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Microbial, Chemical, Antioxidant and GC-MS investigation of Bioactive Compounds in 'Ogiri' Produced from Raw and Fermented *Citrullus lanatus* Seeds

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ABSTRACT

The dominant population pressure in Nigeria has led to a rising need for wild underutilized plant seeds and aesthetically pleasing goods in the daily diet. apart from nutritional attributes. African condiment ('ogiri') is seasoning food condiment that also serve a low protein and calorie food intake for village dwellers who cannot afford protein. Freshly harvested purchased deshelled *Citrullus lanatus* seeds (50 kilograms) were sorted, washed, boiled for 2 h before fermentation to produce 'ogiri' for 14 days. The microbial isolation, chemical measurements were made throughout the fermentation process. were carried out during the fermentation. The microbial ecology (load and population), nutritional, bioactive compounds and antioxidant properties were determined. The microbial successions of Bacteria and Fungi (*Aspergillus niger*, Rhizopus sp., Saccharomyces sp. and *Candida albicans*) were found throughout the fermentation period. The pH increased from 6.1 to 8.2 and TTA decreased from 0.1% to 0.025%. The fermented sample had its mineral elements higher than the raw except on lead; likewise, the proximate and antioxidants had more proximate, and antioxidants compared to the raw sample. Bioactive compounds had the highest area and height at peak 12 with higher concentrations of the following compounds. $C_{18}H_{34}O_2$, $C_{15}H_{30}O$, $C_{18}H_{36}O$, $C_{16}H_{30}O_2$, and $C_{13}H_{24}O_2$ respectively. Therefore, the 'ogiri' produced from *Citrullus lanatus* seeds may be incorporated into the food system.

Keywords: *Citrullus lanatus* seeds, 'Ogiri', Chemical, Antioxidants, Bioactive compounds

Introduction

Traditional medicine relies heavily on plants, and plant extract's therapeutic properties stem from a variety of bioactive and active components in plant extract such as phenolics and high antioxidant capacity free-radical scavenging activities (Nwozo *et al.*, 2023; Rizvi *et al.*, 2022). Plants derived drugs make up significant part of naturally produced pharmaceuticals plants (Aware *et al.*, 2022). Many plants have been reported to produce secondary compounds useful in the management of human diseases and ailments (Kheroda *et al.*, 2019). Seeds of *Citrullus lanatus* have been classified to be underutilized and underexploited plant seeds as people usually concentrate on the pulp, not attaching any significant benefit on the overall fruit seeds. *C. lanatus* belongs to the members of Cucurbitaceae family with bioactive compounds to include cucurbitacin, triterpenes, sterols and alkaloids (Borecka and Karaś, 2025). The seeds have been researched to contribute positively to food security as in serving as a resort to hunger, human health well as climate change (Ofosu *et al.*, 2017). They

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are high antioxidant capacity free-radical scavenging activities (Nwozo *et al*., 2023; Rizvi *et al.,* 2022). Plants derived drugs make up significant part of naturally produced pharmaceuticals plants (Aware *et al*., 2022). Many plants have been reported to produce secondary compounds useful in the management of human diseases and ailments (Kheroda *et al*., 2019). Seeds of *Citrullus lanatus* have been classified to be underutilized and underexploited plant seeds as people usually concentrate on the pulp, not attaching any significant benefit on the overall fruit seeds. *C. lanatus* belongs to the members of Cucurbitaceae family with bioactive compounds to include cucurbitacin, triterpenes, sterols and alkaloids (Borecka and Karaś, 2025). The seeds have been researched to contribute positively to food security as in serving as a resort to hunger, human health well as climate change (Ofosu *et al*., 2017). They have high 35% of crude protein, vitamins like ascorbic acid, minerals, dietary fiber (5%), oil (50%), antioxidants, and phytochemicals such as alkaloids, phenols, tannins and flavonoids (Muhammad *et al*., 2022, USDA, 2020). It also an avenue in wealth creation to alleviate poverty through creation of employment in poor and rural communities especially for women known to have a significant influence on 'ogiri' production and seed have been employed in production of cookies (Adejumo *et al*., 2013). It is considered as valuable economic ingredients with potential applications.

