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Comparative Study of Polyphenol Profile after Stimulated Post-intestinal Digestion of Raw and Dried Ginger Leaves

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Abstract

The present study aimed to determine bioaccessibility (BA) of polyphenols (PPs) and flavonoids (FLs) compounds of Ginger (*Zingiber officinale*) leaves extract after simulated gastrointestinal digestion (GID). One hundred grams of *Z. officinale* leaves (raw and dried) was extracted with 200 mL of distilled water. Determination of PPs and BA index were carried on the extract before digestion (BD) (undigested), post-intestinal digestion without enzymes (PI-DWOEs) and post-intestinal digestion with enzymes (PI-DWEs) using standard methods. The results showed significant decrease in proanthocyanidins (PCs) and anthocyanins (ACs) of dried and *raw Z. officinale* in PI-DWEs when compared to PCs and ACs level in BD. A total of nine major PPs compounds were detected in raw *Z. officinale* leaves in BD and PI-DWOEs, whereas eight PPs were detected in PI-DWEs. In PI-DWOEs, sinapinic acid (6.68 ng/mL) was the most prominent whereas p-coumaric (7.74 ng/mL) was the most protuberant in PI- DWEs. The concentration of total PPs were as follows; BD (41.39 ng/mL) > PI-DWEs (13.6 ng/mL) > PI-DWOEs (19.20 ng/mL). In conclusion, after the in vitro GID of the extract, a good BA index was observed, of which some PPs and FLs contents were near to 100%. However, the reason most PPs and FLs in the extract cannot be available or absorbed intact after digestion could be due to the fact that they undergo a series of reactions with intestinal enzymes. Additionally, not all polyphenols are absorbed equally well. Before it is feasible to predict how PPs will be assimilated by cells, they must first be bioaccessible.

Keywords: Bioaccessibility, Digestion, Enzymes, Flavonoids, Ginger leaves, Polyphenols

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1.0 Introduction

Phenolic compounds are essential components of the human diet since they are present in almost all plant foods (Andriamadio et al., 2015; Otuaga et al., 2020). Phenols are typical chemicals found in plant foods (George and Okpoghono, 2017). Foods supplements include small levels of extra nutritional components are known as nutrition bioactive chemicals (Swallah et al., 2020). They are being evaluated in order to determine how they affect people's comfort. Many bioactive substances have been discovered that exhibit significant changes in chemical structure and activity (Swallah et al., 2020). The beneficial roles that specific dietary components have close link between dietary intake and wellbeing such as decreasing the frequency of persistent maladies like diabetes, cancer and malaria have been the focused of many researchers (Ekakitie et al., 2021; Kadiri and Irene, 2022; Okpoghono et al., 2018; Onyesom et al., 2019).

In-depth knowledge of the effectiveness of gastrointestinal digestion (GID), absorption, and metabolism of bioactive chemicals, studies on the bioaccessibility (BA) and bioavailability (BAV) of phenolic compounds is now of interest to many scientists. The structural changes, digestibility and release of food components under simulated GID in *in vitro* models are usually based on human physiology (Kara et al. 2017). According to Williamson and Clifford (2017) and Ugwu et al. (2021), BA can also be defined as the portion of bioactive compounds that are successfully absorbed into the bloodstream for metabolic use. Bioavailability is the portion of bioactive compounds that are released from the food matrix after digestion and become available for intestinal absorption (Gleichenhagen and Schieber, 2016; Martins et al., 2016).

Worldwide, *Zingiber officinale* (ginger) is extensively used as a spice in flavouring (Okpoghono et al. 2023). It belongs to the family *Zingiberaceae* and the genus Zingiber. The presence of bioactive chemicals gives ginger rhizome its pungent flavour (Wang et al., 2017). Raw ginger leaves are frequently chopped or sliced and added uncooked to salads. Also, ginger leaves can be used in the preparation of maize-plantain pudding delicacy (Okpoghono et al., 2023). Ginger contains powerful antioxidants which may give electrons to other molecules to neutralize free radicals. Polyphenols have been investigated in relation to a number of diseases because of their antioxidant ability (Lecour and Lamont, 2011). About 90–95% of dietary PPs are thought to transit directly from the small intestine to the colon without being absorbed. Non-absorbed PPs metabolites are excreted in the feces or return to the colon via enterohepatic circulation, whereas those that are catabolized by the microbiota in the colon can be absorbed into the enterocytes through passive diffusion and then reach the liver to be further metabolized by phase II enzymes.

