

Comparative evaluation of phenolic content and antioxidant activity of selected fruits and herbs during frozen storage

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ABSTRACT This study evaluates total phenolic content (TPC) and antioxidant activity of mangosteen (*Garcinia mangostana*), bambangan (*Mangifera pajang*), pennywort (*Centella asiatica*), and snake grass (*Clinacanthus nutans*) during frozen storage at -20°C for eight weeks. TPC was determined using the Folin–Ciocalteu method and antioxidant activity was measured by the FRAP assay. Fruits were analyzed on a wet-weight basis and herbs on a freeze dried-weight basis, with measurements taken weekly. By the end of storage, TPC ranged from 37.01 ± 0.22 mg GAE/100 g (wet-weight basis) in fruits to 3783.67 ± 46.28 mg GAE/100 g (freeze-dried weight basis) in herbs. Pennywort exhibited the highest antioxidant activity at week 0 and retained 53153.33 ± 4126 $\mu\text{mol Fe(II)}/100\text{g}$ at week 8. Final FRAP values for snake grass, bambangan, and mangosteen were 1602.49 ± 63.23 , 945.17 ± 28.04 , and 337.86 ± 13.05 $\mu\text{mol Fe(II)}/100\text{g}$, respectively. Across all samples, TPC and FRAP were strongly correlated ($0.836 \leq r \leq 0.971$). At week 8, herbs retained approximately 75%–80% of initial antioxidant activity, while fruits retained about 67%–75%. These data show measurable but matrix-dependent declines in phenolics and reducing capacity during frozen storage. Herbs consistently outperformed fruits in both absolute levels and retention, indicating that pennywort and snake grass are robust sources of phenolics for frozen applications.

KEYWORDS: Frozen storage; Antioxidant activity; Mangosteen; Bambangan; Pennywort; Snake grass

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INTRODUCTION

In recent years, researchers have shown sustained interest in characterizing the bioactive components of food. The primary sources of these compounds in our daily diet are fruits and vegetables, but traditional medicinal herbs also contain numerous bioactive compounds. Polyphenols, including flavonoids, have been widely reviewed for roles in reducing chronic disease risk and maintaining redox homeostasis (Bie *et al.*, 2023). Recent syntheses highlight associations between higher polyphenol intake and lower incidence of cardiovascular disease, certain cancers, type 2 diabetes, and neurodegenerative conditions (Dini *et al.*, 2022). Mechanistically, dietary phenolics donate hydrogen or electrons to neutralize reactive species, chelate pro-oxidant metals, and upregulate endogenous antioxidant enzyme pathways consistent with observed epidemiological effects (Bie *et al.*, 2023). Antioxidant activity of polyphenols is also reflected in inhibition of enzymes such as xanthine oxidase, myeloperoxidase, lipoxygenase, and cyclooxygenase and in synergism with ascorbate (Dini *et al.*, 2022).

Antioxidants may be derived synthetically or naturally. While safety assessments vary by jurisdiction, there is a clear trend toward replacing synthetic additives with plant-derived antioxidants, driven by consumer preference for “clean-label” ingredients and supportive performance data (Gouvêa *et al.*, 2023). This pivot is evident in the sustained growth of research on the extraction, stabilization, and application of polyphenol-rich ingredients (Parveen *et al.*, 2025).

Mangosteen pericarp and pulp contain isoprenylated xanthones (e.g., α -mangostin), anthocyanins and other phenolics with robust in vitro antioxidants and anti-inflammatory activity and growing evidence for broader bioactivities. Recent analytical work has refined quantitative methods to standardize xanthone measurement (Masullo *et al.*, 2022). *Mangifera pajang* (bambangan), widely produced in Borneo, contains phenolics, flavonoids and carotenoids. Current studies confirm substantial antioxidant capacity across pulp, peel, and seed with processing-dependent changes and promising extraction strategies (Chan *et al.*, 2023; Mohd Rosdan *et al.*, 2025). *Centella asiatica* (pennywort) has bioactive triterpenoids (asiaticoside, madecassoside) alongside phenolics. Clinical and preclinical studies support wound-healing, anti-inflammatory and antioxidant effects (Bandopadhyay *et al.*, 2023; Witkowska *et al.*, 2024). *Clinacanthus nutans* (snake grass) exhibits antioxidant and anti-inflammatory activity with schaftoside and related flavonoids as markers (Thongyim *et al.*, 2025).

Despite extensive work measuring plant antioxidants, there are comparatively fewer longitudinal studies on phenolic stability during frozen storage, where degradation depends on tissue, cultivar, and protocol (Neri *et al.*, 2020; Mrázková *et al.*, 2023; Noutfia *et al.*, 2025; Zhao *et al.*, 2025). We therefore investigated TPC and antioxidant activity (FRAP) of mangosteen and bambangan (fruits) and pennywort and snake grass (herbs) during eight weeks of storage at -20°C to clarify stability patterns relevant to frozen products.

METHODOLOGY

Selection Criteria for Fruits and Herbs

Fresh *Garcinia mangostana*, *Mangifera pajang*, *Centella asiatica*, and *Clinacanthus nutans* were obtained from local suppliers in Sabah, Malaysia. Fruits and herbs were visually inspected and only samples that were free from visible defects, bruises, and microbial spoilage were selected. Mangosteen and bambangan fruits at commercial maturity were chosen based on uniform peel colour typical of ripe fruit and firmness appropriate for consumption. Medium sized fruits of similar size were used to minimise variability. For the herbs, only fresh, fully expanded leaves with uniform green colour and without signs of yellowing or wilting were selected.