Fermentation has been in existence dated back BC in the ages past before modernization emerged with the use of technologically based techniques and tools to improve sensory attributes (Sharma *et al*., 2020). It has so many roles that are of health benefits. Legumes and oil seeds are examples of plant that are commonly fermented through enzymatic activity of microbial influence of various indigenous microorganisms. They created to provide benefit -addition to end-products by enhancing their aroma and savory (Ndamitso *et al*., 2020). These seeds possess antinutritional substances and toxic elements in their natural forms and through the process of fermentation detoxifies, enabling them to be edible. Fermented seeds are prepared for seasonings of foods from many ethnics from West Africa (Omafuvbe *et al*., 2004). In addition to lowering the anti-nutritional elements, it significantly enhanced the raw seeds' flavour, nutritional content, and digestibility when they were eaten cooked or roasted snacks (Talabi *et al*., 2023).

Food condiment is one of the products from fermentation and are produced through wild fermentation of plant seeds after several days of submerging in water such as *C. lanatus* seeds. They are characterized with both spoilage and pathogenic microorganisms, which is the major challenge of traditional fermentation (Ashaolu and Reale, 2020). This challenge has reduced consumption rate, and fermented foods have been reported to be safe with production of compounds produced by bacteria including ethyl carbamate, biologically generated amines, and toxins produced by bacteria (Ruiz-Capillas and Herrero, 2019).

A quest for naturally produced condiments with cost effectives have been the major reasons why *C. lanatus* seeds known to be underutilized are now greatly sorted for with several
health benefits.

Material and Methods

Materials collection

Citrullus lanatus seeds (50 kilograms) were used to produce the 'ogiri' in the study and were purchased as a batch in February 2025 from Shasha stall in Akure, Ondo, State Nigeria. The verification of the seeds from Federal University of Technology of was carried out in Crop Department, Akure, Ondo State, Nigeria.

Ogiri Production

The procedure to produce the 'ogiri' sample of Omafuvbe *et al*. (2004) was followed with modification. Seeds of *Citrullus lanatus* were cooked to render softer the outer layer of the seed softer for 9 h were manually dehulled by rubbing between hands

after cooling to remove seed
coats. The dehulled seeds water were drained using sieve and cooked 2 h further tender while
sterilize them. Thereafter, fermentation was carried out for 7 days out in a warm dry enclosure at a temperature of 28 °C in covered containers to create a semi-anaerobic condition. After fermentation, 1% of salt was added to enhance taste and preservation before being packed in airtight containers. The fermented product then pulverized, dried by freezing, and kept in room temperature (27 ± 2°C).

Microbiology Analyses

Microbial agars were employed such as NA and PDA were prepared for isolation during fermentation according to the manufacturer's instruction of microbial isolation. Tenfold serial dilution carried out was aerobically cultured appropriately.

Bacteria isolation

The procedures of Fawole and Oso (2007) was followed for isolating growth of bacteria in 'ogiri' samples, and the isolates were morphologically and biochemically characterized.

Fungi Isolation

The procedure of Gadd *et al* (2018) was followed for isolating fungi growth from the 'ogiri' sample in lactophenol-in-cotton blue solution and morphological features were identified.

Physicochemical Properties

Determination of Chemical Composition

Proximate Composition

The proximate compositions were carried out according to standard (A.O.A.C., 2015) methods. The following difference was used to determine the composition of carbohydrates (CHO) as shown below:

[100- (Moisture + Total ash + Crude fat + Crude fiber + fiber protein)]

The energy value was obtained using the expression below:
(9× Crude fat) + (4× CHO) + (4× Crude protein)

Mineral Determination

Flame photometer was used to analyze sodium and potassium. Phosphovanadomolybdate (yellow method) was used to determine Phosphorus, while calcium, cadmium, lead, iron was assessed by Atomic Absorption Spectrophotometer (AOAC, 2015) protocol.

In-vitro Antioxidant Assays

The free radical scavenging present in 'ogiri' samples were assessed through 2, 2-Diphenyl-1-picryhydrazyl (DPPH) activities by following Aluko and Monu (2003) method.

The equation was used to express the results:

$$\text{DPPH radical scavenging activity } (\%) = \frac{(1 - \text{A517})}{\text{A517}} x\ 100 \qquad (1)$$

The hydroxyl radical present in 'ogiri' samples were assessed by following Girgih *et al* (2011) method to check the scavenging activity. Equation 2 was used to express the results:

Hydroxyl ($\bullet\ OH$) radical scavenging activity (%) =

$$\frac{\left(\Delta\text{A536}\frac{}{\text{min}}\right)\text{b} - \left(\Delta\text{A536}\frac{}{\text{min}}\right)\text{s}}{(\Delta\text{A536}\ /\text{min}\ )\text{b})} x\ 100 \qquad (2)$$

The ABTS [(2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] present in the 'ogiri' samples were assessed for their scavenging ability following of Re *et al*. (1999) method. The following Trolox equivalent antioxidant capacity (TEAC) formula was used to express the results in Trolox as the standard.

