

PHYTOCHEMICAL COMPONENTS, PROXIMATE ANALYSIS, AND ANTIMICROBIAL PROPERTIES OF FERMENTED AND NON-FERMENTED RHIZOMES OF *Zingiber officinale*

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Abstract

The desirability of fermented foods may be attributed to their benefits. This study investigated the pH, *in vitro* antimicrobial assays including Minimum Inhibition Concentration (MIC), Minimum Fungicidal Concentration (MFC), and disc diffusion assay of fermented and non-fermented samples of *Zingiber officinale* against *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli*. The phytochemical screening and proximate compositions were carried out on the fermented and non-fermented samples while High-Performance Liquid Chromatography (HPLC) was carried out only on the non-fermented sample. The pH decreased steadily throughout while the MIC of the fermented samples ranged between 12.5-50.0 mg/mL and that of the non-fermented sample ranged between 25-50.0 mg/mL. The highest zones of inhibition obtained were between 8.0-16.0 mm at 100 mg/mL, 8.0-14.00 mm at 50.0 mg/mL, and 2.00-8.4 mm at 25 mg/mL. Comparatively, the zones were higher (6.8-8.4 mm) for the fermented than for the non-fermented sample (1.8-4.0 mm) and higher against *S. aureus* (4-16.0 mm) than *E. coli* (2.0-12.0 mm) and *C. albicans* (2.0-6.0 mm). The fermented sample contained alkaloids, anthraquinones, flavonoids, phlobatannins, saponins, tannins, and terpenoids, while alkaloids, flavonoids, glycosides, saponins, steroids, tannins, and terpenoids were in the non-fermented sample. The fermented sample contained 87.31% moisture, 1.24% ash, 0.29% fat, 9.73% carbohydrate, 0.24% fiber, and 1.48% protein, while the non-fermented sample contained 83.10% moisture, 1.02% ash, 0.21% fat, 14.46% carbohydrate, 0.18% fiber, and 1.24% protein. HPLC revealed Alpha farnesene (51.77), zingiberene (11.30), zingerone (14.53), 6-gingerol (12.44), and quercetin (35.21) in the fermented sample. Thus, the fermented sample of *Zingiber officinale* is a suitable candidate for the promotion of human health.

Keywords: Alpha farnesene; antioxidant; fermentation; tannins; zingerone

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Introduction

A wide range of ailments is caused by pathogenic microorganisms such as *Escherichia coli* (Hussain *et al.*, 2020). Folk medicines across the globe have been using plants as therapeutic agents against ailments (Imo *et al.*, 2016). This interest may be due to the recognized values of plants' bioactive properties (Gaurav *et al.*, 2010; Imo and Uhegbu, 2015). The phytochemical compositions and biological activities of medicinal plants may vary with geographical origin (Lawal *et al.*, 2016).

Zingiber officinale (Ginger), of the family Zingiberaceae (Sharma *et al.*, 2016) is well known in Nigeria as “atale” in the Yoruba language, “Chitha” in the Hausa language, and “Jinja” in the Igbo language. The rhizome is reported to be a rich source of vitamins and minerals (USDA, 2013). Phytochemical studies of the rhizome of *Z. officinale* showed that it contains active compounds such as phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, tannins, terpenoids, gingerols, shogaols, paradols, zingerone, and zingiberene (Langner *et al.*, 1998; Ali *et al.*, 2008).

Several reports are available on the outstanding medicinal values of its rhizome range from antimicrobial, anti-inflammatory, anticancer, immune-stimulating, cardiovascular, and aphrodisiac properties, treatment of ulcers, antioxidant, anticancer (Bode and Dong 2004; Shukla and Singh, 2007; Ali *et al.*, 2008), etc. However, there is sparing information on the impact of bioprocessing (fermentation) on the properties of *Z. officinale*.

The upturn in the desirability of humans in the bioprocessing of functional foods may be due to improved compounds in the food (Di Cagno *et al.*, 2016). Therefore, the possible bioprocessing of medicinal plants through fermentation may yield new compounds that can be developed into improved drugs for the treatment of infectious diseases (Ali *et al.*, 2017). Fermentation may slightly alter the proximate composition of substrates. Hence, while numerous works of literature documented several reports on the antimicrobial, antioxidants, and proximate composition of different solvent extracts of *Zingiber officinale*, this study improved the rhizome of *Z. officinale* of Nigerian origin through fermentation and investigated the active constituents, nutritional compositions, and the comparative *in vitro* antimicrobial activities of the fermented and non-fermented puree against a few pathogens.

