RESEARCH ARTICLE

In vitro polyphenol content and antioxidant properties of *Corchorus olitorius* stored at 4 °C for 3, 6, and 9 days

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ABSTRACT
Medicinal plants are recognized as rich sources of bioactive compounds with therapeutic potential, particularly due to their antioxidant properties, which are important in combating oxidative stress. *Corchorus olitorius* L., commonly known as jute mallow, is widely consumed as a vegetable and traditional remedy because of its high antioxidant content. This study investigated the in vitro antioxidant activity of *C. olitorius* stored under refrigeration (4 °C) for 3, 6, and 9 days. Standard assays, including total phenolic content (TPC), total flavonoid content (TFC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay, ferric reducing antioxidant potential (FRAP), and total antioxidant capacity (TAC), were used to evaluate antioxidant and polyphenol content. The results showed a gradual, time-dependent increase in antioxidant activity and polyphenol content during storage, highlighting the impact of short-term refrigeration on the bioactive properties of *C. olitorius*. These findings suggest that refrigerated *C. olitorius* may serve as a valuable source of natural antioxidant compounds with potential applications in functional foods, dietary supplements, and pharmaceutical formulations.

Key words polyphenols; antioxidant activity; *Corchorus olitorius*; refrigerated storage; in vitro

Introduction

Plants have historically formed the foundation of highly sophisticated traditional medicine systems that have flourished across different civilizations for thousands of years, and they continue to provide humankind with invaluable therapeutic resources (Nasim et al., 2022; Chaachouay & Zidane, 2024). *Corchorus olitorius* L. (family Malvaceae), commonly known as jute mallow, is a widely consumed leafy vegetable in many parts of Africa and Asia and has traditionally been valued for its nutritional and medicinal properties. The leaves of *C. olitorius* are rich in phenolic compounds, flavonoids, minerals (such as calcium and iron), vitamins, and other phytochemicals that contribute to its antioxidant, anti-inflammatory, and other biological activities (Abdel-Razek et al., 2022; Mokgalaboni & Phoswa, 2023; Okugbo et al., 2024; Sameh et al., 2025). Studies have demonstrated significant antioxidant activity in various extracts of *C. olitorius*, with methanolic and hydrophilic extracts showing strong free-radical scavenging potential in assays like DPPH and ABTS, attributed largely to phenolic constituents (Biswas et al., 2023). Post-harvest processing methods have been employed to break down complex phytochemicals, release bioactive compounds, and make the plant more readily available for therapeutic purposes against oxidative damage. Abiona et al. (2021) in their *in vitro* study used the dried leaf method in the sun and oven with heating temperature stages of 50, 60 and 70 °C to analyze the proximate and mineral compositions of *C. olitorius.* Karigidi and Adebogun (2020) exposed *C. olitorius* to Ultraviolet-C treatment for 10 minutes to enhance its polyphenol content and antioxidant properties.

Post-harvest storage conditions, especially temperature and packaging, have been shown to influence the biochemical and antioxidant properties of leafy vegetables, including *C. olitorius*. A recent study by Maseko et al. (2025) evaluated the effects of different packaging types (non-perforated vs. perforated), storage temperatures (ambient, 4 °C, and 10 °C), and durations (2–10 days) on the phenolic composition, total flavonoid content, and antioxidant activity of *C. olitorius* during storage. The findings revealed that refrigeration at 4 °C and 10 °C consistently enhanced antioxidant activity and preserved phenolic compounds better than ambient storage,

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extending shelf life and consumer acceptability, although total phenolic content generally decreased over time (Maseko et al., 2025). Published research has also reviewed the nutritional and nutraceutical potential of this plant, showing that the leaves are rich in minerals such as calcium and iron, as well as vitamins B1, B2, folic acid, C, and E (Abdel-Razek et al., 2022). Moreover, while some studies have reported increased phenolic and antioxidant values under processing or stress conditions, the specific effects of refrigerated storage over defined time intervals on a range of established antioxidant assays remain underexplored. This gap highlights the need for systematic evaluation of how short-term refrigeration impacts both polyphenol content and antioxidant potential in *C. olitorius*, which is the focus of the present study.