The GCMS was assessed following Turner *et al*. (2020) method.

Analysis of data

Samples of 'ogiri' results with the use of SPSS were investigated, combined and given in mean ± standard deviation (SD). One-way ANOVA was used to analyze the mean values and compared by using New Duncan Multiple Range Test (DMRT). The significance was at $p \leq 0.05$.

Results and Discussion

Microbial load of 'ogiri' during fermentation of *Citrullus lanatus* seeds is presented in **Figure 1**.

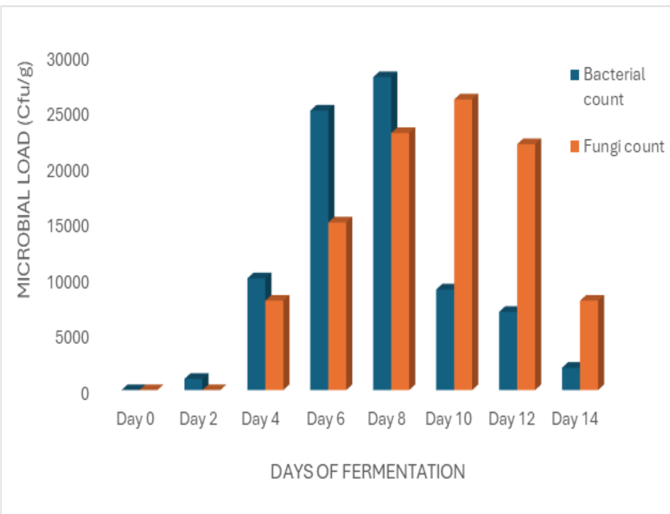


Figure 1: Microbial load of 'ogiri' during fermentation of *Citrullus lanatus* seeds

Legends:

RCLs: Raw *Citrullus lanatus* seeds

FCLs: Fermented *Citrullus lanatus* seeds

The microbial population of *Citrullus lanatus* during fermentation was revealed on day 2, increased from 1 $\times10^3$ cfu/g to 2.8 $\times10^4$ cfu/g on the eighth day, while fungi growth revealed on day 4 from 8 $\times10^3$ sfu/g to 2.6 $\times10^4$ sfu/g of fermentation. When the bacterial growth reached the tenth day, the death phase set in causing rapid decrease in bacterial growth rate from 9 $\times10^3$ cfu/g to 2 $\times10^3$ cfu/g, while fungi growth started from fourth day with 8 $\times10^3$ sfu/g to 2.6 $\times10^4$ sfu/g on the tenth day. The death phase set in from twelfth day causing decrease in the microbial load from 2.2 $\times10^4$ sfu/g to 8 $\times10^3$ sfu/g the end of fermentation. This could be due to release of metabolites during microbial metabolism and proliferation because of simultaneous break down of the substrate by different microorganisms during fermentation causing decrease in their survival and microbial depletion of the substrate (Babatuyi *et al*, 2019; Vermeersch, *et al*. 2019). The microbial successions of Bacteria (Bacillus species, Staphylococcus spp. Lactobacillus sp, and E. coli sp.) and Fungi (*Aspergillus niger*, *Saccharomyces cerevisiae* and *Candida albicans*) were constantly found in all periods of fermentation. Fermented and raw chemical (pH/TTA) of *Citrullus lanatus* seeds during fermentation is shown in Figure 2.

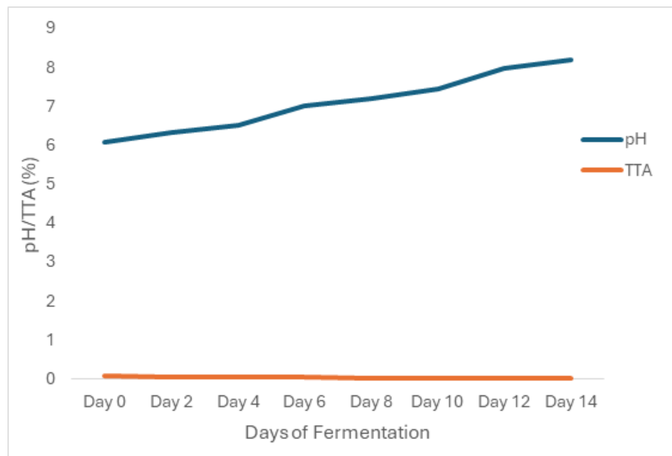


Figure 2: The pH and Total TitraTable Acidity of *Citrullus lanatus* seeds during fermentation.