Materials and Methods

Plant Material

Fresh matured rhizomes of *Zingiber officinale*, bought from Mandate market, Adewole area, Ilorin, Nigeria, were authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, where a voucher specimen was deposited (UILH/001/976/2021).

Preparation of the Plant

The rhizomes were cleaned with distilled water, cut into small sizes, and rinsed with distilled water. They were then crushed using a clean electric blender to form a puree.

Test Organisms

Candida albicans, *Staphylococcus aureus*, and *Escherichia coli* used in this study were obtained from the Microbiology Laboratory of Al-Hikmah University, Ilorin, Nigeria. They were adjusted to 0.5 McFarland standard before use.

Preparation of the Fermented and Non-Fermented Samples of *Z. officinale*

The experimental samples were prepared in 2 Mason jars labeled A and B (for the non-fermented and fermented samples). Each jar held the brine solution (620 mL of distilled water mixed with 17 g of salt) and 200 g of the *Z. officinale* puree in a ratio of 1:3 w/v. The jars were tightly closed and stored at room temperature ($28 \pm 2^\circ\text{C}$). After 24 h, the sample in jar A was filtered using clean cheesecloth and stored in the fridge to prevent fermentation and for further studies, while the sample in jar B was allowed to stand for 14 days fermentation period, with shaking twice daily to promote natural microbial growth (Guarner and Schaafsma, 1998). After the 14-day period, the sample was filtered and stored at 4°C in the fridge to halt fermentation and for further studies. Working concentrations were prepared by successive dilution of the sample with sterile distilled water to give concentrations of 100, 50, and 25 mg/mL.

Determination of the pH for Fermented and Non-Fermented Samples of *Z. officinale*

The pH of the samples was determined using a pH electrode (C. A. 10001, CHAUVIN ARNOUX).

Determination of the MIC/MFC of the Fermented and Non-Fermented *Z. officinale*

The Minimum inhibitory Concentration/

Minimum fungicidal concentration of the sample against the pathogens was assessed by the serial broth dilution technique (Altuner and Akata, 2010). There were 5 sterile tubes set up labeled 100, 50, 25, 12.5, and 6.25. Into each sterile tube, an equal volume of the sample was mixed with nutrient broth, subsequently, 0.1 mL of an 18 h broth culture which had previously been adapted to 0.5 McFarland turbidity level (representing 1.0×10^7 CFU/mL) was introduced into each sterile test tube. There were two control setups in two sterile test tubes; one, containing the sample and the nutrient broth without the standardized inoculum (agent control), and the second, containing the nutrient broth, physiological saline, and the standardized inoculum (test organism control). The tubes were further vortexed and incubation was done at 37°C for 24 h for the bacterial and 28°C for 72 h for the yeast isolate. The procedure was repeated for all the prepared concentrations, test organisms, and test samples. MIC was taken as the concentration of the sample in the tube with no detectable turbidity (representing 99.5% killing of the original inoculum) when compared with the control.

Preparation of Paper Discs and Susceptibility Testing of the Test Organisms

Paper discs (6 mm diameter), prepared from Whatman filter paper (No. 1) were labeled and sterilized for 15 min at 15 lbs pressure. They were left to cool and stored in sterile airtight bottles. The discs were soaked with 20 μ L of the fermented sample using a pipette and allowed to completely dry under a laminar flow cabinet for 24 h before further storage in sterile airtight Bijo bottles at 20°C for further use (Jawetz and Adelberg, 2004; Basri and Fan, 2005). The method of disc diffusion was adopted to determine the susceptibility of the standardized test pathogen to each of the samples (Ncube *et al.*, 2008). The pour plate method was employed using molten agars "Mueller-Hinton" (MHA; Becton-Dickson, USA) for bacteria and potato dextrose (for the propagation of the yeast). The impregnated discs were placed on the inoculated medium and the plates were incubated at 37°C for 24 h for the bacteria and at 30°C for 24 h for the yeast. Triplicate measurements of the diameter of zones of inhibition were taken for each sample.

