

Investigating the blanching effect on antioxidants, phytochemicals, and mineral characters of *Corchorus olitorius* L. cultivars

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Abstract: This study aims to provide an in-depth understanding of the effects of blanching pretreatment on the preservation of antioxidants, phytochemicals, and mineral contents in *Corchorus olitorius*, for optimal retention and utilization. Three distinct varieties of *C. olitorius* seeds, namely Ewedu_Abeokuta, Ewedu_Yaya, and Ewedu_Eleti_Eku, were sourced from farmers and cultivated. The harvested leaves underwent a 2-minute blanching treatment at 60 °C. Subsequent spectrophotometric-based assays were employed to quantify the biochemical contents. At IC₁₀₀, Ewedu_Eleti_Eku (blanched) exhibited the highest 2,2-diphenyl-1-picryl hydrazyl (DPPH) activity (81.87 ± 0.10), which was statistically similar ($p \leq 0.05$) to that of Ewedu_Yaya (blanched) (76.92 ± 3.62). The Ferric ion Reducing Antioxidant Power (FRAP) revealed Ewedu_Abeokuta (blanched) to have the highest antioxidant activity across tested concentrations (IC₂₅ = 17.43 ± 29.83 , IC₅₀ = 20.53 ± 34.98 , IC₇₅ = 25.13 ± 42.82 , and IC₁₀₀ = 24.93 ± 42.11), when compared to the other varieties. Ewedu_Abeokuta (blanched) showed significantly higher ($p \leq 0.05$) Magnesium (Mg) and Selenium (Se) content, with values of 54.60 ± 2.63 and 0.40 ± 0.01 , respectively. In contrast, Ewedu_Yaya (blanched) had the highest Zinc (Zn) content (6.17 ± 0.05 Zn), which was statistically similar ($p \leq 0.05$) to Ewedu_Eleti_Eku (blanched) (5.69 ± 0.84). The FRAP assays (IC₂₅, IC₅₀, IC₇₅, and IC₁₀₀) demonstrated perfect positive correlations ($r = 1$) among themselves, indicating a consistent linear relationship. The treatment further enhanced phenol and tannin content in Ewedu_Yaya and phenol content in Ewedu_Eleti_Eku. These findings suggest that blanching pretreatment can be a valuable technique for improving various quality attributes of *C. olitorius* varieties.

INTRODUCTION

Corchorus olitorius, commonly known as Ewedu in Nigeria and Jute in English, is a widely cultivated and consumed leafy vegetable in Nigeria, as well as other African and Asian countries. Rich in flavor and nutrients, Jute leaves are a popular food source, providing essential nutrients like protein, carbohydrates, and fats, along with a high calorific value. *C. olitorius* is renowned for its rich antioxidant profile, which has been extensively studied for its potential in preventing and managing chronic diseases, including heart disease, cancer, diabetes, and hypertension (Islam, 2013). Alongside other leafy vegetables, *C. olitorius* has demonstrated natural antioxidant activities, offering a promising alternative to synthetic antioxidants. These antioxidants can interfere with oxidative processes, thereby protecting lipids, proteins, and

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DNA from damage (Nefertiti *et al.*, 2018; Upadhyay *et al.*, 2019). The leaves of *C. olitorius* have been found to contain high levels of flavonoids, phenolics, and carotenoids (Kaseke *et al.*, 2021; Kumar *et al.*, 2021; Olagunju *et al.*, 2018), which may confer significant health benefits and enhance overall well-being. Furthermore, the phenolic compounds present in *C. olitorius* leaves have been identified as potential sources of neuroprotection (Wagdy *et al.*, 2019), anti-tumor properties (Taiwo *et al.*, 2016), and anti-inflammatory effects (Yan *et al.*, 2013). Beyond its nutritional value, *C. olitorius* also offers economic benefits, as its stalks are utilized in the production of various industrial products, such as ropes, pulps, fibers, and composites (Doraswamey *et al.*, 2010). However, processing factors, including drying methods, can significantly impact the antioxidant, phytochemical, and mineral properties of *C. olitorius* varieties.

In Nigeria and other African countries, drying methods are employed to preserve the integrity of these valuable compounds. Nevertheless, conventional drying methods can result in significant losses due to excessive burning, while standard drying equipment can be prohibitively expensive. To address these challenges, alternative methods that are cost-effective and efficient are urgently needed to preserve the antioxidant, phytochemical, and mineral properties of *C. olitorius* leaves. One promising approach is hot water treatment, a competitive and commercially accepted blanching technique due to its simplicity, accessibility, and cost-effectiveness (Ben Zid *et al.*, 2015; Nurhuda *et al.*, 2013; Xiao *et al.*, 2017). This blanching pretreatment involves immersing plant materials in hot water at temperatures ranging from 55°C to 100°C for several minutes. Research has shown that this method can effectively maintain flavor, odor, color, texture, and reduce the loss of phytochemicals and nutrients (Magangana *et al.*, 2020; Xiao *et al.*, 2017). For instance, a study reported a 39 % increase in anthocyanin concentration in *Nephelium lappaceum* after blanching at 100 °C for 150 seconds (Nurhuda *et al.*, 2013). Other authors have also recommended blanching pre-treatment for preserving valuable bioactive compounds (Ben Zid *et al.*, 2015; Deylami *et al.*, 2016).

Despite the potential benefits of blanching, there is a notable lack of published data on its effects on the antioxidant, phytochemical, and mineral contents of *C. olitorius* varieties. Therefore, this study aims to bridge this knowledge gap by investigating the impact of blanching on the phytochemicals, antioxidants, and mineral characteristics of *C. olitorius* varieties available in Nigeria. To the best of our knowledge, this is the first reported study to examine the effects of blanching on three specific varieties of *C. olitorius*: Ewedu_Abeokuta, Ewedu_Yaya, and Ewedu_Eleti_Eku. We hypothesize that blanching may not only reduce or inhibit the degradation of antioxidants, phytochemicals, and mineral contents but also reveal variations among the three varieties. This study seeks to provide insights that can inform consumption choices, enhance the extractability of phytochemicals, and potentially lead to stronger antibacterial activity.