Legends:

RCLs: Raw *Citrullus lanatus* seeds

FCLs: Fermented *Citrullus lanatus* seeds

There was steady increase in pH from day 0 (6.1) to day 14 (8.2) and steady decrease in TTA from day 0 (0.1%) to day 14 (0.025%). These trends stated above increased throughout the fermentation could be due to variants proliferation of microorganisms causing breaking down the substrate (Adesanya *et al*., 2021). The proximate composition of the fermented and raw of *Citrullus lanatus* seeds is shown in Table 1. There was increase in the moisture content of fermented *Citrullus lanatus* (47.43%), which was like the report of Makinde (2019).

Table 1: Nutritional Composition (%) of raw and fermented Citrullus lanatus seeds

SAMPLES	RCLs	FCLs
Moisture content	23.97[b]±0.02	47.43[a]±0.00
Total ash	2.71[b]±0.00	3.30[a]±0.01
Crude fat	10.24[a]±0.01	5.45[b]±0.00
Crude fibre	1.54±[b]0.00	1.94[a]±0.00
Crude protein	9.09±[b]0.02	50.49[a]±0.01
Carbohydrate	76.46[a]±0.00	38.00[b]±0.00
Energy value	434.36 [a]±0.01	405[b]±0.01

Values means ± standard deviation of replicate (n = 3). Values with different superscript in the same row are significantly different (P<0.05).

Legends:
RCLs: Raw *Citrullus lanatus* seeds
FCLs: Fermented *Citrullus lanatus* seeds

This could be due to decomposition of the fermenting microorganisms of the products. Fermentation of *Citrullus lanatus* seeds favour high records of ash and fiber contents, these established variants of mineral availability; thereby and suggest the nutritional status. Increased protein value resulting from an increase in activity of proteinase as well as increase in amino acids during fermentation. The low carbohydrate content (38.00%) prone the consumer to the risk of developing diabetes and/ insulin insufficiency, compared to raw sample with high carbohydrate (76.46%), which could be detrimental to health.

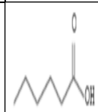
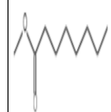
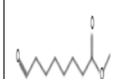
'Ogiri' raw and fermented *Citrullus lanatus* samples seeds of mineral elements are shown in Table 2. The potassium, calcium, sodium, iron, phosphorus ranged from 4.87 to 4.65 mg/100 g, 4.65 to 5.49 mg/100 g,

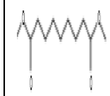
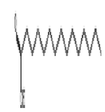
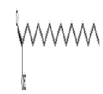
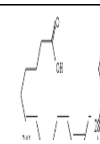
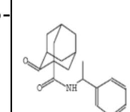
Table 1: Nutritional Composition (%) of raw and fermented Citrullus lanatus seeds

SAMPLES	RCLs	FCLs
K	4.87 [a]±0.00	4.65 [b]±0.02
Ca	4.65 [a]±0.00	5.49 [b]±0.00
Na	7.84 [b]±0.00	8.53 [a]±0.00
Fe	2.48 [b]±0.01	2.64 [a]±0.00
P	3.76 [b]±0.02	7.85 [a]±0.00
Pb	0.12 [a]±0.00	ND
Cd	ND	ND

Values means ± standard deviation of replicate (n = 3). Values with different superscript in the same row are significantly different (P<0.05).
Legends: K = Potassium, Ca = Calcium, Na = Sodium. Fe = Iron, P = Phosphorus, Pb = Lead, Cd = Cadmium, RCLs: Raw *Citrullus lanatus* seeds and FCLs: Fermented *Citrullus lanatus* seeds