Materials and Methods

Plant Material and preparation of extract

Fresh seeds of *C. olitorius* were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, and planted under optimal growth conditions. After a 45-day growth period, the plants were carefully harvested from the screen house at Olusegun Agagu University of Science and Technology (OAUSTECH), Okitipupa. A portion of the harvested leaves was stored in a refrigerator at 4 °C for 3, 6, and 9 days before analysis. Illustration of the appearance after days of refrigerated storage is seen in Figure 1. The plant sample was authenticated at the Department of Biological Sciences, OAUSTECH, and assigned a voucher number of OAUSTECH/H-00799. Representative samples were retained in the university storage unit for record-keeping and future reference.

Leaves of *C. olitorius* were carefully separated from the stems, and 1 g of leaves was weighed and transferred into a homogenizer. The sample was ground until no visible plant particles remained, after which 20 mL of phosphate (PO_4) buffer was added. The homogenate was dispensed into 10 sterile Eppendorf tubes and centrifuged at 10,000 rpm for 10 minutes at 4 °C. The resulting supernatant was carefully collected into sterile tubes to serve as the plant extract for subsequent antioxidant assays.

Total polyphenol contents determination

Total polyphenol content was determined using the Folin–Ciocalteu colorimetric method as described by Biswas et al. (2020). A 10% Folin–Ciocalteu reagent was prepared by diluting 10 mL of the reagent with distilled water to 100 mL, while 7% Na_2CO_3 was prepared by dissolving 7 g of Na_2CO_3 in 100 mL of distilled water. The reaction mixture consisted of 2 mL of sample extract, 2 mL of 10% Folin–Ciocalteu reagent, 10 mL of 7% Na_2CO_3, and 10 mL of distilled water. The mixture was incubated in the dark for 90 minutes, after which absorbance was measured at 750 nm. Total phenolic content was quantified using a gallic acid standard curve and expressed as mg GAE/100 g dry weight (DW).

Total flavonoid contents determination

Total flavonoid content was determined using the colorimetric method described by Ordoñez et al. (2006). A 0.5 M $NaNO_2$ solution was prepared by dissolving 3.448 g in 100 mL of distilled water, while 0.3 M $AlCl_3$ was prepared by dissolving 7.28 g in 100 mL of distilled water. A NaOH solution was prepared by dissolving 4 g in 100 mL of distilled water. The reaction mixture consisted of 0.6 mL of sample extract, 6.8 mL of 30% methanol, 0.3 mL of 0.5 M $NaNO_2$, 0.3 mL of $AlCl_3$, and 2 mL of NaOH. The mixture was allowed to stand for 5 minutes, resulting in a green-colored solution, and absorbance was measured at 506 nm. Total flavonoid content was calculated using a quercetin standard curve and expressed as mg quercetin equivalent per 100 g dry weight (mg QE/100 g DW).

DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of the extract was determined using the method described by Gyamfi et al. (1999). A DPPH solution was prepared by dissolving 36 mg of DPPH in 100 mL of methanol. An aliquot of 2.0 mL of the DPPH solution was added to varying concentrations of the sample extract. The reaction mixtures were incubated in the dark for 30 minutes, after which absorbance was measured at 520 nm. The percentage inhibition of DPPH radicals was calculated as:

$$\text{Inhibition \% of DPPH} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100$$

The antioxidant activity was expressed as percentage inhibition of DPPH radicals; no Trolox standard calibration was used.

$ABTS^{+\bullet}$ radical scavenging activity

The $ABTS^{+\bullet}$ radical scavenging activity of the extract was determined using the method described by Re et al. (1999). The $ABTS^{+\bullet}$ radical cation was generated by reacting ABTS stock solution with potassium persulfate ($K_2S_2O_8$) and incubating the mixture in the dark for 16 hours. The resulting solution was diluted with ethanol (or buffer) to obtain an absorbance of approximately 0.7 at 734 nm. An aliquot of 2.0 mL of the ABTS reagent was added to varying concentrations of the sample extract and incubated in the dark for 30 minutes. Absorbance was then measured at 734 nm. The percentage inhibition of $ABTS^{+\bullet}$ radicals was calculated as:

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Inhibition % of $ABTS^{+}$

$$\bullet = \frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} \times 100$$

The antioxidant activity was expressed as percentage inhibition of $ABTS^{+}\bullet$ radicals; no Trolox standard curve was used.

