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# Antioxidant Properties of Fermented Kolanut husk and Testa of Three Species of Kolanut: *Cola acuminata*, *Cola nitida* and *Cola verticillata*

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author TBF performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Author DJA designed the study and managed the analyses of the study. Both authors read and approved the final manuscript.*

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## ABSTRACT

Excessive free radicals in the body system are known to cause oxidative stress, resulting in some pathological conditions which are fast becoming a challenge that needs urgent attention. Plants are known to be good sources of antioxidants that have the potential of scavenging for free radicals in the body system. In a bid to find solution to the challenge of oxidative stress, three species of kolanut were analysed in this study for their antioxidant properties. The husk and testa of three species of kolanut (*Cola acuminata, Cola nitida* and *Cola verticillata*) were subjected to liquid and solid state fermentation for 10 days, after which it was dried, grounded and analysed. The effect of fermentation was checked quantitatively, comparing the antioxidant properties of the fermented and unfermented (control) samples. Radical scavenging ability (DPPH), Ferric Reducing Antioxidant Property (FRAP), Iron chelation, vitamin C, phenol and flavonoid were determined. The values obtained for the husk and testa of fermented and unfermented (control) samples were within the range of 0.210 to 1.17 mg/g for FRAP, 1.120 to 4.700 mg/g for phenol, 55.740 to 70.230% for

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DPPH, 0.263 to 1.577 mg/g for flavonoid, 88.410 to 97.733% for iron chelation, while that of vitamin C ranged between 1.913 to 4.633 mg/g respectively. The fermented samples had higher antioxidant properties for DPPH, phenol, Iron chelation and vitamin C than unfermented samples. This study has established the fact that the husk and testa of the kolanut species has antioxidant properties and thus can exert several beneficial effects by virtue of these properties.

*Keywords: Antioxidant; kolanut husk; kolanut testa; fermentation; Cola acuminate; Cola nitida and Cola verticillata.*

## 1. INTRODUCTION

Pathological conditions involving cardiovascular disease, cancer, neurological disorder, diabetes, other diseases and aging has been attributed to oxidative stress. Oxidative stress has been observed to induce a cellular redox imbalance which is found to be present in cancer cells and may be related to oncogenic stimulation [1]. Antioxidants consist of vitamins A, C, E, flavonoids, minerals, polyphenols and endogenous enzymes such as superoxide dismutase, catalase and glutathione peroxidase. It has the capability to neutralize unstable molecules called free radicals and help prevent diverse pathologies. Antioxidants work by blocking the process of oxidation and neutralize free radicals, interrupting the chain reaction free radicals form by changing other molecules into free radicals like themselves [2].

Antioxidant is any substance when present at relatively low concentration, compared with those of the oxidisable substrate significantly delays or inhibits oxidation of that substrate scavenging products of respiration [3]. Antioxidant prevents the oxidation of substances until they themselves are transformed into inert products. An example of compounds with antioxidant property include; phenols, quinines, tocopherols, gallic acid and gallates. The most important naturally occurring antioxidant is vitamin E [4]. Study of antioxidant is important because it helps prevent free radicals, primarily highly reactive oxygen and nitrogen species, from damaging human health. The most active free radicals break bonds in DNA molecules and damage the cell's genetic apparatus regulating their growth, which can result in cancerous cells [5].

Plant foods such as vegetables and fruits are good sources of natural antioxidants, containing different antioxidant components. They provide protection against harmful free radicals and are associated with low incidence / mortality rates of cancer and heart diseases in addition to a number of other health benefits. Vitamin C which is also an antioxidant is the most abundant water soluble antioxidant in the human body. It is hypothesized to prevent cancer by inhibiting the formation of N-nitroso compounds in the stomach, and by stimulating the immune system [6]. Different plant/plant wastes have antioxidants in varying quantity and types with potency to cure a number of pathological diseases.

Kola nut is an indigenous plant with a number of Nigerian populations involved in its farming, trading and industrial utilization [7,8]. *Cola acuminata* and *Cola nitida* are the main and common types of kolanut, with red, pink or white coloured cotyledons often observed among nuts extracted from same pod. *Cola verticillata* is another specie of kolanut but of lesser importance as compared with the afore mentioned because of its slimy nature when chewed [9]. They are used in the pharmaceutical industries, food industries for the production of soft drinks, chocolate, jam, jelly, wines and candles, activated carbon, liquid soap, replacement of up to sixty percent of maize used in poultry feed formulations. The testa can be used as ingredient in the formulation of fertilizer because of its high potassium content. Locally, the husk can be used as traditional medicine to reduce labour pains, treating swellings and fresh wounds. The root of the tree can be used as chewing stick for cleaning the teeth and can also be used for carving [7]. The presence of antioxidants/bioactive compounds in *kolanut* plant is a logical explanation that can be given for their medicinal properties.

The objective of this study is to analyse for the antioxidant properties of dried fermented husk and testa of three species of kolanut, which is to harness for its possible use as supplement in antioxidant deficient foods.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Preparation

The *kola nut* species were obtained in bulk from a farm in owena, Osun state, Nigeria and authenticated at the department of Crop Science and Production, Federal University of Technology, Akure. Ondo State and transported to Microbiology laboratory in the same institution for analyses. The samples were rinsed to get rid of sand particles, the husk and testa were fermented separately for ten days in different bowls with lid after which the samples were dried, grounded, sieved for analyses.