Table 3: Bioactive Compounds found in Fermented Citrullus lanatus seed

PEAK	R-TIME	AREA%	HEIGHT %	MOLECULAR WEIGHT	FORMULAR	COMPOUND NAME	STRUCTURE
1	4.175	1.89	2.48	HIT 1- 116 2- 116 3- 116 4- 102 5- 130	$C_6H_{12}O_2$ $C_6H_{12}O_2$ $C_6H_{12}O_2$ $C_5H_{10}O_2$ $C_7H_{14}O_2$	Hexanoic acid Caproic acid n- caproic acid Pentanoic acid Heptanoic acid	
2	5.808	0.54	1.93	HIT 1- 158 2- 158 3- 158 4- 158 5- 116	$C_9H_{18}O_2$ $C_9H_{18}O_2$ $C_9H_{18}O_2$ $C_9H_{18}O_2$ $C_6H_{12}O_2$	Octanoic acid Methyl ester Caprylic acid methyl ester Methyl 6- methyl heptanoate Pentanoic acid	
3	7.550	0.56	1.53	HIT 1- 128 2- 198 3- 272 4- 442 5- 214	C_9H_{20} $C_{14}H_{30}$ $C_{15}H_{28}O_4$ $C_{22}H_{45}Cl_3Si$ $C_{16}H_{30}O_4$	Octane tridecane Oxalic acid Silane Oxalic acid	
4	8.683	0.46	1.48	HIT 1- 172 2- 268 3- 170 4- 256 5- 310	$C_9H_{16}O_3$ $C_{17}H_{32}O_2$ $C_{10}H_{18}O_2$ $C_{16}H_{32}O_2$ $C_{20}H_{38}O_2$	Methyl 8- oxooctanoate Cyclopentaneundecanoic acid 8-Nonenoic acid Tetradecanoic acid Cyclopropanepentanoic acid	
5	8.950	0.67	1.83	HIT 1- 168 2- 168 3- 168 4- 126 5- 140	$C_{12}H_{24}$ $C_{12}H_{24}$ $C_{12}H_{24}$ C_9H_{18} $C_9H_{16}O$	1- Undecene 4- Undecene 2- Undecene 1- Nonene 3-Octen-2-one	
6	10.050	3.43	9.30	HIT 1- 186 2- 268 3- 256 4- 310 5- 238	$C_{10}H_{18}O_3$ $C_{17}H_{32}O_2$ $C_{16}H_{32}O_2$ $C_{20}H_{38}O_2$ $C_{15}H_{26}O_2$	Nonanoic acid Cyclopentaneun decanoic acid Tetradecanoic acid Cyclopentaneun decanoic acid Tetradecynoic acid	
7	10.992	1.83	2.65	HIT 1- 254 2- 188 3- 282 4- 240 5- 126	$C_{16}H_{30}O_2$ $C_9H_{16}O_4$ $C_{18}H_{34}O_2$ $C_{15}H_{28}O_2$ $C_8H_{14}O$	Cyclopentaneundecanoic acid Azelaic acid Oleic acid Z-8-Methyl-9-tetradecenoic acid 2- Octenal	