Additionally, the bioavailability and metabolization of PPs in the body determines their impact on health. In order to assess the effects of digestion and the bio-accessibility of the PPs, a requirement for further physiological processes in the body, an *in vitro* study was carried out. The *in vitro* models are reliable instruments for evaluating the phenolic profile alterations before absorption. These are more reliable, morally neutral, less expensive, and quicker than *in vivo* models in simulating the digestive process (Kamiloglu et al., 2017). Due to their effectiveness in forecasting the outcomes of *in vivo* digestion studies, these models are now increasingly being used by scientists (Bohn et al., 2018). A promising environmentally friendly extraction method for extracting polyphenols from plant matrix is hot water extraction of polyphenols from plant material. In comparison to conventional extraction techniques, using hot water as the solvent has a number of benefits. Water is nonflammable, nontoxic, easily accessible, and environmentally benign, in contrast to routinely used solvents. By raising the temperature, which improves the polyphenols' water solubility and the diffusion process, it can significantly improve its solvating capabilities (Plaza et al., 2013). These factors led us to conduct bioaccessibility and *in vitro* digestion tests of the PPs compounds that were extracted from ginger leaves using the aqueous extract method. In addition, this study compared the BA index of PPs and FL*s* compounds of raw and dried *Z. officinale* leaves aqueous extract after stimulated *in-vitro* gastrointestinal digestion.

2.0 Materials and Methods

Plant materials

The *Z. officinale* leaves used in this study was harvested in Umuosele, Amai, Delta State and it was identified in Department of Plant Biology and Biotechnology, University of Benin with voucher number of UBH dt-174 deposited in their herbarium.

Extraction of Z. officinale

Extraction of raw sample (sample A) was done by homogenising raw sliced *Z. officinale* leaves (100 g) in 200 mL of distilled water (50°C) and then filtered (grade 1 filter paper). The concentration of sample was obtained by subtracting residue from 100 g of fresh of fresh sliced *Z. officinale* leaves. The dried sample (sample B) extract was obtained by dissolving hot air dried (70°C) *Z. officinale* leaves (100 g) in water (200 mL, 50°C) for 24 hours and then filtered (grade 1 filter paper). The water extracts were evaporated under reduced temperature of 37°C using water bath until no moisture was visible. Exactly 10 g of the dried extract was reconstituted in 30 mL distilled water. The concentration of extracts was calculated to 0.3 g/mL.

In vitro GID stimulation

The *in vitro* GID stimulation of the extracts was performed as described by Tagliazucchi et al. (2016). Briefly, 2 mL of *Z. officinale*

extract was brought to pH 2.5 with 40 mM HCl. After addition of 5 mL of manufactured gastric liquid (300 U/mL pepsin and 2 g/L NaCl), the blend was brooded in a water bath for 2 hours at 37 °C. From that point, the pH was brought to 7.5 with 0.1 M $NaHCO_3$ and 4 mL of synthetic intestinal liquid (0.8 g/L pancreatin and 5 g/L bile extract) was included. The blend was brooded in a bath for 2 hrs at 37 °C. The finishing of the enzymatic response in each stage occurred by cooling the test for 10 mins in ice bath. After intestinal stage, 1 mL aliquot was pulled out from the mixture, then100 mL of methanol was added and centrifuged at 10,000 RPM for 10 mins at 4 °C. The supernatant was acidified to pH 2.5 to safeguard the stability of the phenolic compounds contemporary in the mixture and kept at -20 °C for further analysis. DWOEs were done to distinguish the result of the chemical environment from that of the digestive enzymes. This process was also performed using quercetin standards for assessment. Sample treatment was as follows: Before digestion (BD), Post-intestinal digestion with enzymes (PI-DWEs) and Post-intestinal digestion without enzymes (PI-DWOEs). BA index was determined for better empathetic relationship between phenolic compounds and their BA in organic systems. Polyphenol compounds bioaccessibility (PCB) which represents the portion of bioaccessible phenolic compounds from *Z. officinale* extract was calculated using equation 1:

PCB = PCx/PCy×100 ----------1

Where PCy was the concentration of polyphenols originally contained in *Z. officinale* aqueous extract and PCx was the concentration of polyphenols after in vitro digestion.