## 2.2 Fermentation Procedure

Five and three hundred grams of husk and testa were weighted into clean lid bowls appropriately labeled. The samples were subjected to liquid and solid state fermentation. For the liquid state fermentation, one thousand five hundred (1500 ml) and one thousand (1000 ml) milliliters of distilled water was used to ferment the husk and testa respectively while samples subjected to solid state fermentation was fermented without water. The samples were left to ferment for ten days.

## 2.3 Determination of Total Phenol of kolanut Husk and Testa

The total phenol content of the sample was determined using the method of [10]. The reaction mixture was incubated at 45ºC for 40mins, and the absorbance was measured at 700nm in the spectrophotometer (Gulfex Medical and Scientific England, Spectrumlab 23A, model number 23A08215).

## 2.4 Determination of Vitamin C of Kolanut Husk and Testa

The vitamin C content was determined using the ascorbic acid as the reference compound. Two grams (2 g) of kolanut husk and white shell and five milliliters (5 ml) of the infusion was pipette and mixed with 30ml of 13.3% of TCA and 75 microliter of DNPH. The mixture was incubated at 37ºC for 3 hrs and 50 ml of $H_2SO_4$ was added and the absorbance was read at 520 nm.

## 2.5 Determination of Total Flavonoid of Kolanut Husk and Testa

The total flavonoid content of the extract was determined using a colorimeter assay developed by [11] Absorbance was read at 510 nm against the blank.

## 2.6 Determination of Free Radical Scavenging Ability of Kolanut Husk and Testa

Free radical scavenging ability of the extract against DPPH (1, 1- diphenyl-2-picryhydrazyl) was carried out using [12] method. Absorbance was measured at 516 nm.

## 2.7 Determination of $Fe^{2+}$ Chelation of Kolanut Husk and Testa

The ability of the extract to chelate iron was determined using a modified method of [13].

## 2.8 Statistical Analysis

Mean and standard errors of the mean of triplicate readings obtained in the study were subjected to statistical analysis using SPSS version 16 Microsoft windows and descriptive one way analysis of variance (ANOVA).

## 3. RESULTS

Antioxidant property of fermented *Cola acuminata, Cola nitida and Cola verticillata* husk and testa were compared with unfermented (just dried; JD) husk and testa of the three species which served as the control. The following parameters were measured in each sample; FRAP (Ferric Reducing Antioxidant Property), phenol, flavonoid and vitamin C were measured in mg/g while DPPH (1, 1- diphenyl-2-picryhydrazyl) and Iron chelation were measured in %. The measurement of antioxidant properties of fermented and unfermented (control) dried husk and testa of the three species of kolanut understudy, subjected to liquid and solid state fermentation for ten days are summarized and presented on appropriately labeled graphs below. Each of the graphs compares effect of fermentation methods employed on the samples against the controls and also compares which of the samples has highest antioxidant properties.

Figs. 1 and 2 shows the FRAP content of unfermented and fermented dried husk and testa of the three species of kolanut respectively. For samples subjected to liquid state fermentation, husk of *Cola nitida* was observed to have the highest Ferric Reducing Antioxidant Property (FRAP) of 1.17±0.03 and unfermented *Cola acuminata* husk had the lowest reading of 0.21±0.01. The testa of *Cola verticillata* had highest value of 0.87±0.01 and lowest for unfermented *Cola nitida* testa, measuring 0.31±0.01. The FRAP (Ferric Reducing Antioxidant Property) of the solid fermented samples was observed to follow similar trend with the liquid fermented samples. *Cola acuminata* husk was observed to have the highest value of 0.85±0.01 and lowest value of 0.21±0.01 for unfermented *Cola verticillata* husk while the testa had highest reading of 0.84±0.01 and 0.31±0.01 for solid state fermented and unfermented samples of *Cola nitida.*

The values of the radical scavenge ability of unfermented and fermented dried husk and testa of the three kolanut species is represented on Figs. 3 and 4. The free radical scavenging ability of the samples measured using DPPH (1, 1-diphenyl-2-picryhydrazyl) showed that, both methods of fermentation employed followed similar trend. Thus, revealing liquid and solid fermented *Cola verticillata* husk and *Cola nitida* testa to have low values of 55.74±0.15, 58.74±0.16 and 57.75±0.13, 62.73±0.21 respectively. Meanwhile, unfermented *Cola nitida* husk and testa had the highest readings of 66.73±0.06 and 94.30±0.30 respectively for both methods of fermentation. Furthermore, Liquid fermented *Cola verticillata* husk and testa exhibited premier reading of 4.70±0.12 and 4.69±0.10 when the phenol content of the samples were measured, with relative trend observed for the solid fermented samples as presented in Figs. 5 and 6. While, *Cola acuminata* subjected to liquid and solid state fermentation were observed to have same values of 1.31±0.21 and 1.12±0.06 for husk and testa respectively.

Figs. 7 and 8 shows the Flavonoid content of unfermented and fermented dried husk and testa of the three species of kolanut.

The presence of flavonoid is more evident in unfermented samples as compared to the liquid fermented samples. Unfermented *Cola verticillata* husk and *Cola nitida* testa read 0.97±0.012 and 1.57±0.066 respectively, making them samples with the highest flavonoid content as shown on Figs. 7 and 8, while lowest values of 0.45±0.015 and 0.30±0.001 were recorded for liquid fermented and unfermented *Cola nitida* husk and *Cola acuminata* testa respectively. The flavonoid content of solid fermented samples is comparative to that of the liquid fermented samples, with unfermented *Cola verticillata* husk and *Cola nitida* testa having same readings as those liquidly fermented while, the lowest value was recorded for solid fermented and unfermented *Cola acuminata* husk and testa.