8	12.233	4.85	8.16	HIT 1- 216 2- 216 3- 198 4- 198 5- 212	$C_{11}H_{20}O_4$ $C_{11}H_{20}O_4$ $C_{12}H_{22}O_2$ $C_{12}H_{22}O_2$ $C_{13}H_{24}O_2$	Nonanedioic acid Dimethyl ester 10- Undecenoic acid 10- Undecenoic acid Cyclopropanenonanoic acid	
9	14.258	3.12	5.83	HIT 1- 228 2- 256 3- 242 4- 228 5- 158	$C_{14}H_{28}O_2$ $C_{16}H_{32}O_2$ $C_{15}H_{30}O_2$ $C_{14}H_{28}O_2$ $C_9H_{18}O_2$	Tetradecanoic acid n- Hexadecanoic acid Pentadecanoic acid Tetradecanoic acid Nonanoic acid	
10	15.808	0.45	0.82	HIT 1- 242 2 -256 3- 284 4- 228 5- 298	$C_{15}H_{30}O_2$ $C_{16}H_{32}O_2$ $C_{18}H_{36}O_2$ $C_{14}H_{28}O_2$ $C_{19}H_{38}O_2$	Pentadecanoic acid n- Hexadecanoic acid Octadecanoic acid Tetradecanoic acid Nonadecanoic acid	
11	18.058	24.96	18.93	HIT 1- 256 2- 256 3-298 4- 284 5- 242	$C_{16}H_{32}O_2$ $C_{16}H_{32}O_2$ $C_{19}H_{38}O_2$ $C_{18}H_{36}O_2$ $C_{15}H_{30}O_2$	n- Hexadecanoic acid n- Hexadecanoic acid Nonadecanoic acid Octadecanoic acid Pentadecanoic acid	
12	20.900	37.67	20.44	HIT 1- 282 2- 226 3- 268 4- 254 5- 212	$C_{18}H_{34}O_2$ $C_{15}H_{30}O$ $C_{18}H_{36}O$ $C_{16}H_{30}O_2$ $C_{13}H_{24}O_2$	Oleic acid Z-10- Pentadecen-1 -ol E-2- Octtadecadecen-1 -ol 9- Hexadecenoic acid Tridecanoic acid	
13	21.133	11.42	16.36	HIT 1- 284 2 -256 3- 372 4- 256 5- 270	$C_{18}H_{36}O_2$ $C_{16}H_{32}O_2$ $C_{22}H_{44}O_4$ $C_{16}H_{32}O_2$ $C_{17}H_{34}O_2$	Octadecanoic acid n- Hexadecanoic acid Octadecanoic acid Hexadecanoic acid Heptadecanoic acid	
14	23.783	3.21	4.55	HIT 1- 266 2- 210 3-338 4- 210 5- 238	$C_{18}H_{34}O$ $C_{14}H_{26}O$ $C_{22}H_{42}O_2$ $C_{14}H_{26}O$ $C_{16}H_{30}O$	9 -Octadecenal 9- Tetradecenal 13- Docosenoic acid 3,11- Tetradecadien- 1-ol Cis- 11- Hexadecenal	
15	26.367	4.93	3.69	HIT 1- 297 2- 121 3- 313 4- 178 5- 121	$C_{19}H_{23}NO_2$ $C_6H_7N_3$ $C_{21}H_{42}O_2$ $C_{20}H_{27}NO_2$ $C_{12}H_{18}O$	2- Oxoadamantane- 1-carboxamide 2- Amino-4- methylpyrrole-3-carbonitrile 2-Hydroxy-2- adamantane-1-carboxamide methyl4,2,8-Ethanylylidene-2H-1-benzopyran Pyridine, 2-ethyl-6-methyl-	

7.84 to 8.53 mg/100 g, 2.48 to 2.64 mg/100 g and 3.76 to 7.85 mg/100 g.

Lead was only detected in raw sample with 0.12 mg/100 g. The fermented sample had higher values in calcium, sodium, iron and phosphorus than raw sample, which could be due to fermentation, and this was due to breakdown of the component of the seed by activity of microbiomes (Sharma *et al*., 2020).
The In-vitro antioxidant assays of the 'ogiri' samples of raw and fermented *Citrullus lanatus* seeds are shown in **Figure 3**.

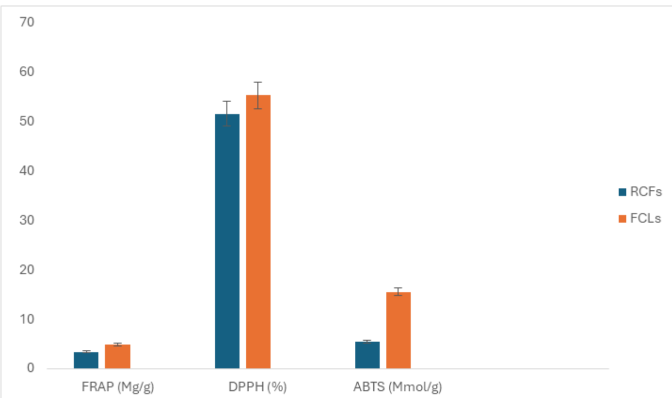


Figure 3: In-vitro Antioxidant Assays of raw and fermented *Citrullus vulgaris* seeds
Legends:
RCLs: Raw *Citrullus lanatus* seeds
FCLs: Fermented *Citrullus lanatus* seeds

All the fermented values had higher antioxidant recorded signifying good scavenging capacity of free radicals. The GCMS carried out is presented in Table 3. This was revealed that the fermented sample had appreciable bioactive compounds, which can serve as function foods and therapeutics such as anti-inflammation, hypocholesterolemic, cancer preventive, hepatoprotective, etc.

Conclusion

The study revealed the microbial ecology, proximate, antioxidant and bioactive compounds of *C.lanatus* seed. The fermented sample showed better improved quality in all the parameters investigated, especially bioactive compounds. Therefore, the fermented sample might act to be functional and therapeutic food in amelioration of oxidative related stress diseases addition to nutritional quality.

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