The percentage of flavonoids bioaccessibility (FB)in the extract of *Z. officinale* were estimated using equation 2:

FB = Fa/Fb×100 ----------2

Where Fb is the concentration of FLs initially present in Z. officinale aqueous extract and Fa is the concentration of phenolic compounds following in vitro digestion.

Analyses of polyphenols and flavonoids

Biochemical analyses of polyphenols (PC, AC and TPC) in the samples (*in-vitro*) were determined. The PCs was estimated according to the method of Sun et al. (1998). The test tube was filled with 0.5 mL of sample, 3 mL of 4% vanillin solution in methanol, and 1.5 mL of Conc. HCl. After 15 minutes of standing time, the mixture was tested for absorbance at 500 nm using methanol as a reference. The calibration curve was generated using a catechin solution. The PCs was expressed in mg (+)-catechin/g.

Determination of AC was based on the strategy depicted by Iland et al. (1996). Sample (200 µL) was mixed with 3.8 mL of 1 M HCl, and it was then let to sit for 3 hours at room temperature. Using 1 M HCl as the reference solution, the absorbance (X) of the acidified, diluted extract was measured at 520 nm. Following the absorbance (P) of a 1% w/v solution of malvidin-3-glucoside, ACs were estimated as mg/mL of extract solution as shown in equation 3:

Anthocyanins (mg/mL) = X x 1000/ Dilution factor× 1000/ P ---------3

The determination of TPC was carried out according to the method described by Dewanto et al. (2002). A sample volume of five millilitres (0.5 ml) was dissolved in 100 µL of Folin-Ciocalteau reagent and six millilitres of distilled water. After 1 minute of vortexing, 2 mL of 15% Na_2CO_3 was added, and the mixture was vortexed once more for 30 seconds. The solution was diluted up to 10 mL with distilled water. Absorbance was read using spectrophotometer at 750 nm after one hour and thirty minutes. Calibration curve was created using a solution of gallic acid. TPC of samples were expressed as milligrams of gallic acid equivalent (mg GAE)/100 g of dry weight.

Quantitative determination of flavanones was accomplished using a spectrophotometric method of 2,4-Dinitrophenylhidrazin (DNP) proposed by Nagy and Grancai (1996). Sample (40 µL) was dissolved in 80 µL of DNP solution (For 5 mL: 50 mg of DNP in 100 µL of 96% sulphuric acid and 4850µL of methanol), mixed, and incubated at 50 °C for 50 min in a water bath before being cooled to room temperature and having 280µL of 10% potassium hydroxide (KOH) in water added. The absorbance was finally measured at 486 nm. A calibration curve based on pinocembrin (0.5-5.0 mg/mL) 0.5, 1, 2, 3,4 and 5 mg/mL with absorbance of 0.123, 0.243, 0.323, 0.477, 0.524 and 0.832 correspondingly was used to measure the concentration of flavanones that was expressed as µg of pinocembrin equivalent per milligram of sample.

Flavanonols (Dihydroquercetin: Taxifolin) and Flavones (Luteolin) were assayed by adding 0.1 mL of sample, 0.3 mL of 5% $NaNO_2$ aqueous solution and 5 mL of water in test tube. After 5 minutes, 1.5 mL of 1M NaOH was added, followed by 5 µL of acetic acid and 0.2 mL of 10% $AlCl_3$. The UV spectrum was calibrated between 300 and 600 nm on a white background. Taxifolin and Luteolin was calculated using equation 4:

X (mg/mL) = (10×V×A)/(p × E) ----------4

Where, X is the total content of taxifolin or luteolin. V = 8 mL, A– absorption at 517 nm for luteolin, and 507 nm for taxifolin, p– sample volume (mL). E= taxifolin specific absorbance = 134.5 at 507 nm; or luteolin specific absorbance = 63.9 at 517 nm.

The method described by Ebrahimzadeh et al. (2008) was used to determine the TFC. The sample was combined with five millilitres (5 mL) of 2% aluminium (III) chloride (AlCl3) in methanol. After 10 minutes, the absorption was measured at 415 nm in comparison to a blank sample made up of a 5 mL sample solution and 5 mL of methanol without $AlCl_3$. Rutin (0-100 mg/l) served as the standard for the standard curve used to determine the total flavonoid content.