METHOD

Cultivars Growth

Three distinct varieties of *C. olitorius* seeds, namely Ewedu_Yaya, Ewedu_Abeokuta, and Ewedu_Eleti Eku, were sourced from Omu Panu farm settlement in Osogbo local government area, Osun state, Nigeria (N 7° 46.147, E 4° 36.701). These seeds were cultivated in the same soil and subjected to identical irrigation treatments for three months in the screen house of the Department of Botany, Lagos State University, Ojo, Lagos, Nigeria. The planting followed a complete randomized design with 3 replicates for the *C. olitorius* seed varieties.

The selection of these varieties was based on their distinct characteristics, including leaf color, size, shape, edge, and venation (Figure 1). To ensure optimal phytochemical contents, the *C. olitorius* leaves were harvested at the peak stage of maturity. It is well-established that the phytochemical contents of plants vary across different growth stages, with mature leaves

typically exhibiting the highest concentration (Osundinakin *et al.*, 2025). Consequently, only fully grown leaves were harvested for this study.



Figure 1. The three harvested *Corchorus olitorius* (showing distinct morphological characters) varieties used for blanching.

Blanching Procedures

The collected leaves underwent a thorough cleaning process, involving washing under running tap water, followed by a 24-hour soak in distilled water to remove surface contaminants. This step ensured that subsequent analyses were not influenced by external factors. The leaves were then blanched using a standardized procedure (Osundinakin *et al.*, 2025) with modifications. The blanching process involved submerging the samples in water at 60 °C for 2 minutes, followed by immediate cooling in a granulated ice block for 30 seconds to halt residual heat transfer and preserve texture and color.

A 100 g sub-sample of the blanched *C. olitorius* was then dried in an oven at a controlled temperature of 60 °C, relative humidity of 18.25 %, and air velocity of 1.0 m/s for 6 hours. Drying removed excess moisture, allowing for preservation and further analysis. The dried samples were subsequently ground into a fine powder using a ceramic mortar and pestle. The powdered samples were stored in airtight containers and refrigerated at -20 °C to prevent contamination until further analysis.

Antioxidant Activity

DPPH radical scavenging assay

The antioxidant activity of the extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, with slight modifications to the procedure described by Magangana *et al.*, 2021. A 0.1 mM solution of DPPH in ethanol was prepared, and 1 ml of this solution was added to 1 ml of extract in water at varying concentrations (25-100 µg/ml). The mixture was vigorously shaken and allowed to stand at room temperature for 30 minutes. The absorbance was then measured at 517 nm using a UV-Visible Spectrophotometer.

A lower absorbance value indicated higher free radical scavenging activity. The percentage of DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH SCAVENGING EFFECT (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \quad \text{Equation 1}$$

The absorbance of the control sample was denoted as A₀, while the absorbance of the standard sample or extract was represented as A₁. The IC₁₀₀ value, which represents the concentration

of the compounds required to inhibit 100 % of DPPH radical formation, was calculated from the obtained data. This value served as a measure of the antioxidant activity of the extract.

Ferric ion reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay was performed following the modified procedure of (Benzie & Strain, 2016). Briefly, 2 mL of *C. olitorius* extract was mixed with 20 mL of 50 % methanol and homogenized in cold water. The mixture was then centrifuged at 4000 rpm (4 °C) for 6 minutes.

A 150 µL aliquot of the supernatant was carefully taken and added to 2850 µL of freshly prepared FRAP reagent. The mixture was vortexed for 60 seconds and incubated in the dark for 30 minutes. The absorbance was measured at 593 nm using a UV-Visible spectrophotometer.

Quantitative Analysis of Phytochemical Compounds

To separate and identify the individual components of the extracts, thin-layer and paper chromatographic procedures were employed. Preliminary phytochemical analysis was conducted using standard methods outlined by Sofowora (1993) to detect the presence of phytochemical compounds. All analyses were performed in triplicate, and the mean absorbance value was calculated for each sample. This approach ensured the reliability and accuracy of the results, providing a comprehensive understanding of the phytochemical composition of the extracts.

Estimation and detection of flavonoids

To detect the presence of flavonoids, a few drops of lead acetate solution were added to the extracts. The formation of a yellow-colored precipitate confirmed the presence of flavonoids. The flavonoid content was quantitatively determined using the aluminum chloride colorimetric method (Chang *et al.*, 2002) with slight modifications. The absorbance was measured at 415 nm, and quercetin was used as the standard. The flavonoid content is expressed as quercetin equivalent (QE) in milligrams per gram of extracted compound (mg g⁻¹).

Estimation and detection of phenols

To detect the presence of phenols, 5 mL of the extract was treated with a few drops of ferric chloride solution. The formation of a bluish-black color indicated the presence of phenols. The phenol content was quantitatively determined using Folin's procedure described by Agbor *et al.* (2014) with modifications. The absorbance value was measured at a wavelength of 765 nm using a spectrophotometer.

Estimation and detection of saponins

To detect the presence of saponins, 0.5 mg of the extract was mixed with 5 mL of distilled water and shaken. The formation of a creamy mass of small bubbles, characterized by frothing, indicated the presence of saponins. Saponin content was quantitatively determined using the method described by Osundinakin *et al.* (2025). The absorbance was read at a wavelength of 528 nm using a spectrophotometer.