HPLC Analysis

The HPLC analysis was done using a modular chromatographic system Shimadzu (Nexeramx) made up of an LC-10AD pump, a column oven (CTO-10A), a UV-DAD detector (SPD-10A), an interface (CBM-10A), and a Workstation (LC-10).

The sample (1.0 g), suspended in acetonitrile/water (1:1 v/v) was centrifuged for 10 min at 3,000 rpm and filtered. Then, analysis was performed on a 250 mm $\times$ 4.6 mm ID $\times$ 5 mm at 30°C. Using the mobile phase acetonitrile comprising of water (40:60 v/v) at a flow rate of 1 mL/min., separations of the constituents were done in the isocratic mode at a mobile phase of constant composition during the evaluation period (Springfield *et al.*, 2005).

Qualitative Phytochemical Screening

The samples were screened for alkaloids, anthraquinones, flavonoids, glycosides, saponins, tannins, and terpenoids in the samples (Kenneth-Oboji, 2017):

Test for Alkaloids: An equal volume of the sample was added to concentrated hydrochloric acid, and a few drops of Mayer's reagent were included. The presence of alkaloids was reported as the production of green color or white precipitate.

Anthraquinones: One mLs of the sample was placed in a dry test tube, 20 mL of chloroform was added and the solution was heated in a water bath for 5 min. Then, the sample was filtered while hot and allowed to cool. The filtrate was added an equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink colouration as indicative of the presence of Anthraquinones.

Test for Flavonoids: The sample (1 mL) was diluted with 1 mL of Sodium hydroxide (NaOH) and hydrochloric acid (HCL). The development of a yellow solution indicated the presence of flavonoids.

Test for Glycoside: One mL of the sample was added to 20 cm³ of water, heated for 5 min in a water bath, and filtered through Gem filter paper (12.5 cm). Then 0.2 cm³ of Fehling's solutions A and B were mixed with 5 cm³ of the filtrate until it became alkaline (tested with litmus paper). A brick-red coloration on heating showed a positive

Phlobatannins: The sample (2 mL) was added into dilute HCl and observed for red precipitate that was indicative of the presence of phlobatannins.

Test for Saponins: The sample (1 mL) was diluted with 3 mL of distilled water and this was shaken for 15 min. The formation of a 1 cm layer of foam indicated the presence of saponin. Steroids: The second portion of the solution above was mixed with concentrated sulphuric acid carefully so that

the acid formed a lower layer and the interface was observed for a reddish-brown colour indicative of a steroid ring.

Test for Tannins: The sample (1 mL) was mixed with 2 mL of distilled water followed by a few drops of ferric chloride (10%). A blue or green color indicated a positive result.

Test for Terpenoids: The sample (1 mL) was mixed with 2 mL of chloroform and 2 mL of concentrated sulfuric acid. The formation of a reddish-brown color at the interface indicated the presence of terpenoids.

Proximate Analysis

The test samples were analyzed for moisture, ash, protein, lipids, fat, fiber, and carbohydrate contents using methods (AOAC, 2001). The moisture and ash were determined using the weight difference method. Crude fat was determined by extracting the samples with petroleum ether in a soxhlet extractor.

Results

The pH values of the sample between 0 hours - 14 days ranged between 6.8-6.0 (Figure 1).

The antimicrobial assay of the fermented sample ranged between 12.5-50.0 mg/mL against the bacteria strains and 50.0 mg/mL against the yeast strain, while the value ranged between 25.0-50.0 mg/mL against the bacteria strains and 50.0 mg/mL against the yeast strain (Table 1).

The diameter of zones of inhibition (mm) of the fermented sample was higher (16 mm) at 100 mg/mL against *S. aureus*, while the least zone of inhibition (7 mm) was produced against *C. albicans* at 25 mg/mL (Figure 2). The non-fermented sample produced higher zones of inhibition (4 mm) at 100 mg/mL against *E. coli* while the least zone of inhibition (2 mm) was produced at 25 mg/mL against other organisms.