Raw *Z. officinale* leaves aqueous extract, which had the highest TPC and TFC, was subjected to HPLC-DAD analysis. The cornerstone of HPLC and the chromatographic process in general is the distribution of the sample between a mobile phase and stationary phase, which

allows for the separation of various components in a sample. This separation was accomplished through a number of equilibrium stages where the components of the injected sample interact by partitioning or adhering to one another as they pass through the system. According to the substances examined, the photo diode array UV detector was used to identify the HPLC chromatograms at three different wavelengths: 272, 280, and 310 nm. Based on their retention time and by spiking with the standards under the same circumstances, the compounds were identified. Evaluation of the integrated peak area served as the basis for sample quantification. Total polyphenol profile standard concentration was 20.80 ng/mL while the standard peak area was 4486.1575 ng/mL. Individual polyphenol profile standard concentration was 1.847 ng/mL with standard peak area of 494.9361 ng/mL.

Statistical analysis

All the results were expressed in simple percentage, mean bars and mean ±SD. All data were analysed using Analysis of variance (ANOVA). Significant difference between means were determined at $P < 0.05$ confidence level using post-hoc: least significant difference (LSD).

Results and discussion

In vitro gastrointestinal digestion (GID) is a broadly used method to determine the BA of consumed bioactive compounds. In the present study, BA of phenolic compounds in the dried and raw *Z. officinale* leaves aqueous extract was subjected to an *in vitro* stimulated model assay to mimic the digestion in human. The changes that occur in PCs, ACs, FLs (flavonone, luteolin and taxifolin), TPC and TFC of dried and raw *Z. officinale* leaves aqueous extract after stimulated GID (*in-vitro*) at different digestion stages are presented in Table 1, 2 and 3. The results revealed significant decrease in PCs and ACs of the dried and raw *Z. officinale* in PI-DWEs when compared to before digestion (BD). However, BA of PCs, ACs, flavonone, luteolin and taxifolin were lower in PI-DWEs when compared to PI- DWOEs.

There were significant decrease in the values of TPC and TFC of dried and raw *Z. officinale* leaves aqueous extract in PI-DWEs when compared to the values obtained in BD. However, TPC and TFC had lower BA percentage in PI-DWEs in relative to PI-DWOEs. Interestingly, TPC and TFC raw *Z. officinale* leaves BA percentage were higher compared to that of dried *Z. officinale* leaves extract. In raw plant materials, drying generally led to a loss of naturally existing antioxidants. The majority of natural antioxidants may be lost as a result of intense and/or extended heat treatment (Rababah et al., 2015). According to Lim and Murtijaya's (2007) research, oven drying resulted in a significant reduction in the antioxidant activity and TPC of *Phyllanthus amarus*, which accelerated the enzymatic oxidation of the plant's natural antioxidants. Torti et al. (1995) reported that homogenizing with a homogenizer proved to be more efficient and dependable for removing phenolics compounds from both tender and tough fresh leaves. Temperatures between 20 and 50 °C are commonly used for traditional polyphenol extraction and concentration in a water bath; temperatures above 70 °C promote fast polyphenol destruction (Rababah et al., 2015). Intense thermal processes at high temperatures have the potential to significantly reduce the amount of antioxidants found in plants naturally, as well as to inactivate enzymes and degrade phytochemicals (Rababah et al., 2015). The dried *Z. officinale* leaves extract TPC was reduced due to the unpredictable nature of small molecules created during digestion as a result of the hydrolysis of large molecules, but for the raw *Z. officinale* leaves extract, there was a negligible increase in TPC. This increase may be attributed to the release of bound phenolics into the digestive juice caused by the activity of digestible enzymes on the matrix. Due to the short contact period with the enzyme, this finding suggested that both phenolic acids and FLs were less effectively released after a chemical reaction. Regarding the fluctuation of phenolic acids and FLs in the extract following simulated gastrointestinal transit, the results were in consistent with those of Chait et al. (2020). The decrease in phenolic compound concentration following stomach digestion may be brought on by the compounds' sensitivity to high pH, a proteolytic enzyme, or complex formation with other components (Chait et al., 2020). Following intestinal digestion, TPC (83.40%) had a higher percentage of BA than TFC (41.60%). As a result, it is possible to hypothesize that PPs may interact with dietary components to modify their molecular weight, solubility, and undergo other chemical changes that affect their availability. The complex and porous structure of dietary components traps the PPs and transports them to the colon, where they enhance the levels of phenolic acids (Gleichenhagen and Schieber, 2016; Kara et al., 2017). According to Gleichenhagen and Schieber (2016), bile salts' action and the pH of the intestinal environment can modify chemical structures, resulting in new molecules with varied biological functions.