Estimation and detection of tannins

To detect the presence of tannins, the extract was mixed with water and heated in a water bath. The mixture was then filtered, and Iron (III) chloride was added to the filtrate. The appearance of a dark green coloration indicated the presence of tannins. The tannin content was quantitatively estimated using the Folin-Denis colorimetric method described by Kirk and Sawyer (1998). The absorbance was measured at 765 nm using a spectrophotometer, with the reagent blank used to calibrate the instrument to zero.

Estimation of Minerals Content

Estimation of magnesium

The Ammonium phosphate test was conducted to determine the presence of Magnesium in the extract. The addition of Ammonium phosphate to the extract resulted in a white precipitate of magnesium ammonium phosphate (MgNH_4PO_4), indicating the presence of magnesium. The magnesium content was quantitatively determined by measuring the absorbance using a spectrophotometer. The results were expressed as milligrams per 100 grams (mg/100g) of the extract.

Estimation of selenium

The extract was digested in nitric acid and subsequently diluted (Al-Rmalli, 2012). A colorimetric assay was then performed using 2,3-diaminonaphthalene to determine the selenium content. The absorbance was measured using a spectrophotometer and compared to a calibration curve generated from known selenium standards. The selenium content was expressed as milligrams per 100 grams (mg/100g) of the extract.

Estimation of zinc

The leaf extract was digested in nitric acid and diluted (Al-Rmalli, 2012). A colorimetric test was then performed using zincon reagent to determine the zinc content. The absorbance was measured at a wavelength of 213.85 nm using a spectrophotometer. The absorbance value was then compared to a calibration curve generated from known zinc standards, allowing for a quantitative determination of the zinc content.

Statistical Analysis

Statistical analysis was performed using SPSS. A one-way Analysis of Variance (ANOVA) was conducted to compare the means of phytochemicals among groups, followed by Duncan's Multiple Range Test (DMRT) at $p < 0.05$ to identify significant differences. Additionally, a two-way ANOVA was performed to evaluate the antioxidant activity across varying concentrations. To reduce dimensionality and identify key variables influencing cluster formation, Principal Component Analysis (PCA) was conducted. Furthermore, a two-tailed Pearson correlation coefficient analysis was performed to examine the relationships between all studied variables.

RESULTS

DPPH Radical Scavenging Assay of Blanched and Unblanched *C. olitorius* Varieties

The 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay was conducted on various *C. olitorius* (Ewedu) varieties, examining the effects of blanching on antioxidant activity at different concentrations (IC_{25} , IC_{50} , IC_{75} , and IC_{100}). The results, presented in Table 1, revealed distinct trends. Notably, at IC_{25} , blanching significantly ($p \leq 0.05$) reduced DPPH activity in the three blanched varieties compared to their unblanched counterparts. In contrast, at higher concentrations, the blanched variety Ewedu_Eleti_Eku exhibited significantly ($p \leq 0.05$) higher DPPH activity at IC_{50} (54.41 ± 0.05) and IC_{75} (68.31 ± 0.02). At IC_{100} , the DPPH activity was statistically similar (81.87 ± 0.10).

Table 1. DPPH radical scavenging assay of blanched and unblanched *Corchorus olitorius* varieties

<i>Corchorus olitorius</i> Varieties	DPPH 25 μg	DPPH 50 μg	DPPH 75 μg	DPPH 100 μg
Ewedu_Abeokuta (Blanched)	18.38 ± 0.02^c	32.37 ± 10.46^c	50.05 ± 10.14^c	60.79 ± 0.72^c
Ewedu_Abeokuta (Unblanched)	30.47 ± 2.64^{ab}	50.83 ± 1.73^a	64.86 ± 1.74^{ab}	80.39 ± 2.65^b
Ewedu_Eleti_Eku (Blanched)	27.69 ± 0.07^b	54.41 ± 0.05^a	68.31 ± 0.02^a	81.87 ± 0.10^a
Ewedu_Eleti_Eku (Unblanched)	30.62 ± 1.37^a	50.68 ± 1.40^{ab}	61.24 ± 1.12^{ab}	82.96 ± 0.87^a
Ewedu_Yaya (Blanched)	25.19 ± 4.59^b	49.77 ± 2.66^{cb}	63.65 ± 2.62^a	76.92 ± 3.62^{ab}
Ewedu_Yaya (Unblanched)	32.13 ± 3.62^a	56.86 ± 3.61^a	69.08 ± 2.00^a	86.43 ± 0.99^a

Different letters mean that they differ significantly at $p \leq 0.05$. Means $\pm$ standard errors followed by different superscripts (a, b, and c) on the same columns and rows are significantly different

FRAP Radical Scavenging Assay of Blanched and Unblanched *C. olitorius* Varieties

The Ferric ion reducing antioxidant power (FRAP) results, presented in Table 2, showed significant enhancements in antioxidant activity due to blanching. Specifically, blanched Ewedu_Abeokuta exhibited substantial increases ($p \leq 0.05$) at all concentrations: IC₂₅ (17.43 ± 29.83), IC₅₀ (20.53 ± 34.98), IC₇₅ (25.13 ± 42.82), and IC₁₀₀ (24.93 ± 42.11), compared to its unblanched counterpart. Blanched Ewedu_Eleti_Eku and Ewedu_yaya showed statistical similarity to their unblanched controls at IC₂₅. However, blanching significantly enhanced FRAP activities for Ewedu_Eleti_Eku at higher concentrations (IC₅₀, IC₇₅, and IC₁₀₀) compared to the unblanched sample. Interestingly, blanched Ewedu_yaya demonstrated a consistent reduction in FRAP activity at IC₅₀ and IC₇₅, followed by a significant increase at IC₁₀₀.

Table 2. FRAP Radical Scavenging assay of blanched and unblanched *Corchorus olitorius* varieties.