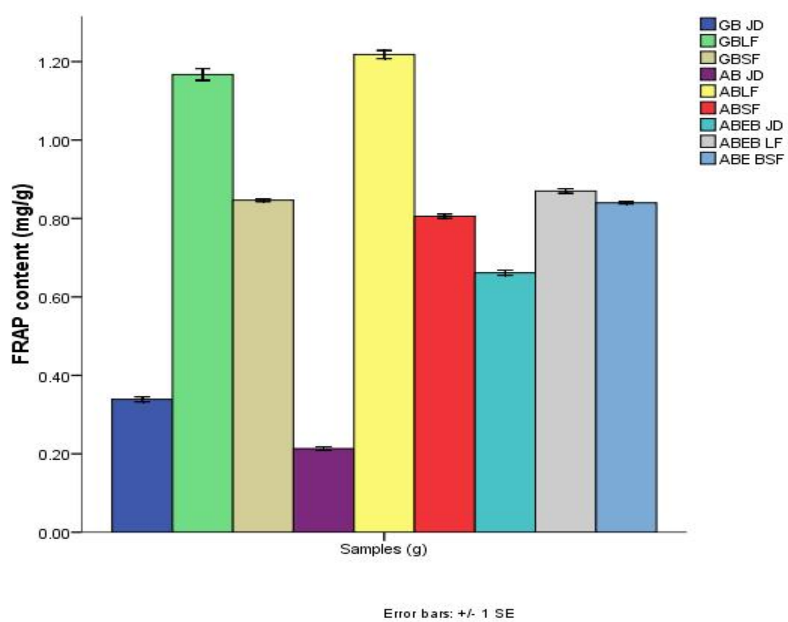


**Fig. 1. FRAP content of unfermented and fermented dried husk of the three species of kolanut**
***Key: AB*** *- Cola acuminata husk;* ***AW****- Cola acuminata white shell;* ***GB****- Cola nitida husk;* ***GW****- Cola nitida white shell;* ***ABEB****- Cola verticillata husk;* ***ABEW****- Cola verticillata white shell;* ***JD*** *– just dried (unfermented);* ***LF****- liquid state fermentation;* ***DPPH****- 2,2- diphenylpicrylhydrazyl;* ***FRAP****- Ferric reducing antioxidant power;* ***Fe*** *– Iron*

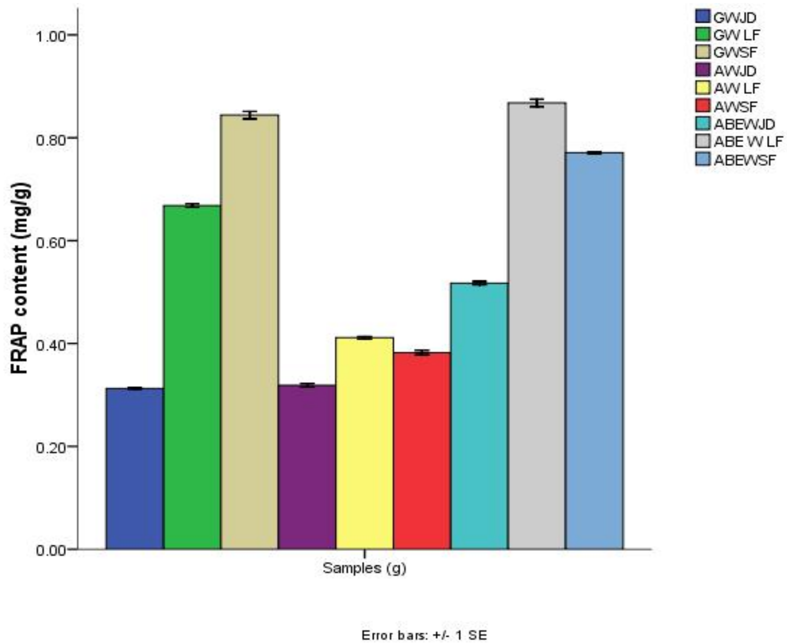


**Fig. 2. FRAP content of unfermented and fermented dried testa of the three species of kolanut**

***Key: AB*** *- Cola acuminata husk;* ***AW****- Cola acuminata white shell;* ***GB****- Cola nitida husk;* ***GW-*** *Cola nitida white shell;* ***ABEB****- Cola verticillata husk;* ***ABEW****- Cola verticillata white shell;* ***JD*** *– just dried (unfermented);* ***DPPH****- 2,2- diphenylpicrylhydrazyl;* ***LF****- liquid state fermentation;* ***SF –*** *Solid state fermentation;* ***FRAP****- Ferric reducing antioxidant power;* ***Fe*** *– Iron*