Ferric reducing antioxidant potential (FRAP)

The ferric reducing antioxidant power of the sample extract was determined using the method of Benzie & Strain (1996). The acetate buffer was prepared by dissolving 0.31 g of sodium acetate ($C_2H_3NaO_2$) and 0.4 mL of acetic acid (CH_3COOH) in 100 mL of distilled water. A hydrochloric acid solution was prepared by diluting 0.144 mL of HCl in 100 mL of distilled water, and 0.031 g of 2,4,6-tripyridyl-s-triazine (TPTZ) was dissolved in 10 mL of the prepared HCl solution. Additionally, 0.324 g of ferric chloride ($FeCl_3 \cdot 6H_2O$) was dissolved in 100 mL of distilled water. The FRAP working reagent was prepared by mixing acetate buffer, TPTZ solution, and $FeCl_3 \cdot 6H_2O$ solution in a ratio of 10:1:1. An aliquot of 0.050 mL of the sample extract was mixed with 1.4 mL of the FRAP reagent and incubated at room temperature for 30 minutes. Absorbance was measured at 593 nm. A freshly prepared $FeSO_4 \cdot 7H_2O$ solution was used as the standard, and results were expressed as mg Fe^{2+} equivalents per 100 g dry weight (mg Fe^{2+}E/100 g DW).

Total antioxidant capacity

Total antioxidant capacity of the sample extract was determined using the phosphomolybdenum method described by Prieto et al. (1999). The TAC reagent was prepared by dissolving 1.643 g of sodium phosphate ($NaPO_3$), 0.494 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$), and 3.37 mL of sulfuric acid (H_2SO_4) in 100 mL of distilled water. An aliquot of 0.2 mL of the sample extract was mixed with 2.0 mL of the prepared TAC reagent and incubated in a water bath at 95 °C for 90 minutes. After cooling, absorbance was measured at 695 nm using a spectrophotometer. Total antioxidant capacity was expressed as mg ascorbic acid equivalent per 100 g (mg AAE/100 g DW).

Statistical analysis

Data were expressed as mean ± standard deviation (SD) of triplicate determinations (n = 3). Statistical differences among storage periods were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Differences were considered significant at $p < 0.05$. Graphs were plotted as percentage inhibition versus concentration.

Results

Polyphenol content and antioxidant capacity of C. olitorius during refrigerated storage

In Table 1, the TPC of the leaf extract ranged from 54.78 ± 1.60 mg GAE/100 g DW at 0 day to 91.89 ± 3.18 mg GAE/100 g DW at 6 days, before declining to 64.21 ± 1.56 mg GAE/100 g DW at 9 days. This indicates an initial increase in polyphenol content during storage, peaking at day 6, followed by a slight reduction at day 9. TFC showed a significant increase from 25.71 ± 1.75 mg QE/100 g DW at 0 day to 34.63 ± 3.43 mg QE/100 g DW at 3 days, after which it declined steadily to 18.77 ± 1.71 mg QE/100 g DW and 17.21 ± 1.56 mg QE/100 g DW at 6 and 9 days, respectively. FRAP activity increased from 35.51 ± 2.37 mg Fe^{2+}E/100 g DW at 0 day to a maximum of 50.53 ± 3.82 mg Fe^{2+}E/100 g DW at 3 days, before slightly decreasing at 6 days (45.93 ± 2.92 mg Fe^{2+}E/100 g DW) and returning to near initial levels at 9 days (32.44 ± 1.41 mg Fe^{2+}E/100 g DW). TAC showed a pronounced increase from 6.25 ± 0.19 mg AAE/100 g DW at 0 day to 22.94 ± 2.51 mg AAE/100 g DW at 3 days and further increased sharply to 85.39 ± 3.72 mg AAE/100 g DW at 6 days, maintaining a high value of 82.65 ± 4.13 mg AAE/100 g DW at 9 days. TPC, FRAP, and TAC generally

Table 1. *Changes in total polyphenol content, total flavonoid content, ferric reducing antioxidant power, and total antioxidant capacity of C. olitorius leaf extract during refrigerated storage at 4 °C.*