<i>Corchorus olitorius</i> Varieties	FRAP 25 µg	FRAP 50 µg	FRAP 75 µg	FRAP 100 µg
Ewedu_Abeokuta (Blanched)	17.43 ± 0.83^a	20.53 ± 0.98^c	25.13 ± 0.82^a	24.93 ± 0.11^a
Ewedu_Abeokuta (Unblanched)	0.21 ± 0.00^b	0.27 ± 0.00^{ab}	0.42 ± 0.00^b	0.56 ± 0.00^c
Ewedu_Eleti_Eku (Blanched)	0.13 ± 0.00^c	0.35 ± 0.00^a	0.40 ± 0.00^b	0.46 ± 0.00^{bc}
Ewedu_Eleti_Eku (Unblanched)	0.14 ± 0.00^{bc}	0.26 ± 0.01^b	0.35 ± 0.01^c	0.35 ± 0.00^{cd}
Ewedu_Yaya (Blanched)	0.20 ± 0.00^b	0.25 ± 0.00^b	0.35 ± 0.00^c	0.47 ± 0.00^c
Ewedu_Yaya (Unblanched)	0.18 ± 0.00^{bc}	0.30 ± 0.00^a	0.37 ± 0.00^{bc}	0.41 ± 0.00^d

Different letters means that they differ significantly at $p \leq 0.05$. Means $\pm$ standard errors followed by different superscripts (a, b, and c) on the same columns and rows are significantly different

Phytochemicals in Blanched and Unblanched *Corchorus olitorius* Varieties

The phenol content varied significantly among the blanched Ewedu varieties (Table 3). Blanched Ewedu_Eleti_Eku (33.33 ± 0.08) and Ewedu_Yaya (36.44 ± 1.15) showed significant ($p \leq 0.05$) increases in phenol content compared to their unblanched counterparts. On the other hand, blanched Ewedu_Abeokuta (29.43 ± 1.72) had a significant decrease.

Blanching had a positive effect on tannin content in Ewedu_Abeokuta (60.45 ± 2.66) and Ewedu_Yaya (43.76 ± 3.01), although the increases were not statistically significant ($p \leq 0.05$) compared to their unblanched varieties. However, blanching significantly ($p \leq 0.05$) reduced the tannin content in Ewedu_Eleti_Eku (50.54 ± 0.05).

Blanching significantly decreased the flavonoid content in Ewedu_Abeokuta (33.67 ± 3.13) and Ewedu_Yaya (42.42 ± 2.66), while having no significant ($p \leq 0.05$) effect on Ewedu_Eleti_Eku (48.38 ± 0.02). The saponin content remained statistically similar in all three blanched varieties compared to their respective unblanched varieties.

Table 3. Phytochemicals of blanched and unblanched *Corchorus olitorius* varieties.

<i>Corchorus olitorius</i> Varieties	Phenol mg GAE/g DM	Tannin mg GAE/g DM	Flavonoid CE per g DM	Saponin mg DM/100g
Ewedu_Abeokuta (Blanched)	29.43 ± 1.72^c	60.45 ± 2.66^a	33.67 ± 3.13^c	17.49 ± 1.00^{ab}
Ewedu_Abeokuta (Unblanched)	35.44 ± 2.66^{ab}	45.60 ± 2.63^{ab}	41.51 ± 3.60^b	21.07 ± 1.72^a
Ewedu_Eleti_Eku (Blanched)	33.33 ± 0.08^b	50.54 ± 0.05^b	48.38 ± 0.02^{ab}	20.01 ± 0.01^{ab}
Ewedu_Eleti_Eku (Unblanched)	31.07 ± 0.9^c	61.63 ± 0.89^a	45.96 ± 0.87^b	18.46 ± 0.75^{ab}
Ewedu_Yaya (Blanched)	36.44 ± 1.15^a	43.76 ± 3.01^c	42.42 ± 2.66^c	21.86 ± 1.72^a
Ewedu_Yaya (Unblanched)	34.11 ± 1.74^b	41.79 ± 2.01^c	54.05 ± 2.63^a	20.27 ± 2.65^a

Different letters means that they differ significantly at $p \leq 0.05$. Means $\pm$ standard errors followed by different superscripts (a, b, and c) on the same columns and rows are significantly different

Mineral Contents in Blanched and Unblanched *C. olitorius* Varieties

Blanching significantly increased the Magnesium (Mg) content in Ewedu_Abeokuta (54.60 ± 2.63) and Ewedu_Eleti_Eku (37.31 ± 0.08) (Table 4). In contrast, Ewedu_Yaya (34.29 ± 2.65) showed no significant difference in Mg content compared to its unblanched variety. Blanching had no significant effect on Selenium (Se) content in Ewedu_Abeokuta and Ewedu_Yaya, whereas it significantly decreased the Se content in Ewedu_Eleti_Eku (0.14 ± 0.00).

The Zinc (Zn) content of blanched Ewedu_Abeokuta (5.79 ± 0.19) was significantly higher than its unblanched counterpart. However, the Zn content of the other two varieties, Ewedu_Eleti_Eku and Ewedu_Yaya, remained statistically similar to their respective unblanched controls.

Table 4. Mineral content of blanched and unblanched *Corchorus olitorius* varieties.