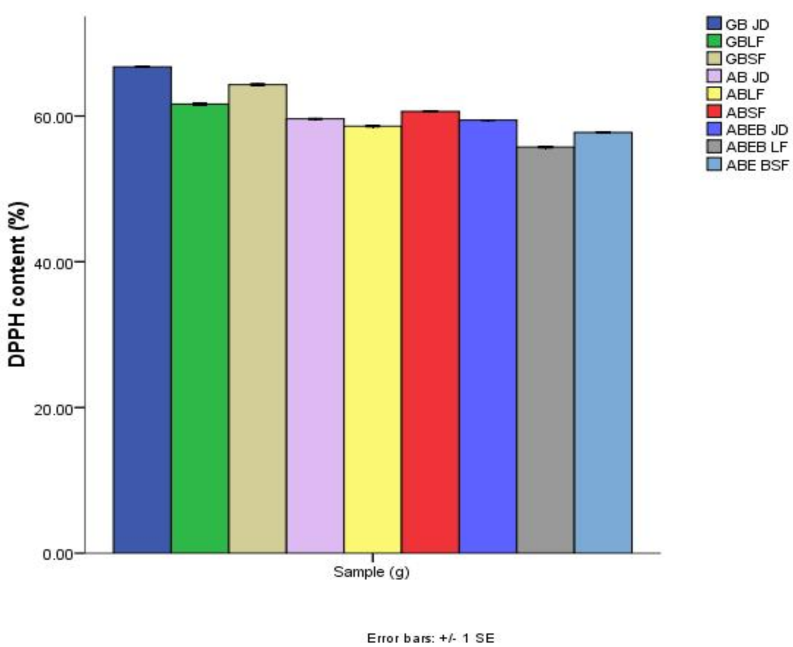


**Fig. 3. Radical scavenge ability of unfermented and fermented dried husk of the three species of kolanut**

***Key: AB*** *- Cola acuminata husk;* ***AW****- Cola acuminata white shell;* ***GB****- Cola nitida husk;* ***GW-*** *Cola nitida white shell;* ***ABEB****- Cola verticillata husk;* ***ABEW****- Cola verticillata white shell;* ***JD*** *– just dried (unfermented);* ***DPPH****- 2,2- diphenylpicrylhydrazyl;* ***LF****- liquid state fermentation;* ***SF –*** *Solid state fermentation;* ***FRAP****- Ferric reducing antioxidant power;* ***Fe*** *– Iron*

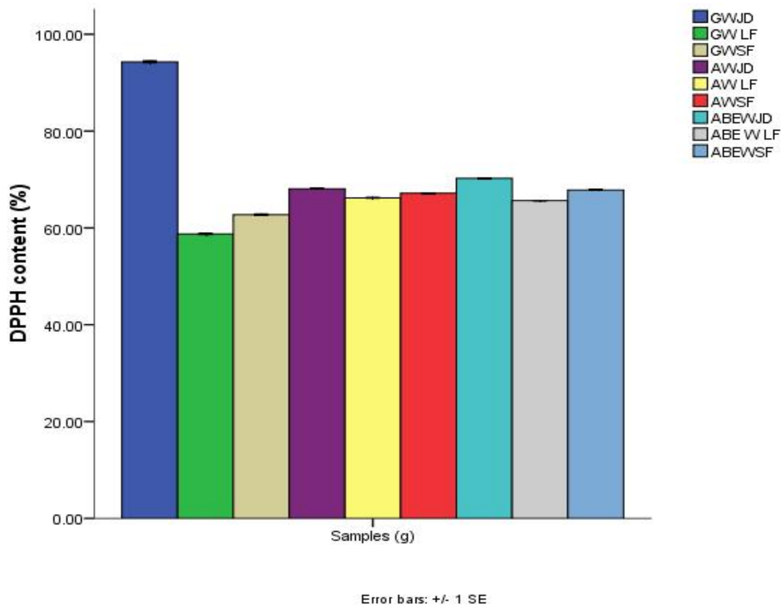


**Fig. 4. Radical scavenge ability of unfermented and fermented dried testa of the three species of kolanut**

***Key: AB*** *- Cola acuminata husk;* ***AW****- Cola acuminata white shell;* ***GB****- Cola nitida husk;* ***GW****- Cola nitida white shell;* ***ABEB****- Cola verticillata husk;* ***ABEW****- Cola verticillata white shell;* ***JD*** *– just dried (unfermented);* ***DPPH****- 2,2- diphenylpicrylhydrazyl;* ***LF****- liquid state fermentation;* ***SF –*** *Solid state fermentation;* ***FRAP****- Ferric reducing antioxidant power;* ***Fe*** *– Iron*

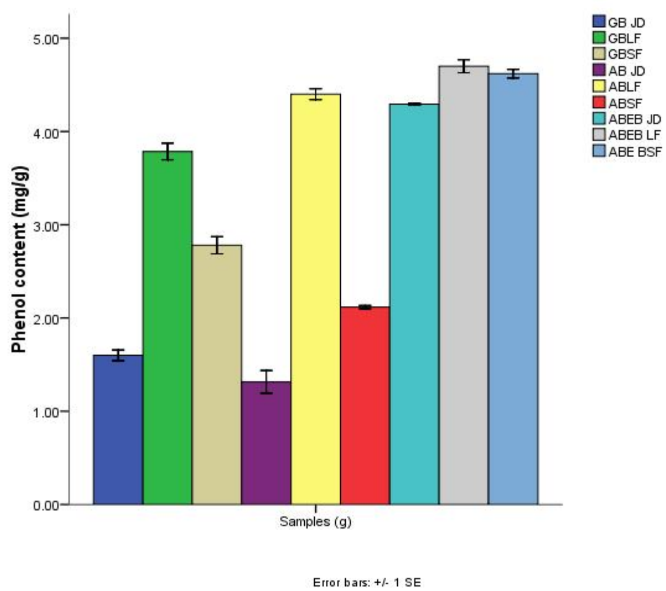


**Fig. 5. Phenol content of unfermented and fermented dried husk of the three species of kolanut**

***Key: AB*** *- Cola acuminata husk;* ***AW****- Cola acuminata white shell;* ***GB****- Cola nitida husk;* ***GW-*** *Cola nitida white shell;* ***ABEB****- Cola verticillata husk;* ***ABEW****- Cola verticillata white shell;* ***JD*** *– just dried (unfermented);* ***DPPH****- 2,2- diphenylpicrylhydrazyl;* ***LF****- liquid state fermentation;* ***SF –*** *Solid state fermentation;* ***FRAP****- Ferric reducing antioxidant power;* ***Fe*** *– Iron*