Eight (8) constituents were present in the fermented while seven (7) constituents were present in the non-fermented samples (Table 2).

Proximate compositions were slightly higher in the fermented than non-fermented *Z. officinale* (Table 3).

Five constituents, retained at different times and peak heights, were identified in the non-fermented sample of *Z. officinale*. Alpha farnesene was detected at the highest peak height while the least was zingiberene (Figure 3).

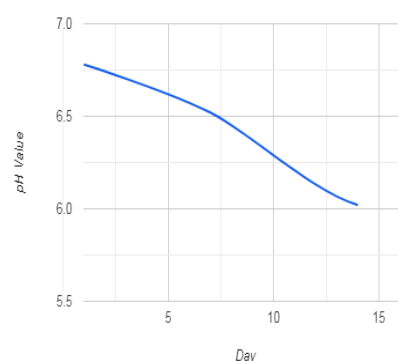


Figure 1. pH values of *Z. officinale* during the period of fermentation

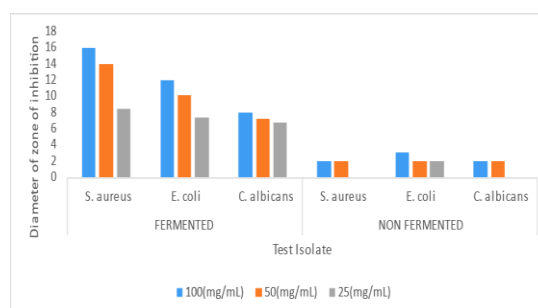


Figure 2. Diameter of zones of inhibition (mm) of the fermented and non-fermented samples of *Z. officinale* against the test pathogens

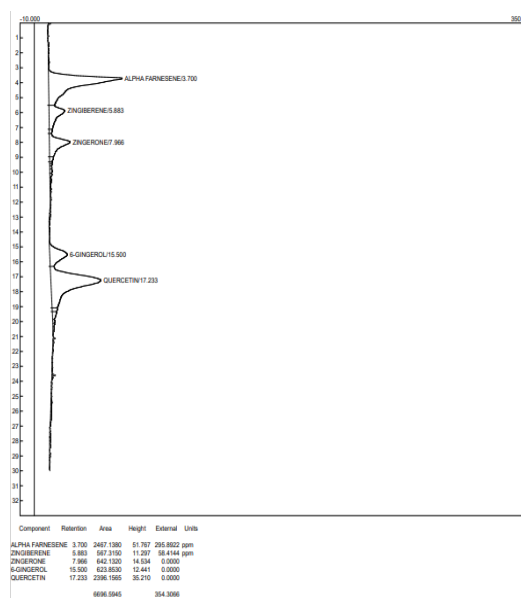


Figure 3. HPLC chromatogram of the non-Fermented sample of *Z. officinale*

Table 1. Antimicrobial assay of the fermented and non-fermented samples of *Z. officinale* sample

	Concentration (mg/mL) of the sample	
	Fermented	Non-fermented
<i>S. aureus</i>	12.5	50.0
<i>E. coli</i>	50.0	25.0
<i>C. albicans</i>	50.0	50.0

Table 2. Phytochemical constituents of the fermented and non-fermented samples of *Z. officinale*

Phytochemical components	Fermented	Non-fermented
Alkaloids	+	+
Anthraquinones	+	-
Flavonoids	+	+
Glycoside	+	+
Phlobatannin	-	-
Saponin	+	+
Steroids	-	-
Tannin	+	-
Terpenoid	+	+

Keys: + = present; - = absent

Table 3. Comparative proximate composition of the fermented and non-fermented *Z. officinale* samples

Proximate composition	Proximate values	
	Non-fermented	Fermented
Moisture content (%)	83.10±0.01	87.31±0.21
Ash (%)	1.02±0.10	1.24±0.12
Fat (%)	0.21±0.44	0.29±0.62
Carbohydrate (%)	14.46±0.32	9.73±0.21
Fiber (%)	0.18±0.23	0.24±0.13
Protein (%)	1.24±0.12	1.48±0.24