<i>Corchorus olitorius</i> Varieties	Magnesium mg/100 gm	Selenium mg/100 gm	Zinc mg/100 gm
Ewedu_Abeokuta (Blanched)	54.60 ± 2.63^a	0.40 ± 0.01^a	5.79 ± 0.19^b
Ewedu_Abeokuta (Unblanched)	33.02 ± 2.01^b	0.34 ± 0.00^a	5.13 ± 0.04^c
Ewedu_Eleti_Eku (Blanched)	37.31 ± 0.08^b	0.14 ± 0.00^c	5.69 ± 0.84^{ab}
Ewedu_Eleti_Eku (Unblanched)	27.94 ± 0.87^c	0.27 ± 0.01^{ab}	5.07 ± 1.01^b
Ewedu_Yaya (Blanched)	34.29 ± 2.65^b	0.14 ± 0.00^c	6.17 ± 0.05^a
Ewedu_Yaya (Unblanched)	39.37 ± 2.65^{ab}	0.11 ± 0.00^c	6.67 ± 1.01^a

Different letters means that they differ significantly at $p \leq 0.05$. Means $\pm$ standard errors followed by different superscripts (a, b, and c) on the same columns and rows are significantly different

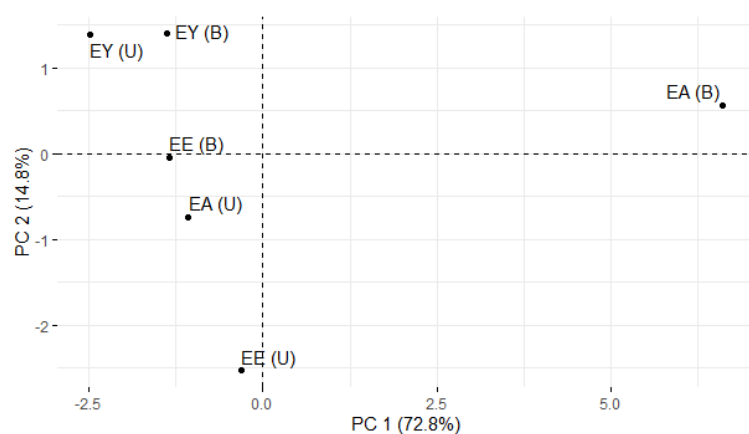
Chemotype Differences

Principal Component Analysis (PCA) was performed to categorize the *C. olitorius* varieties based on their phytochemical composition, antioxidant activity, and mineral constituents (Figure 2). The analysis included four secondary compounds: total flavonoid content (TFC), total phenol content (TPC), total tannin content (TTC), and total saponin content (TSC), two antioxidant activity measurements (DPPH and FRAP), and three mineral constituents (Mg, Se, and Zn).

The loading plot of PC1 and PC2 revealed the main dependencies and relationships between variables. The sample score plot for PC1 versus PC2 (Figure 2 & Figure 3) grouped the six populations into clusters based on their phytochemical similarity and antioxidant activity. The populations included blanched Ewedu_Abeokuta (EA(B)), unblanched Ewedu_Abeokuta (EA(U)), blanched Ewedu_Eleti_Eku (EE(B)), unblanched Ewedu_Eleti_Eku (EE(U)), blanched Ewedu_Yaya (EY(B)), and unblanched Ewedu_Yaya (EY(U)).

The right half cluster, comprising EA(B), EE(B), and EY(B), exhibited the most desirable chemical profile, being rich in flavonoids, polyphenols, and tannins, and displaying excellent antioxidant activities. In contrast, EA(U) and EE(B) clustered together, indicating similarities in their phytochemical composition and antioxidant activity.

The contributory effect of phytochemicals, antioxidants, and mineral composition to the formation of each PCs was assessed (Figures 4C, D and E). In decreasing order, DPPH_50µg, Frap_100µg, Frap_50µg, Frap_25µg, Frap_75µg, DPPH_100µg, and DPPH_25µg had the highest contributory effect to the formation of PC1 (Figure 4D). Other contributory traits below the reference lines are flavonoid, magnesium, selenium, phenol, saponin, tannin, and zinc. Contrastingly, zinc had the biggest impact on the formation of PC2, followed by tannin, magnesium, selenium, and saponin, while the remaining traits fall below the reference line (Figure 4D).



Key: EA (B)- Ewedu_Abeokuta Blanched; EA (U)- Ewedu_Abeokuta Unblanched; EE (B)- Ewedu_Eleti_Eku Blanched; EE (U)- Ewedu_Eleti_Eku Unblanched; EY (B)- Ewedu_Yaya Blanched; EY (U)- Ewedu_Yaya Unblanched

Figure 2. Principal component bi-plot of blanched and unblanched varieties of *C. olitorius* (Ewedu_Abeokuta, Ewedu_Eleti_Eku, and Ewedu_Yaya).

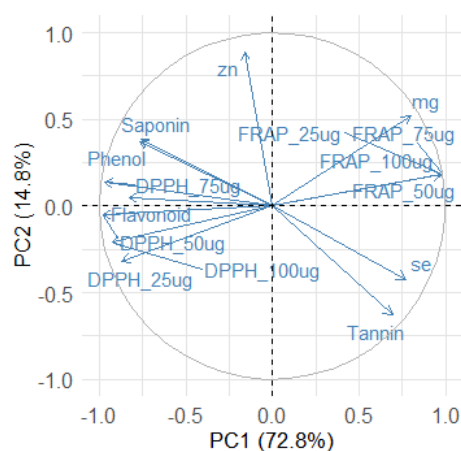


Figure 3. Principal component bi-plot of antioxidants, phytochemicals, and minerals present in *C. olitorius* varieties (Ewedu_Abeokuta, Ewedu_Eleti_Eku and Ewedu_Yaya).