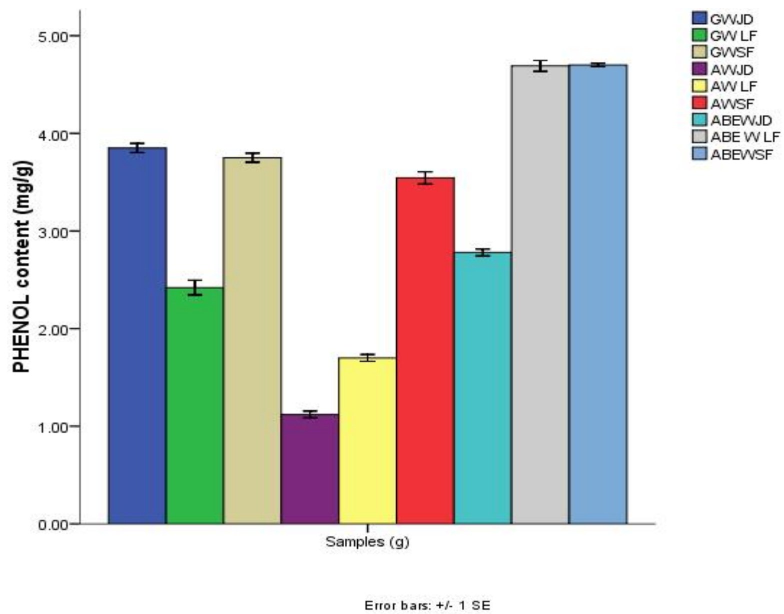


**Fig. 6. Phenol content of unfermented and fermented dried testa of the three species of kolanut**

***Key: AB*** *- Cola acuminata husk;* ***AW****- Cola acuminata white shell;* ***GB****- Cola nitida husk;* ***GW****- Cola nitida white shell;* ***ABEB****- Cola verticillata husk;* ***ABEW****- Cola verticillata white shell;* ***JD*** *– just dried (unfermented);* ***DPPH****- 2,2- diphenylpicrylhydrazyl;* ***LF****- liquid state fermentation;* ***SF –*** *Solid state fermentation;* ***FRAP****- Ferric reducing antioxidant power;* ***Fe*** *– Iron*

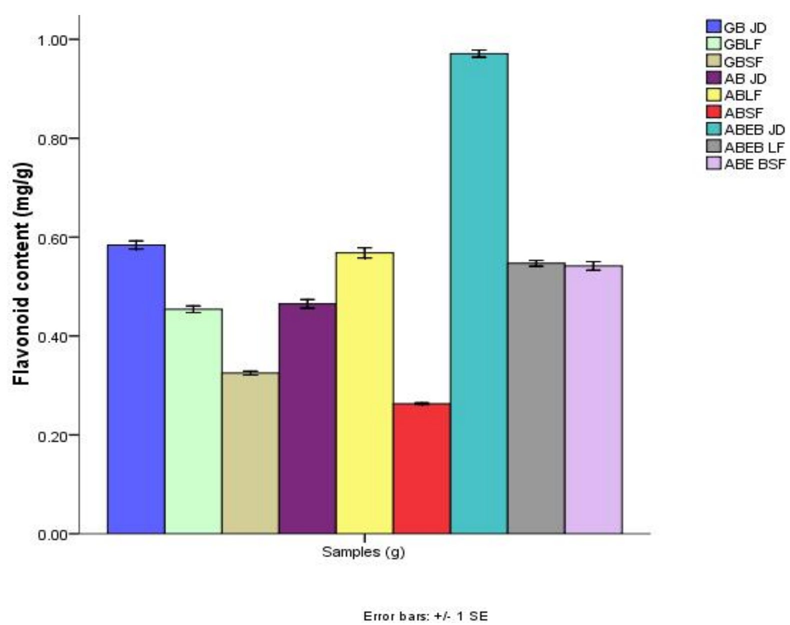


**Fig. 7. Flavonoid content of unfermented and fermented dried husk of the three species of kolanut**

***Key: AB*** *- Cola acuminata husk;* ***AW****- Cola acuminata white shell;* ***GB****- Cola nitida husk;* ***GW****- Cola nitida white shell;* ***ABEB****- Cola verticillata husk;* ***ABEW****- Cola verticillata white shell;* ***JD*** *– just dried (unfermented);* ***DPPH****- 2,2- diphenylpicrylhydrazyl;* ***LF****- liquid state fermentation;* ***SF –*** *Solid state fermentation;* ***FRAP****- Ferric reducing antioxidant power;* ***Fe*** *– Iron*