Parameters	0 day	3 days	6 days	9 days
TPC (mg GAE/100 g DW)	54.78 ± 1.60^{c}	68.98 ± 1.77^{b}	91.89 ± 3.18^{a}	64.21 ± 1.56^{b}
TFC (mg QE/100 g DW)	25.71 ± 1.75^{b}	34.63 ± 3.43^{a}	18.77 ± 1.71^{c}	17.21 ± 1.56^{c}
FRAP (mg Fe^{2+}E/100 g DW)	35.51 ± 2.37^{c}	50.53 ± 3.82^{a}	45.93 ± 2.92^{b}	32.44 ± 1.41^{c}
TAC (mg AAE/100 g DW)	6.25 ± 0.19^{c}	22.94 ± 2.51^{b}	85.39 ± 3.72^{a}	82.65 ± 4.13^{a}

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Figure 1. *Visual appearance of C. olitorius leaves under refrigerated storage at 4 °C. A (0 day), B (3 days), C (6 days), and D (9 days).*

increased during the initial stages of refrigerated storage, reaching their peak between days 3 and 6, before slightly decreasing or stabilizing at day 9. In contrast, TFC exhibited an early peak at day 3, followed by a continuous decline during the later storage period.

DPPH Radical Scavenging Assay

The DPPH radical scavenging assay is based on the ability of antioxidants to donate electrons or hydrogen atoms, thereby neutralizing DPPH free radicals and converting them to a stable, colorless reduced form (Ben Yakoub et al., 2020). The DPPH radical scavenging activity of *C. olitorius* leaf extract at varying concentrations (20–100 mg/mL) during refrigerated storage is presented in Figure 2. Panel A (0 day) showed an increase in radical scavenging activity from 8% at 20 mg/mL to 20% at 100 mg/mL. Panel B (3 days) exhibited an increase from 12% at 20 mg/mL to 46% at 100 mg/mL, while Panel C (6 days) ranged from 9.8% at 20 mg/mL to 32.1% at 100 mg/mL. Panel D (9 days) showed the highest activity, increasing from 17.8% at 20 mg/mL to 49.8% at 100 mg/mL. These results indicate an overall concentration-dependent increase in DPPH radical scavenging activity, with 100 mg/mL at 3 and 9 days showing the highest percentage inhibition.

$ABTS^{+\bullet}$ radical scavenging activity

The $ABTS^{+\bullet}$ radical scavenging activity of *C. olitorius* leaf extract at varying concentrations during refrigerated storage is presented in Figure 3. Panel A (0 day) showed an increase in radical scavenging activity from 37.4% at 20 mg/mL to 77% at 100 mg/mL. Panel B (3 days) exhibited a moderate increase from 42% at 20 mg/mL to 66% at 100 mg/mL. Panel C (6 days) ranged from 64% at 20 mg/mL and remained relatively constant at 80.1% across 40–100 mg/mL. Panel D (9 days) increased from 43.8% at 20 mg/mL, remained steady at 49.8% across 40–80 mg/mL, and peaked at 78.5% at 100 mg/mL. These results indicate a concentration-dependent increase in $ABTS^{+\bullet}$ radical scavenging activity, with the highest activity observed at 100 mg/mL across the storage period.