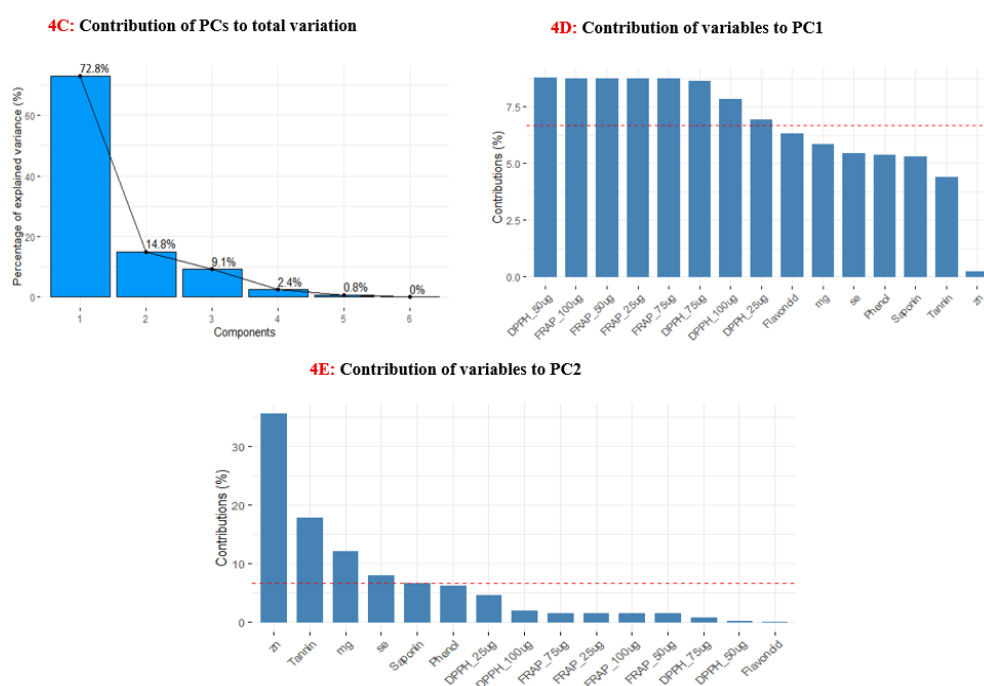


Figure 4. C: Bar plots show the contribution of individual PC to the total variation. D & E: The Red dotted lines across the bar plots indicate reference lines. The variable bars above the dotted red lines are considered most important to the formation of PC1 and PC2.

Hierarchical Clustering Analysis of Blanched and Unblanched *C. olitorius* Varieties

Hierarchical Cluster Analysis (HCA) was performed to investigate differences among the six Ewedu populations and identify similarities based on secondary metabolites and antioxidant capacities (Figure 5). Ward's method and squared Euclidean distance were used to measure similarity between samples. The HCA grouped the six populations into five clusters at the lowest level (20) based on the phytochemicals (Figure 5).

Cluster I and II comprised blanched Ewedu_Abeokuta (EA (B)) varieties, characterized by low levels of flavonoids, phenols, saponins, and minimal DPPH radical scavenging activity. Cluster III consisted of unblanched Ewedu_Eleti_Eku samples, distinguished by high tannin content. Cluster IV grouped blanched Ewedu_Eleti_Eku and unblanched Ewedu_Yaya, sharing high flavonoid content and similar high DPPH scavenging activity at IC₅₀, IC₇₅, and IC₁₀₀. Cluster V contained blanched Ewedu_Yaya and unblanched Ewedu_Abeokuta, characterized by the highest mean values of total polyphenols and saponins.

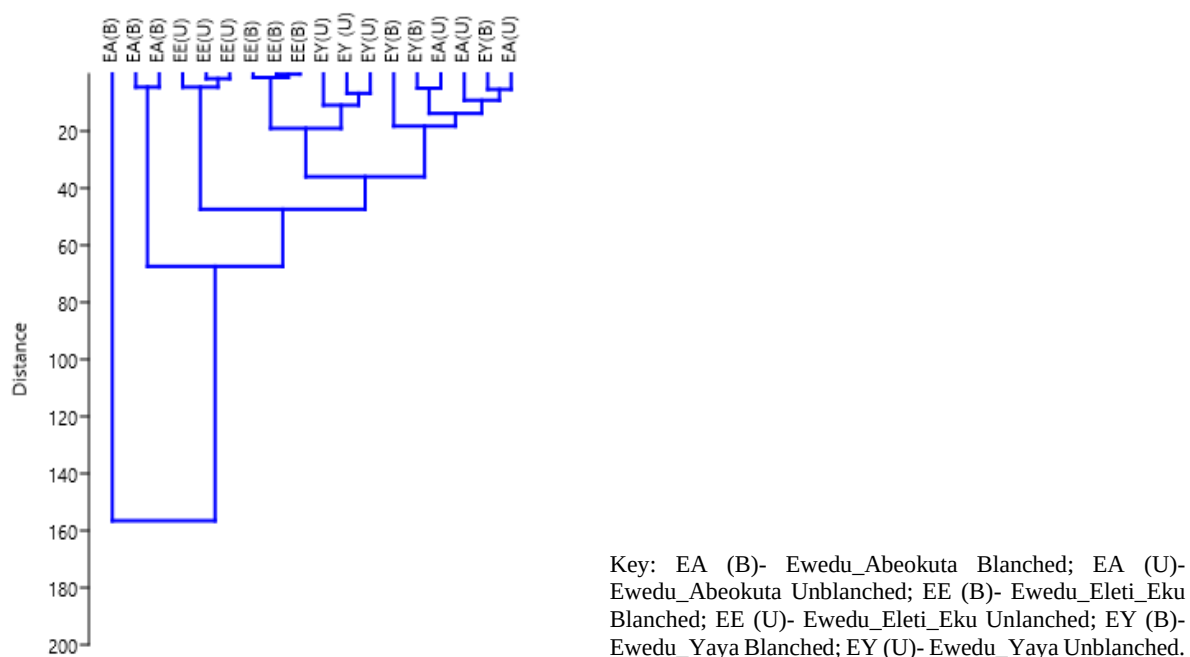


Figure 5. Dendrogram showing the clustering of blanched and unblanched *C. olitorius* varieties based on their antioxidants, phytochemicals, and minerals.