Discussions

Z. officinale was reported as being rich in bioactive constituents (Kizhakkayil and Sasikumar, 2012) thus, this study on *Z. officinale* was aimed to improve its quality and properties. Fermentation is reported to improve the properties of food nutritional composition, reduce the naturally occurring toxic compounds (Foroutan, 2012; Sanlier *et al.*, 2019), and break down complex compounds into their simple counterparts. While numerous reports are available on the properties and composition of *Z. officinale*, this work provides new findings on the fermented sample *Z. officinale*. The addition of common salt during this study was aimed to promote the growth of beneficial microorganisms and improve the nutritional composition of the sample as reported by Breidt *et al.* (2007). Xiong *et al.* (2006) also reported that a typical range of 2 to 10% salt concentrations was to enhance Sauerkraut fermentation.

The decline in pH values observed in this study may be attributed to the activity of fermenting lactic acid bacteria (LAB). LAB may help to create stressful conditions in a medium thereby inhibiting the growth of spoilage-causing microorganisms (Perez-Diaz *et al.*, 2019).

The nutritional compounds reported in this study are in line with a previous scientific study where carbohydrates, fats, and vitamins were reportedly present in *Z. officinale* (Shukla and Singh, 2007). Wang (1968) also stated that fermentation may impact the proximate composition of substrates. Hence, the impact of fermentation on this study may be linked to the higher carbohydrate content recorded for the fermented than the non-fermented samples of *Z. officinale*. This agrees with the report that fermentation significantly improves the concentration of nutritional compounds (Di Cagno *et al.*, 2016). A similar report given by Das *et al.* (2005) also reported an increase in the nutritional quality of fermented foods.

Phytochemicals play notable roles in the biological properties of therapeutic plants (Aboaba *et al.*, 2011). Thus, the results of the secondary metabolites reported in this study are similar to the reports obtained in the organic and aqueous extracts of *Z. officinale* (Shirin-Adel and Prakash, 2010). In corroboration with our findings, fermented foods, fruits, and vegetables have been reported to contain flavonoids (Kaur and Kapoor, 2001). Terpenoids, saponins, flavonoids, and alkaloids have been found to possess antimicrobial properties (Tapas *et al.*, 2008; Mir *et al.*, 2013) and this was evident in the present study. The constituents (Alpha farnesene, zingiberene, zingerone, 6-gingerol, and quercetin) detected in the fermented sample in this work conform with the report on extracts of *Z. officinale* containing gingerols, paradols, shogaols, and zingerones, with a wide range of biological activities (Shukla and Singh, 2007; Kizhakkayil and Sasikumar, 2012).

Also in this study, *Z. officinale* exhibited an array of activities against the pathogens which aligns with the safety of the extract of *Z. officinale* in the treatment of digestive diseases as reported by Al-ani and Al-Shahwany (2018). The lower MIC/MFC of the fermented than the non-fermented sample is a function of higher antimicrobial potentials. This corroborates the report of Tripathi *et al.* (2013) who stated that lower MIC is indicative of higher antimicrobial activities.

The activity of different extracts of *Z. officinale* against Gram-positive bacteria was also reported by Ahmed *et al.* (2022). However, a broad spectrum of biological activities reported by Shukla and Singh (2007) supports the findings of this study where similar activity was observed for the fermented and

non-fermented samples of *Z. officinale* against multiple pathogens. Although this study reported higher activity of the fermented sample against Gram-positive bacteria. The higher antimicrobial activities displayed by the fermented sample in the present study corroborate the report of Oyarekua (2013) which stated that fermentation plays an important role in reducing antinutritional factors and increasing antimicrobial activities in fermented products. Hence, this study reveals the active components, nutritional compositions, and antimicrobial efficacies of *Zingiber officinale*.

Conclusions

This study revealed that the fermented rhizome of *Z. officinale* has inhibitory activities against *S. aureus*, *E. coli*, and *C. albicans*. It also showed that the sample, having a significant antioxidant property, also harbor compounds with proven health-promoting activities. Thus, this study generally indicates that fermentation improved the health-benefiting properties of the rhizome of *Z. officinale* and is a reliable alternative for the preparation of plants for medicinal purposes. However, further studies are needed on the safety profile of different doses of the fermented rhizome of *Z. officinale*.

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Conflict of interest

The authors declare no conflict of interest.

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