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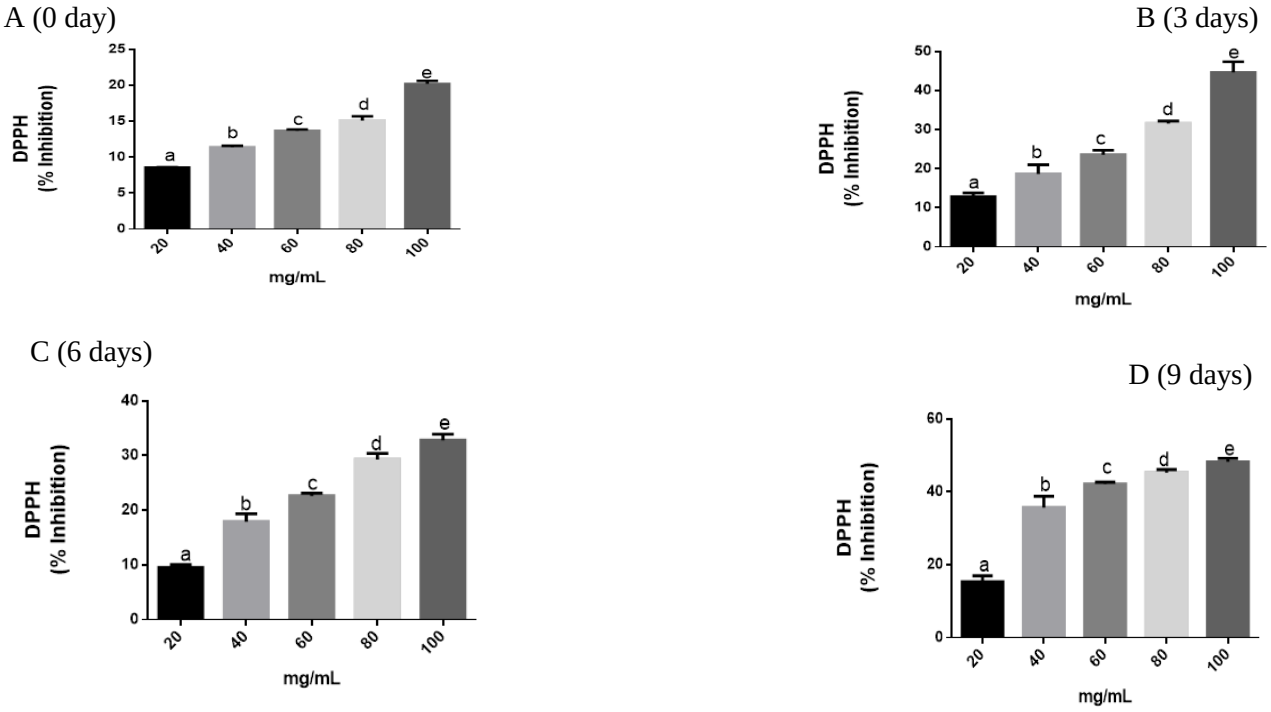

Figure 2. DPPH radical scavenging activity of *C. olitorius* leaf extract at different storage periods under refrigeration (4 °C). Panel A: 0 day; Panel B: 3 days; Panel C: 6 days; Panel D: 9 days. Values represent mean ± SD (n = 3).