Pearson's Correlations Among 3 Studied Varieties of *Corchorus olitorius*

The Pearson correlation coefficients (Table 5) revealed the strength and direction of linear relationships among the studied parameters. A strong positive relationship between DPPH_IC₅₀ and DPPH_IC₇₅ ($r = 0.957^{**}$) was seen, indicating a robust linear association. Perfect positive correlations ($r = 1$) among FRAP_IC₂₅, FRAP_IC₅₀, FRAP_IC₇₅, and FRAP_IC₁₀₀ were recorded, suggesting a consistent linear connection. A relatively weaker negative correlation between Tannin and Flavonoid ($r = -0.453$), as shown, implies a moderate inverse relationship. These correlations provide insight into the interdependencies among the measured variables, highlighting the complex relationships between phytochemical composition and antioxidant activity.

Table 5. Pearson's correlations among 3 studied traits of *C. olitorius*. (Considered variables: TFC, TPC, TTC, TSC, Mg, Zn, Se, DPPH, and FRAP).

	TPC	TTC	TFC	TSC	DPPH IC ₂₅	DPPH IC ₅₀	DPPH IC ₇₅	DPPH IC ₁₀₀	FRAP IC ₂₅	FRAP IC ₅₀	FRAP IC ₇₅	FRAP IC ₁₀₀	Mg	Se	Zn
TPC	1														
TTC	-.781**	1													
TFC	0.29	-0.453	1												
TSC	.749**	-.738**	0.223	1											
DPPH_ IC ₂₅	0.343	-0.365	.678**	0.358	1										
DPPH_ IC ₅₀	0.411	-.505*	.721**	0.384	.712**	1									
DPPH_ IC ₇₅	0.449	-.558*	.659**	0.366	.646**	.957**	1								
DPPH_ IC ₁₀₀	0.246	-0.308	.656**	0.187	.686**	.916**	.894**	1							
FRAP_ IC ₂₅	-0.165	0.205	-0.309	-0.171	-0.43	-.797**	-.817**	-.903**	1						
FRAP_ IC ₅₀	-0.167	0.206	-0.307	-0.172	-0.43	-.796**	-.816**	-.902**	1	1					
FRAP_ IC ₇₅	-0.166	0.205	-0.308	-0.171	-0.43	-.796**	-.816**	-.902**	1	1	1	1			
FRAP_ IC ₁₀₀	-0.166	0.206	-0.311	-0.171	-0.431	-.798**	-.818**	-.903**	1	1	1	1			
Mg	-0.436	0.215	-.474*	-0.356	-.646**	-.710**	-.584*	-.662**	.537*	.537*	.537*	.539*	1		
Se	-.498*	.616**	-.781**	-0.451	-0.432	-.693**	-.681**	-.532*	0.36	0.359	0.36	0.362	0.379	1	
Zn	0.248	-0.421	0.217	0.368	0.056	0.076	0.157	0.79	-0.53	-0.53	-0.53	-0.53	.306	-.442	1

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed)

DISCUSSION

The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay is a colorimetric method that detects the reduction of DPPH free radicals by antioxidants. The assay relies on the ability of antioxidants to donate electrons, which pair off with the unpaired electrons of DPPH radicals, resulting in the formation of the corresponding hydrazine. The antioxidant activities of the three blanched *C. olitorius* varieties were confirmed through DPPH radical scavenging and FRAP assays. While blanching generally reduced the DPPH scavenging activity of Ewedu_Abeokuta, Ewedu_Yaya, and Ewedu_Eleti_Eku varieties across all concentrations (IC₂₅, IC₅₀, IC₇₅, and IC₁₀₀), all the extracts still demonstrated high antioxidant capacity at IC₁₀₀ as compared to the other concentrations. On the other hand, the varieties exhibited the weakest DPPH scavenging activities at IC₂₅ and IC₅₀. These findings are consistent with previous reports, where low scavenging activity was observed at IC₅₀ in *Rhus* species (Zammouri *et al.*, 2013). However, other studies have noted increased antioxidant activity in blanched *Aspalathus linearis* (Bhebhe *et al.*, 2015), highlighting the variability in antioxidant responses across different plant species and concentrations.

Contrary to expectations, our study did not reveal a strong correlation between DPPH scavenging activity and flavonoid content. This finding diverges from a previous report (Zheng *et al.*, 2019), which observed a positive correlation between flavonoid concentration and DPPH radical inhibition in *Plumula nelumbinis*. Our study did not find a positive correlation between DPPH and FRAP assays. Additionally, the results on blanched *C. olitorius* varieties diverged from those reported by Magangana *et al.* (2022), who investigated the effects of hot water blanching on pomegranate cultivars. Specifically, they observed a strong positive correlation between DPPH and FRAP assays, along with a significant increase in antioxidant activity in the blanched cultivars compared to their unblanched counterparts. However, these findings did align with theirs in one aspect: the significant increase in FRAP assay values. Ewedu_Abeokuta exhibited a significant increase in FRAP assay values at all concentrations compared to its unblanched counterpart, mirroring the trend observed in the pomegranate cultivars.

The FRAP values obtained for both blanched and unblanched *C. olitorius* leaf extracts in this study were relatively low compared to those reported for other plants, vegetables, fruits, fruit waste, and peels (Deng *et al.*, 2018; Magangana *et al.*, 2022; Ojha *et al.*, 2018; Zammouri *et al.*, 2013). Previous studies have established a strong relationship between total phenolic content and antioxidant activity, including radical scavenging and reducing potential (Farvin & Jacobsen, 2013). However, this study did not find a strong correlation between high phenolic content values and FRAP values. The discrepancies observed in phenolic content and FRAP values may be attributed to variations in phenolic content among extracts and differences in cultivar sensitivity. These findings suggest that the antioxidant activity of *C. olitorius* leaf extracts may be influenced by factors beyond just phenolic content.

