	86.62^{a}±0.26	85.62^{a}±0.61
1.0	72.10^{b}±0.34	84.55^{a}±0.01	84.61^{a}±0.28

Table 5: The H_2O_2 Radical Scavenging Activity of n-Hexane extracts of *Zingiber officinale*, *Curcuma longa* and Cell Gevity.

Sample conc. (mg/ml)	CellGevity	*Zingiber officinale*	*Curcuma longa*
0.2	56.17^{c}±0.51	69.12^{b}±0.01	76.35^{a}±0.71
0.4	55.36^{c}±0.01	67.24^{b}±0.42	73.33^{a}±0.32
0.6	55.35^{b}±0.22	68.33^{a}±0.41	72.05^{a}±0.63
0.8	53.62^{b}±0.02	66.86^{a}±0.53	70.19^{a}±0.53
1.0	50.03^{c}±0.61	64.51^{b}±0.01	69.77^{a}±0.16

DISCUSSION

The experiment described in this study was designed to profile the phytochemical constituents n-hexane extracts of two indigenous antioxidant-reach plants; *Zingiber officinale* and *Curcuma longa*, more so,

to determine their anti-radical scavenging capacities, in comparison to CellGevity; a known nutraceutical antioxidant supplement. The phytochemical profile of the plants showed the presence of tannins, flavonoids, phenols, alkaloids, saponins and terpenoids. Steroids was absent in both extracts while glycoside was absent only in *Curcuma longa*, as presented in Table 1. Tannin is one of the phytochemicals that is heavily present in the extracts of *Zingiber officinale* and *Curcuma longa*. Tannin is an active ingredient in plant based medicine.[40] Several studies have reported antioxidant activities of plant tannins;[41-43] it is included in beverages to serves as antioxidants.[44] It has been established that tannin exhibits its antioxidant role by donating either hydrogen atom or electron, suggesting that tannin do not function solely as primary antioxidant but also as secondary antioxidant.[45] Tannin exacts its antioxidant mechanism by chelating metal ions such as Fe (II), Zn (II) and Cu (II) and more so, interfering with the reaction steps in the Fenton reaction, thereby retarding oxidation.[46] Other phytochemical constituents found in the extracts of *Zingiber officinale* and *Curcuma longa* include flavonoids which is known to possess antioxidant properties[47,48] as a result of their ability to inhibit and scavenge free radicals. Phenols are antioxidants in human and plants.[49] Phenols exhibits their antioxidant activity by reducing the rates of oxidation through the mechanism of transferring hydrogen atom from their OH groups to the chain-carrying $ROO^{\bullet}$ radicals.[50] Saponin also possesses antioxidant activity. It has been established that some plant saponins have powerful antioxidant activity; therefore, are potential and novel antioxidant candidates.[51,52] The antioxidant potential of alkaloids has been reported. The biological activity; mainly antioxidant effects of many plants used in folk medicine are attributed to the presence of alkaloids.[53-55] As a result of their antioxidant activity, terpenoids have been shown to be protective against oxidative stress conditions leading to different diseases such as liver, renal, neurodegenerative and cardiovascular diseases, cancer, diabetes as well as in ageing processes.[56,57] Similarly, the antioxidant potential of glycosides has been reported. The antioxidant of Quercetin monoglycosides and diglycoside; flavonol glycosides as potent inhibitors of lipid peroxidation have been reported.[58]

Quantitatively, appreciable quantities of the phytochemicals were obtained as shown in Table 2. From our findings, significant difference ($p<0.05$) was observed in the total antioxidant capacity (TAC), with *Zingiber officinale* possessing the highest TAC of 0.92±0.02, followed by *Curcuma longa* 0.84±0.42, while Cellgevity had the least TAC of 0.76±0.61. This is suggesting that *Zingiber officinale* would possess more anti radical scavenging power than *Curcuma longa* and Cellgevity. The higher TAC associated with *Zingiber officinale* could be attributed to more abundant glycoside it possesses compared to *Curcuma longa* as shown in Table 1. Results obtained from evaluating the free radical scavenging activity of the extracts using DPPH showed that increase in the concentration of the extracts had no significant effect in the activity of the extracts (Table 3) and Cellgevity, the known antioxidant. This is suggesting that the extracts are concentration-bound, which implies that at low concentration, the extract would be effective enough to mop up a given radical. Similar trend was observed for free radical scavenging activity of the extracts using ABTS radical (Table 4). Based on the results obtained, *Zingiber officinale* and *Curcuma longa* possesses more scavenging power more than the nutraceutical. The antioxidant activity of the extracts against hydrogen peroxide (H_2O_2) as presented in Table 5 revealed scavenging activity was a peak at a low extract concentration between 0.2 mg/ml and 0.6 mg/ml. This followed the same trend as in DPPH where it was shown that the extracts exhibited excellent scavenging ability at low extract concentrations. It shows that the extracts are efficacious at low concentrations.

In this study, the secondary phenolics from the indigenous *Zingiber officinale* and *Curcuma longa* exhibited more antioxidant and chelating capacity than nutraceutical; a known antioxidant supplement. This suggests that incorporation of these phenolics in the diets would mop up free radicals produced during the body's oxidation process, which in turn results to other health benefits such as reducing the risk of developing cancer, cardiovascular and other diseases. In fact, it has been reported that tannin forms a complex with protein (tannin-protein complex) which provided persistent antioxidant activity.[45,59]

The antiradical activities observed in this *in vitro* study as a result of the antioxidation properties of the phenolic compounds could convincingly inhibit lipid peroxidation, improve the activity of antioxidant enzymes and attenuate oxidative stress *in vivo*.

CONCLUSION

Free radical scavenging activities of n-haxane extracts of indigenous *Curcuma longa* and *Zingiber officinale* was investigated and their phytochemical profile was qualitatively and quantitatively determined. One of the effective strategies for preventing oxidative damage caused by reactive oxygen species is the use of molecules with antioxidant properties. Based on the results of this study, *Zingiber officinale* and *Curcuma longa* are good reservoirs for such molecules. These molecules could act as direct antioxidants through free radical scavenging mechanisms and/or as indirect antioxidants through the induction of enzymes system responsible for antioxidant activity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay; **ABTS:** 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid; **H_2O_2:** Hydrogen peroxide; **FRAP:** Ferric reducing antioxidant power.

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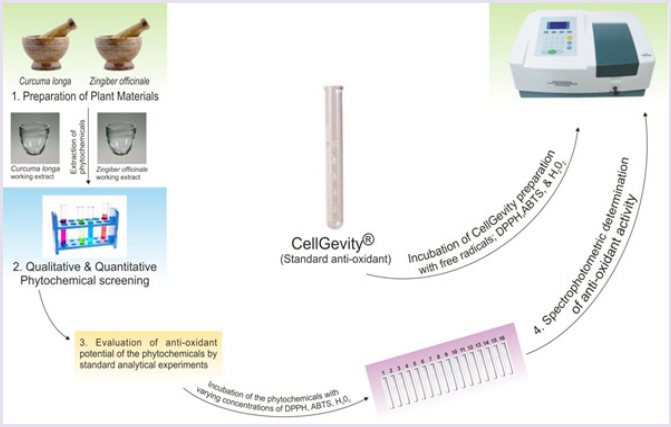
GRAPHICAL ABSTRACT



SUMMARY

- *Curcuma longa* and *Zingiber officinale* are Nigeria's native flora that could be beneficial to the pharmaceutical and food industries, following their reach antioxidant properties.

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