Tables 4 depict the PP profile of raw Z. officinale leaves aqueous extract BD, PI-DWEs and PI-DWOEs. Nine major PPs compounds; p-coumaric acid, o-salicyclic acid, protocatechuic acid, caffeic acid, p-anisic acid, vanillic acid, gallic acid, ferulic acid, and sinapinic acid were found in the extract BD and PI-DWOEs, whereas eight PPs compounds (p-coumaric acid, o-salicyclic acid, protocatechuic acid, caffeic acid, p-anisic acid, vanillic acid, gallic acid and sinapinic acid) were detected in the PI- DWEs. However, sinapinic acid was the most prominent PPs found before the stimulated digestion and PI-DWOEs with the value 18.88 ng/mL and 6.68 ng/mL respectively. Nevertheless, p-coumaric (7.74 ng/mL) was the most prominent in PI-DWEs. Through the ability to scavenge free radicals, sinapinic acid and p-coumaric acid has been identified to fight against tumour, stomach, colon, and cardiac illnesses (Lou et al., 2012).

Table 1. Bioaccessibility of polyphenols (Proanthocyanidins and Anthocyanins) of dried and raw *Z. officinale* leaves aqueous extract after simulated gastrointestinal digestion (*in-vitro*)

Group		Proanthocyanidins (Units/g sample)	PCB (%)	Anthocyanins Units/g sample)	PCB (%)
Dried *Z. officinale* leaves aqueous extract	BD	10.47 ± 2.35 [a]		0.92 ± 0.19 [a]	
	PI-DWEs	4.42 ± 1.96 [b]	42.21	0.23 ± 0.05 [a]	25.00
	PI-DWOEs	7.44 ± 1.98 [a, b]	71.06	0.50 ± 0.01 [a]	54.35
Raw *Z. officinale* leaves aqueous extract	BD	18.22 ± 2.44 [c]		1.41 ± 0.01 [a]	
	PI-DWEs	8.54 ± 1.70 [a]	46.87	0.51 ± 0.15 [a]	36.17
	PI-DWOEs	10.48 ± 2.70 [a]	57.52	0.81± 0.10 [a]	57.44
Standard	BD	25.12 ± 5.17 [d]		8.17 ± 2.01 [b]	
	PI-DWEs	18.25 ± 2.08 [c, d]	72.65	4.40 ± 1.01 [c]	53.85
	PI-DWOEs	20.23 ± 5.94 [d]	80.53	4.97 ± 0.11 [c]	60.80

Triplicate values are given in mean ±SD. Mean values in the same column with different letter differ p< 0.05

Table 2. Bioaccessibility of flavonoids (flavonone, luteolin and taxifolin) of dried and raw *Z. officinale* leaves aqueous extract after simulated gastrointestinal digestion (*in-vitro*)

Group	Flavonone (Units/g sample)	FB (%)	Luteolin (Units/g sample)	FB (%)	Taxifolin (Units/g sample)	FB (%)
Dried *Z. officinale* leavesaqueous extract						
BD	10.50 ± 2.95 [a]		1.01± 0.52 [a]		4.53 ± 1.17 [a]	
PI-DWEs	4.20 ± 0.06 [b]	40.00	0.48 ±0.32 [a]	47.52	0.85 ± 0.43 [b]	18.76
PI-DWOEs	8.25 ± 1.20 [a]	78.57	0.85 ± 0.42 [a]	84.15	2.16 ± 0.28 [c]	47.68
Raw *Z. officinale* leaves aqueous extract						
BD	16.27 ± 2.15 [c]		8.16 ± 5.20 [b]		9.53 ± 0.57 [d]	
PI-DWEs	8.34 ± 1.22 [a]	51.25	3.33 ± 2.75 [a]	40.80	4.85 ± 0.45 [a]	50.89
PI-DWOEs	10.17 ± 3.20 [a]	62.50	5.16 ± 3.25 [a,b]	63.23	6.53 ± 1.10 [a,d]	68.52
Standard						
BD	22.26 ± 2.15 [d]		14.16 ± 5.20 [b,c]		12.33 ± 0.57 [e]	
PI-DWEs	17.33 ± 3.25 [c]	77.85	10.33 ± 2.75 [b]	72.95	6.85 ± 0.65 [e,d]	55.56
PI-DWOEs	15.87 ± 3.25 [c]	71.29	11.16 ± 3.25 [b]	78.81	6.33 ± 1.05 [a,d]	51.33