The secondary metabolites extracted in this study, including flavonoids, tannins, saponins, and phenols, exhibited notable variations among the three *C. olitorius* varieties. Flavonoids, known for their antioxidant properties, are influenced by the number and location of free hydroxyl (OH) groups (Panche *et al.*, 2016). Tannins are a distinct category of polyphenolic compounds, while saponins are high-molecular-weight glycosides.

An important marker for any effective extraction process is the yield obtained for producing high-quality products (Wang *et al.*, 2011). Hot water blanching did not significantly ($p < 0.05$) affect the yield content of tannins, flavonoids, and saponins in the three varieties. However, it caused a significant increase in the total phenolic content of Ewedu_Yaya and Ewedu_Eleti_Eku compared to their unblanched counterparts. The extract yield values obtained for tannin, flavonoid, saponin, and phenolic content were higher than those reported in previous studies (Biswas *et al.*, 2020; Noha *et al.*, 2017) for *C. olitorius*. This suggests that the blanching extraction process used in this study was effective.

The dendrogram analysis revealed that secondary metabolites and blanching treatment influenced the quantitative differences observed among the three *C. olitorius* cultivars. Specifically, unblanched Ewedu_Eleti_Eku had high tannin content; blanched Ewedu_Eleti_Eku and unblanched Ewedu_Yaya were characterized by high flavonoid content and DPPH scavenging activity; blanched Ewedu_Yaya and unblanched Ewedu_Abeokuta possessed high total polyphenol and saponin content. These findings highlight the impact of blanching and variety on the phytochemical composition of *C. olitorius* extracts.

Magnesium (Mg) is an essential mineral that plays a crucial role in various physiological processes, including insulin secretion, oxidative metabolic enzymatic reactions, blood pressure regulation, and energy production (Kartika *et al.*, 2011). It also contributes to the development of healthy teeth and bones. A study conducted in Africa (Kamga *et al.*, 2013) reported a higher Mg content (87 mg/100 g) in *C. olitorius* compared to our findings. In contrast, another study (Biswas *et al.*, 2020) reported a lower Mg value (37.84 mg/100 g) compared to the highest value (54.60 mg/100 g) recorded in our blanched Ewedu_Abeokuta sample. Blanching significantly increased the Mg content of Ewedu_Abeokuta and Ewedu_Eleti_Eku, but had no significant effect on Ewedu_Yaya. The variations in Mg content could be attributed to factors such as cultivar response to blanching, harvest maturity, and duration of the blanching treatment. Magnesium also had a positive effect on FRAP across all concentrations. The relationship between Mg and FRAP indicated that an increase in Mg content strongly influenced and caused an increase in FRAP. This suggests that Mg is a key contributor to the antioxidant activity of *C. olitorius*, as measured by FRAP.

Zinc (Zn) is an essential mineral that plays a crucial role in enzymatic reactions, cell growth, and multiplication. It is particularly important for individuals with HIV, as it supports their immune system. A deficiency in Zn can lead to various symptoms, including appetite loss, reduced growth, and delayed wound healing. Previous studies have reported varying levels of Zn accumulation in *C. olitorius*, ranging from 0.91 ± 0.28 g/100gm to 7.35 ± 0.61 g/100gm (Biswas *et al.*, 2020), and 3.05 to 4.14 g/100gm (Baiyeri *et al.*, 2022). These findings are comparable to these reports. Pearson correlation coefficient analysis revealed that Zn had an influence on DPPH scavenging activity at IC₁₀₀, although this influence was not significant. The relationship between DPPH and Zn was unclear, and no clear upward or downward regulation relationship could be established. However, another study on *C. olitorius* reported a significant, strong effect of Zn on crude protein content (Baiyeri *et al.*, 2022), suggesting that Zn plays a role in increasing crude protein levels in *C. olitorius*.

Selenium (Se) is an essential micronutrient for humans and animals, closely linked to the antioxidant properties of alpha-tocopherol (vitamin E) and involved in the biosynthesis of thyroid hormones. The Se content determined in different populations of *C. olitorius* in a previous study (Biswas *et al.*, 2020) ranged from 99.01 to 121.24 µg/100 g, which is similar to the range found in our study. However, blanched and unblanched Ewedu_Abeokuta samples in our study showed higher Se concentrations. As reported by Aziz *et al.* (2016), the variations observed in mineral uptake among different studies may be influenced by factors such as plant type and age, soil conditions, agricultural practices, and industrial activities. The mineral concentrations obtained in our study are within reasonable limits that could support healing and preemptory processes in humans.

CONCLUSION

To the best of our knowledge, this study is the first to investigate the effects of hot water blanching pretreatment on *Corchorus olitorius*. Our findings suggest that this treatment can enhance the extraction of various quality attributes, including phenols and tannins in Ewedu_Yaya, phenols in Ewedu_Eleti_Eku, magnesium (Mg) concentration in Ewedu_Abeokuta and Ewedu_Eleti_Eku, zinc concentration in Ewedu_Abeokuta, and Ferric Reducing Antioxidant Power (FRAP) activity in Ewedu_Abeokuta.

This study also demonstrates that *C. olitorius* cultivars in Nigeria are rich in phytochemical constituents, including phenols, flavonoids, tannins, and saponins, and exhibit strong radical scavenging activity and antioxidant power. Given these findings, hot water blanching pretreatment may be a valuable technique for processing *C. olitorius* cultivars on a commercial scale, particularly for the creation and fortification of value-added products in the nutraceutical and pharmaceutical industries.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Data Availability Statement

Research data is available and can be sent with e-mail upon request.

Authorship Contribution Statement

Michael Osundinakin: Conceptualization, Writing review and editing, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Itunuoluwa Adekoya:** Methodology, Supervision, and Editing. **Michael Olugbade:** Supervision and Editing.

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