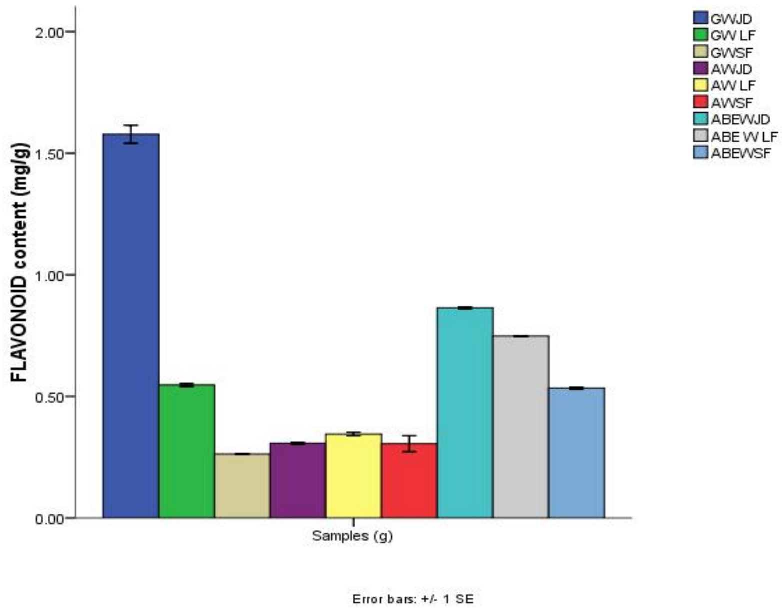


**Fig. 8. Flavonoid content of unfermented and fermented dried testa of the three species of kolanut**

***Key: AB*** *- Cola acuminata husk;* ***AW****- Cola acuminata white shell;* ***GB****- Cola nitida husk;* ***GW****- Cola nitida white shell;* ***ABEB****- Cola verticillata husk;* ***ABEW****- Cola verticillata white shell;* ***JD*** *– just dried (unfermented);* ***DPPH****- 2,2- diphenylpicrylhydrazyl;* ***LF****- liquid state fermentation;* ***SF –*** *Solid state fermentation;* ***FRAP****- Ferric reducing antioxidant power;* ***Fe*** *– Iron*

Iron Chelation of unfermented and fermented dried husk and testa of the three species of kolanut is presented on Figs. 9 and 10. Liquid fermented husk of *Cola acuminata* had the highest iron cheating ability of 98.11±0.01 and the testa of unfermented *Cola nitida* with a reading of 97.22±0.204. Unfermented samples of *Cola acuminata* husk and testa were observed to have low iron chelating property of 88.41±0.115 and 84.35±0.250 respectively. But in the case of solid fermented samples, only *Cola nitida* husk had the highest value of 97.73±0.115 and 97.22±0.204 for unfermented *Cola nitida.* Low values were recorded for unfermented *Cola acuminata* husk and testa.

In addition, the vitamin C content of the sample was measured to be present but relatively low. The vitamin C content of unfermented and fermented dried husk and testa of the kolanut is shown in Figs. 11 and 12. *Cola acuminata* husk and testa subjected to liquid state fermentation was observed to have the highest reading of 4.23±0.116 and 3.54±0.578 respectively while, the unfermented *Cola verticillata* husk and testa both had lowest readings of 2.76±0.130 and 1.91±0.083. Relatively, *Cola acuminata* husk subjected to solid state fermentation had highest reading of 4.63±0.346 and 3.50±0.080 for unfermented *Cola nitida* testa.

## 4. DISCUSSION

The need to rebuild and maintain a healthy defense system has led numerous researchers in recent years around the world to search for natural and health supporting properties of plants and microorganisms that have not yet been examined thoroughly enough. The obtained results suggest significant antioxidant potency of the analyzed kolanut husk/testa and their possible use as natural sources of antioxidants.

Ferric Reducing Antioxidant Property (FRAP) is the ability of antioxidant to reduce $Fe^{3+}$ to $Fe^{2+}$. The liquid fermented samples showed a better ability to reduce $Fe^{3+}$ to $Fe^{2+}$ from the result presented above could be attributed to the breakdown of the lignocelluloses walls. Nature of the phenolics and their hydrogen donating ability could also be a contributing factor [14]. Liquid fermented samples had higher FRAP as compared to solid fermented and unfermented samples, this therefore shows that a solvent is required to release the antioxidant of the plant materials. This also indicate that if other solvents that have better extracting power is used, the value of FRAP could have been higher than that recorded in this study. Ferric reducing power activity might be due to their hydrogen donating ability as reported by [15]. Phenolic compounds have been reported to be a major antioxidant component as compared with ascorbic acid, lycopene, tocopherol and carotene in plant materials such as mushrooms [16].