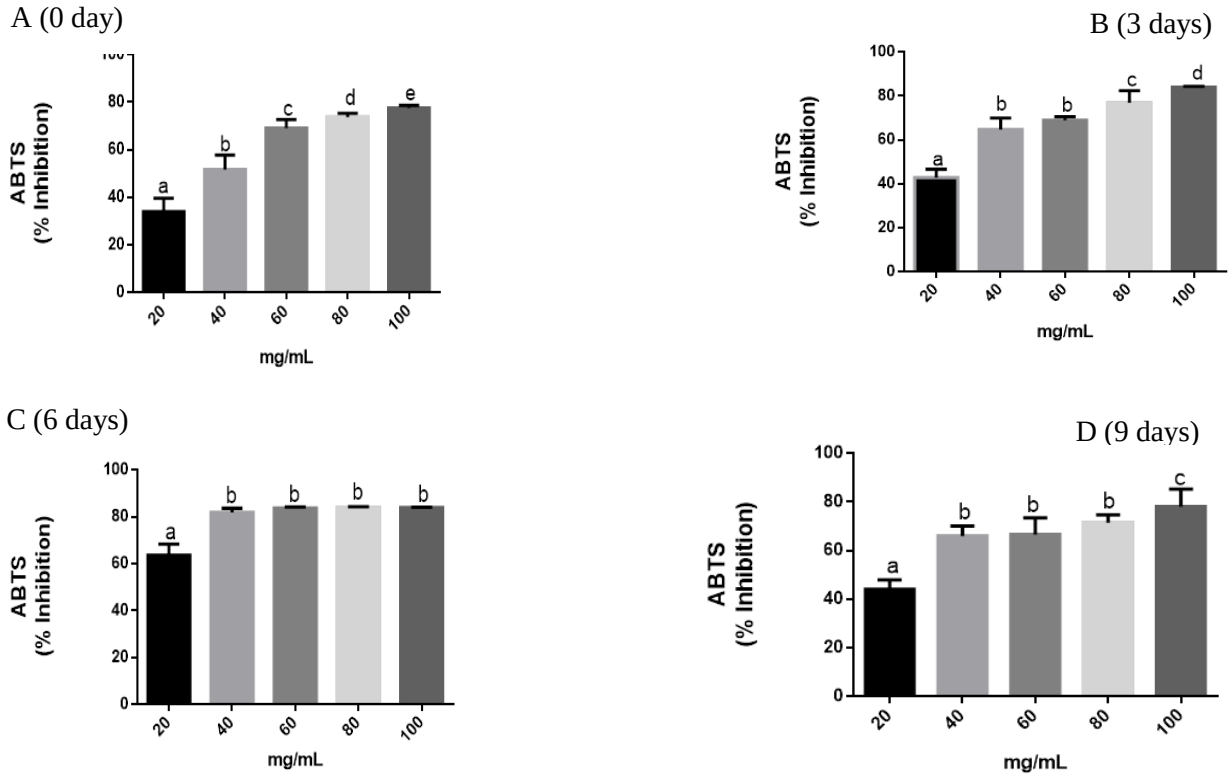

Figure 3 *$ABTS^{+}$• radical scavenging activity of C. olitorius leaf extract at different storage periods under refrigeration (4 °C). Panel A: 0 day; Panel B: 3 days; Panel C: 6 days; Panel D: 9 days. Values represent mean ± SD (n = 3).*

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Discussion

Plants synthesize and accumulate a wide range of secondary metabolites, including polyphenols and flavonoids, which are known to exhibit antioxidant properties by acting as reducing agents, hydrogen donors, and radical scavengers (Kasote et al., 2015). The accumulation of these phytochemicals is influenced by both intrinsic factors, such as plant organs and age, and extrinsic factors, including storage conditions and environmental stressors. In this study, refrigerated storage of *C. olitorius* leaf extract resulted in dynamic changes in polyphenol, flavonoid, and antioxidant profiles, reflecting the stability and activity of these compounds under low-temperature conditions. The observed increase in total polyphenol content and antioxidant capacity during the early stages of storage suggests that refrigerated conditions may favor the release or preservation of phenolic compounds in the leaf extract. Similar trends have been reported in other leafy vegetables and plant extracts, where low-temperature storage slowed degradation and allowed for continued enzymatic or non-enzymatic accumulation of bioactive compounds (Giro & Ferrante, 2018; Maseko et al., 2025). Conversely, the slight decline in polyphenols and flavonoids at later storage periods may be attributed to oxidation, polymerization, or interaction with other phytochemicals, which can reduce their availability and free-radical scavenging potential (Martinez et al., 2025).

The antioxidant activities measured by DPPH and ABTS radical scavenging assays mirrored the trends observed in polyphenol and flavonoid content. The extract demonstrated concentration-dependent radical scavenging activity, indicating that the bioactive compounds contribute significantly to neutralizing free radicals. This relationship aligns with previous reports highlighting a positive correlation between total phenolics, flavonoids, and antioxidant activity in plant extracts (Biswas et al., 2020). The FRAP assay further confirmed that the leaf extract possesses reducing power, supporting the notion that phenolic compounds are critical in electron transfer and radical quenching. The variations in antioxidant activity over the storage period may also reflect structural transformations of polyphenols and flavonoids, as well as changes in their solubility or interactions with other constituents in the extract. These findings are consistent with prior studies that have documented changes in phytochemical content and antioxidant activity during storage or maturation stages of plant materials (Tulio et al., 2002; Martinez et al., 2025; Maseko et al., 2025). These results suggest that *C. olitorius* leaves are a promising source of bioactive compounds, and that storage conditions can modulate the extent of their antioxidant potential.

Conclusion

This study demonstrated that *C. olitorius* leaf extract contains significant phytochemical content and antioxidant potential that vary with storage duration under refrigeration. The results indicate that polyphenols and flavonoids are major contributors to the observed radical scavenging and reducing activities, highlighting their importance in maintaining the antioxidant capacity of the extract over time. Refrigerated storage was shown to preserve or even enhance antioxidant activity during the early stages, with peak activity observed at intermediate storage periods, followed by slight reductions at later stages. The findings underscore the potential of *C. olitorius* leaves as a natural source of bioactive compounds with therapeutic and functional benefits. These results support their inclusion in the human diet as a source of natural antioxidants and suggest that proper storage conditions can help maximize their phytochemical content and antioxidative efficacy.

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