Triplicate values are given in mean ± SD. Mean values in the same column with different letter differ at p < 0.05

Additionally, it has been suggested that p-coumaric acid may help prevent stomach cancer by averting the production of cancerous nitrosamines. P-coumaric acid has been shown to possess both chemoprotective and antioxidant properties (Lou et al., 2012). Total PP concentrations of the raw *Z. officinale* leaves were as follows: BD 41.39 ng/mL > PI-DWOEs 19.24 ng/mL > PI-DWEs 13.60 ng/mL. The decrease in raw *Z. officinale* leaves aqueous extract total PP of PI-DWEs might be due to sequence of responses of the PPs with the digestive enzymes which might have led to PPs forming complex with the digestive enzymes.

Table 3. Total phenol and total flavonoids of dried and raw Z. *officinale* leaves aqueous extract after simulated gastrointestinal digestion (*in-vitro*)

Group	Total phenol content (TPC) (mg GAE/g sample)	TPB (%)	Total flavonoids content (TFC) (mg Rutin/g sample)	TFB (%)
Dried *Z. officinale* leaves aqueous extract				
BD	70.45 ± 1.36 [a]		42.50 ± 0.39 [a]	
PI-DWEs	60.42 ± 3.96 [b]	85.76	35.20 ± 0.15 [c]	82.82
PI-DWOEs	65.44 ± 8.96 [a,b]	92.88	38.00 ± 0.30 [c]	89.41
Raw *Z. officinale* leaves aqueous extract				
BD	78.21 3.95 [c]		47.05± 0.50 [a]	
PI-DWEs	66.83 ± 9.90 [b]	85.44	36.50 ± 0.55 [c]	77.58
PI-DWOEs	71.44 ± 11.73 [a]	91.34	41.50± 0.10 [a]	88.20

Table 4. Polyphenol profile of raw *Z. officinale* leaves aqueous extract

Polyphenol compounds	**BD Conc. (ng/mL)**	**PI-DWOEs Conc. (ng/mL)**	**PI-DWEs Conc. (ng/mL)**
p-coumaric acid	11.03	10.08	7.70
O-Salicyclic Acid	1.09	0.49	0.06
Protocatechuic Acid	1.09	0.26	0.06
Caffeic Acid	1.19	0.63	0.41
p-Anisic Acid	0.82	0.56	0.03
Vanillic Acid	1.00	0.03	0.36
Gallic Acid	1.76	0.19	0.09
Ferulic Acid	4.53	0.32	ND
Sinapinic Acid	18.88	6.68	4.79
Total	41.39	19.24	13.60

Before digestion (BD), post-intestinal digestion without enzymes (PI-DWOEs), post-intestinal digestion with enzymes (PI-DWEs)

Conclusion

In conclusion, after the *in vitro* gastrointestinal digestion of the extract, a good BA index of PPs was observed, some of which levels were near to 100%. However, comparisons of PPs properties revealed that digestion appeared to be an important factor in maintaining and increasing this property. The raw *Z. officinale* leaves aqueous extract had significantly higher PPs and FLs in comparison to dried *Z. officinale* leaves aqueous extract. In the PI-DWEs, decrease in PPs and FLs bioaccessibility were observed in comparison to PI-DWOEs. However, the fact that the majority of PPs and FLs in the extract go through a series of interactions with the digestive enzymes may be the reason why they cannot be available or absorbed intact after digestion.

Authors Contribution

JO, OJ Conceptualize the work
JO Designed the research
OJ Conducted the field work and experimental analysis
JO, UBI Analyzed and interpreted the data
JO, OJ, IP, EFI, SUO, QOS wrote and proofread the manuscript

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Not applicable.

Conflict of interest

There is no conflict of interest to declare by the authors.

Availability of data and materials

The datasets of this current study are available on appeal from the corresponding author.

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