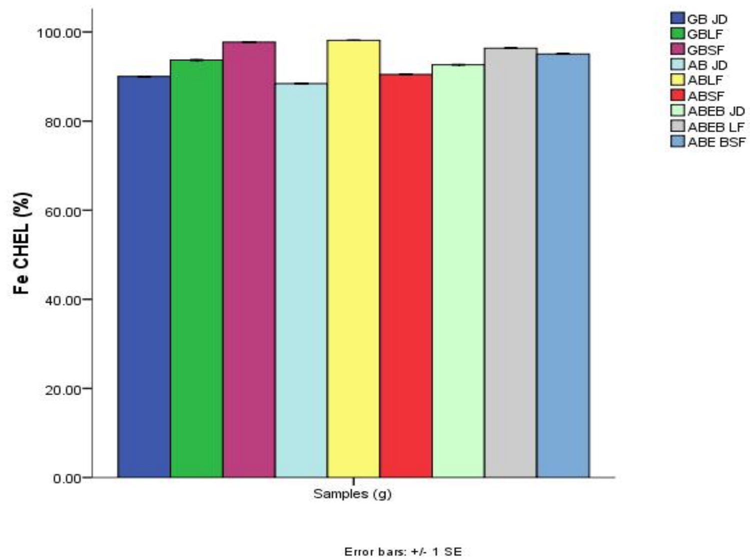


**Fig. 9. Iron Chelation of unfermented and fermented dried husk of the three species of kolanut**

***Key: AB*** *- Cola acuminata husk;* ***AW****- Cola acuminata white shell;* ***GB****- Cola nitida husk;* ***GW****- Cola nitida white shell;* ***ABEB****- Cola verticillata husk;* ***ABEW****- Cola verticillata white shell;* ***JD*** *– just dried (unfermented);* ***DPPH****- 2,2- diphenylpicrylhydrazyl;* ***LF****- liquid state fermentation;* ***SF –*** *Solid state fermentation;* ***FRAP****- Ferric reducing antioxidant power;* ***Fe*** *– Iron*

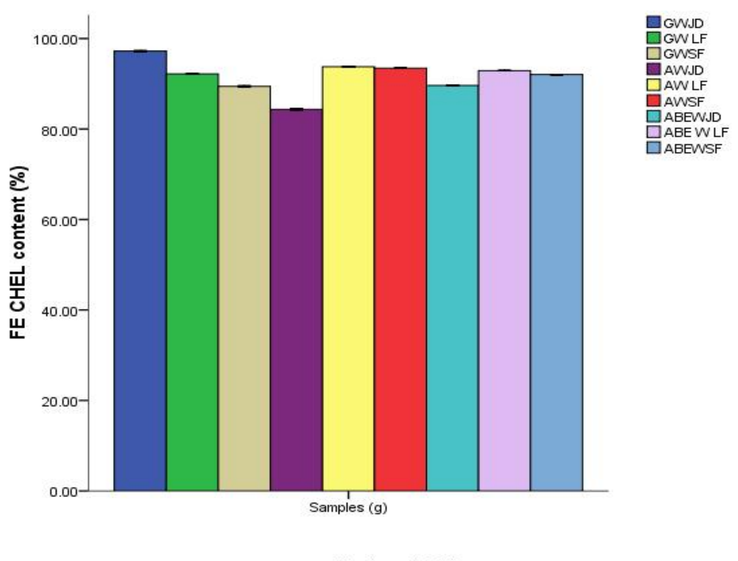


**Fig. 10. Iron Chelation of unfermented and fermented dried testa of the three species of kolanut**

***Key: AB*** *- Cola acuminata husk;* ***AW****- Cola acuminata white shell;* ***GB****- Cola nitida husk;* ***GW****- Cola nitida white shell;* ***ABEB****- Cola verticillata husk;* ***ABEW****- Cola verticillata white shell;* ***JD*** *– just dried (unfermented);* ***DPPH****- 2,2- diphenylpicrylhydrazyl;* ***LF****- liquid state fermentation;* ***SF –*** *Solid state fermentation;* ***FRAP****- Ferric reducing antioxidant power;* ***Fe*** *– Iron*

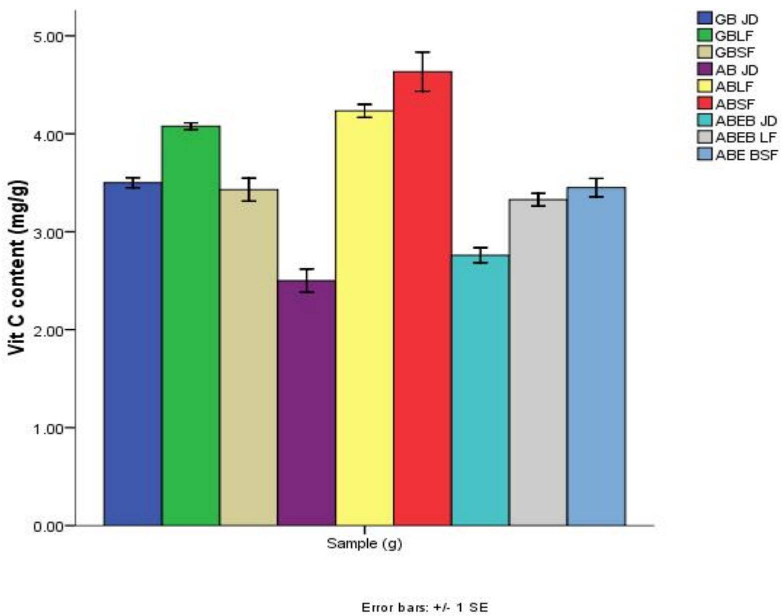


**Fig. 11. Vitamin C content of unfermented and fermented dried husk of the three species of kolanut**

***Key: AB*** *- Cola acuminata husk;* ***AW****- Cola acuminata white shell;* ***GB****- Cola nitida husk;* ***GW****- Cola nitida white shell;* ***ABEB****- Cola verticillata husk;* ***ABEW****- Cola verticillata white shell;* ***JD*** *– just dried (unfermented);* ***DPPH****- 2,2- diphenylpicrylhydrazyl;* ***LF****- liquid state fermentation;* ***SF –*** *Solid state fermentation;* ***FRAP****- Ferric reducing antioxidant power;* ***Fe*** *– Iron*

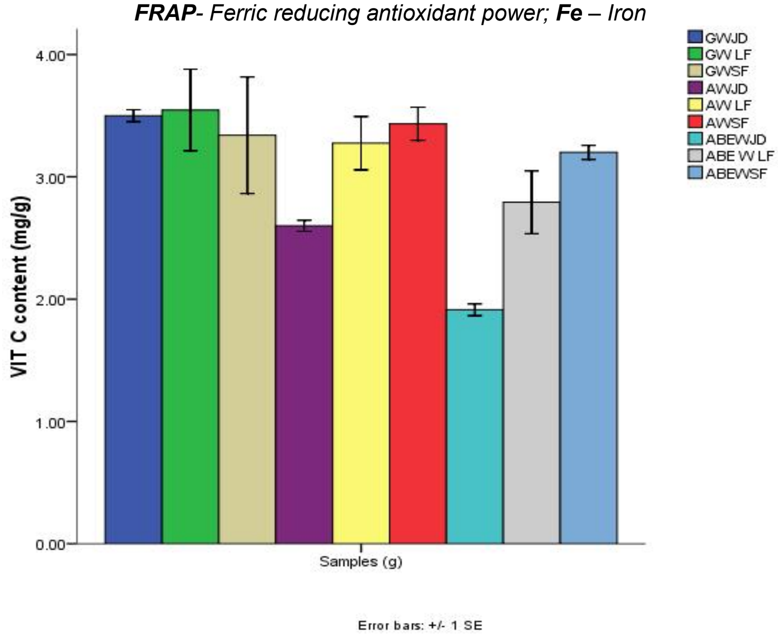


**Fig. 12. Vitamin C content of unfermented and fermented dried testa of the three species of kolanut**

***Key: AB*** *- Cola acuminata husk;* ***AW****- Cola acuminata white shell;* ***GB****- Cola nitida husk;* ***GW****- Cola nitida white shell;* ***ABEB****- Cola verticillata husk;* ***ABEW****- Cola verticillata white shell;* ***JD*** *– just dried (unfermented);* ***DPPH****- 2,2- diphenylpicrylhydrazyl;* ***LF****- liquid state fermentation;* ***SF –*** *Solid state fermentation;* ***FRAP****- Ferric reducing antioxidant power;* ***Fe*** *– Iron*

DPPH (2, 2-diphenylpicrylhydrazyl) assay is stable and significantly decreases on exposure to radical scavengers by donating hydrogen atom to become a diamagnetic molecule. The principle of the reduction is based on the reaction of antioxidant with stable free radical DPPH and converts it to 1, 1-diphenylpicrylhydrazine [17]. The result obtained in this study indicated that the samples had good radical scavenging activity which corresponds in value with the works of [15] and [18]. The extent of decrease in the absorbance of DPPH in the presence of antioxidant correlates with the free radical scavenging potential of the antioxidant [18]. The reducing capacity of the samples may be serving as a significant suggestion of its plausible antioxidant capacity. These reducing properties of antioxidants are generally associated with presence of reductones [17].

Important ingredients in human diet such as plant diets exert biological effects which have been attributed to the presence of polyphenols/ phenolic compounds. These compounds have inhibitory effects on carbohydrate hydrolyzing enzymes and antioxidant activity as a result of their ability to bind with protein. Flavonoid is an example of polyphenolic compound that has anti-inflammatory action, scavenging for free radical, inhibit hydrolytic and oxidative enzyme [19] Flavonoid has been shown to have antioxidant activity through their scavenging and chelating mechanism, thus making them beneficial health wise and nutritionally [20] Compounds such as flavonoid, which contain hydroxyl functional groups are responsible for antioxidant effects in plants. The phenolic compounds are the main agents that can donate hydrogen to free radicals, thereby breaking the chain reaction of lipid peroxidation at the first initiation stage. The ability of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups [21]. The basic mechanism of Folin Ciocalteau assay is a reduction/oxidation reaction based on redox properties of antioxidant compounds that can react with the Folin Ciocalteau reagent enabling for the measurement of phenolic concentration [22].

All the samples exhibited ability to chelate metal ions and this ability might be due to high concentration of phenolic compounds that can chelate metal ions. The metal ions could chelate with antioxidants, which might result in the suppression of OH generation and inhibit peroxidation process of biological molecules [14]. Ferric ions have been shown to reduce active $Fe^{2+}$ depending on some set conditions such as pH. Iron cheating agents are expected to inhibit metal dependent oxidative processes and have potential in combating reactive oxygen species mediated diseases [23].

From the study, it can be deduced that fermented samples had higher antioxidant properties (DPPH, phenol, iron chelation and vitamin C) than the unfermented which could be due to some microorganisms' ability especially fungi to produce antioxidant in form of their secondary metabolite [24]. Venskutonis and Gruzdiene [25] reported that extracts possessing good radical scavenging activity would be promising as ingredients of functional foods and nutraceuticals for example with cancer preventing and/or anti-aging properties.

## 5. CONCLUSION

Regular consumption of foods and beverages exhibiting antioxidant activity can help reduce the harmful effects of free radicals and oxidative stress. The study has shown the three species of kolanut husk and testa to have antioxidant properties. Further studies should be carried out to check for presence of aflatoxin. Suitable form in which the samples can be consumed either in diet or as decoction should be studied.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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