Preparation of Fruits

After washing the fruits under tap water, pulps were separated, blended to purees, and stored at -20°C until analysis. Processing was completed within 24 h of purchase. Aliquots were prepared to avoid repeat freeze-thaw cycles (Coskun & Isbilir, 2024). All samples were prepared in triplicate.

Preparation of Herbs

Roots of herbs were removed, leaves and petioles were washed and blotted dry. Samples were freeze-dried (Alpha 2-4 LD Plus, Christ), milled, and stored in airtight containers at -20°C . Freeze-drying was selected as it retains higher flavonoid and phenolic content than hot-air or vacuum drying in *C. asiatica* and similar botanicals (Babaei *et al.*, 2025; Thongyim *et al.*, 2025).

Extraction of Samples

Ten grams of samples were extracted with 90 mL methanol:water (80:20, v/v). Mixtures were shaken 1 h at room temperature and kept in the dark for 24 h, then centrifuged ($13,680 \times g$, 10 min) and filtered ($0.45 \mu\text{m}$) (Karaman *et al.*, 2014). The 80:20 methanol-water system provides broad spectrum recovery of phenolics, $0.45 \mu\text{m}$ clarification reduces turbidity for spectrophotometry. Extracts were protected from light and stored at -20°C .

Determination of Total Phenolic Content (TPC)

The total phenolic content was determined using the Folin–Ciocalteu assay according to Karaman *et al.* (2014). A 0.1 mL of the extract was mixed with 0.75 mL of Folin–Ciocalteu reagent and vortexed for 15 s. Then, 0.75 mL of sodium carbonate solution (60 g/L) was added to the mixture. The reaction mixture was incubated at room temperature for 120 min and the absorbance was measured at 760 nm using a spectrophotometer. Results were expressed as mg GAE/kg, reported as mg GAE/100 g fresh weight for fruits or per freeze-dried weight for herbs. The assay operates via electron transfer under alkaline conditions. Calibration used gallic acid ($R^2 \geq 0.99$) with reagent blanks (Perez *et al.*, 2023). The total phenolic content of the samples was calculated as milligrams of gallic acid equivalents (GAE/kg) of samples.

Ferric Reducing Antioxidant Power (FRAP)

FRAP followed Benzie & Strain (1996) method, where reagent was prepared as follows; 300 mM acetate buffer pH 3.6 : 10 mM TPTZ in 40 mM HCl : 20 mM FeCl_3 ; 10:1:1. Cuvette blanked with 3 mL FRAP at 593 nm, then 0.1 mL extract + 0.3 mL water added, and ΔA_{593} at 4 min was compared to FeSO_4 standards (0–1.0 mM; $R^2 \geq 0.99$). Results are expressed as mM/100 g or $\mu\text{mol Fe(II)}/100 \text{ g}$, readings were taken within a ≤ 6 min linear window (Benzie & Strain, 1996).

Statistical Analysis

All analyses were carried out in triplicate, and the results are expressed as mean \pm standard deviation. Data was analyzed using the Statistical Package for the Social Sciences (SPSS, version 23, IBM Corp., NY, USA). The effect of frozen storage time on TPC and FRAP for each sample was evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Differences among the four plant materials at each storage week were also assessed using one-way ANOVA. Pearson correlation analysis was used to determine the relationships between total phenolic content and FRAP values. Statistical significance was set at $p < 0.05$.

RESULT AND DISCUSSION

Total Phenolic Content

Fruits

Fruits analyzed were mangosteen and bambangan. As shown in Figure 1, at week 0 (day 1) the phenolic content in mangosteen was $55.31 \pm 1.17 \text{ mg GAE}/100 \text{ g}$ and, with slight fluctuations, decreased to $37.01 \pm 0.22 \text{ mg GAE}/100 \text{ g}$ (wet weight) by week 8. The total phenolic content decreased by 33.09%. Mangosteen was collected out of season, but pre-purchase handling history (for example, time since harvest, transport duration, temperature, and light exposure) was not documented in this study. Therefore, the lower TPC cannot be directly attributed to storage or transport conditions based on the present data. Nevertheless, the observed values may reflect seasonal variability and/or postharvest handling effects reported in prior work. In addition, direct comparison with literature should consider basis differences, as some reports present phenolics on a dry-weight basis (Naczek *et al.*, 2011), whereas the current values are on a wet-weight basis. Off-season mangosteen has also been reported to show lower extractable phenolics than peak-season fruit (Matan *et al.*, 2024), which is consistent with the present pattern.

Bambangan recorded $104.52 \pm 2.35 \text{ mg GAE}/100 \text{ g}$ (wet weight) at week 0 and decreased steadily to $78.71 \pm 2.96 \text{ mg GAE}/100 \text{ g}$ by week 8 (–24.69%). Compared with mangosteen, bambangan started higher and retained a greater percentage of its phenolics. This suggests relatively greater frozen-

state stability of bambangan phenolics (e.g., mangiferin-rich profiles) compared with more labile classes in mangosteen (Zhang *et al.*, 2021; Zhao *et al.*, 2025).

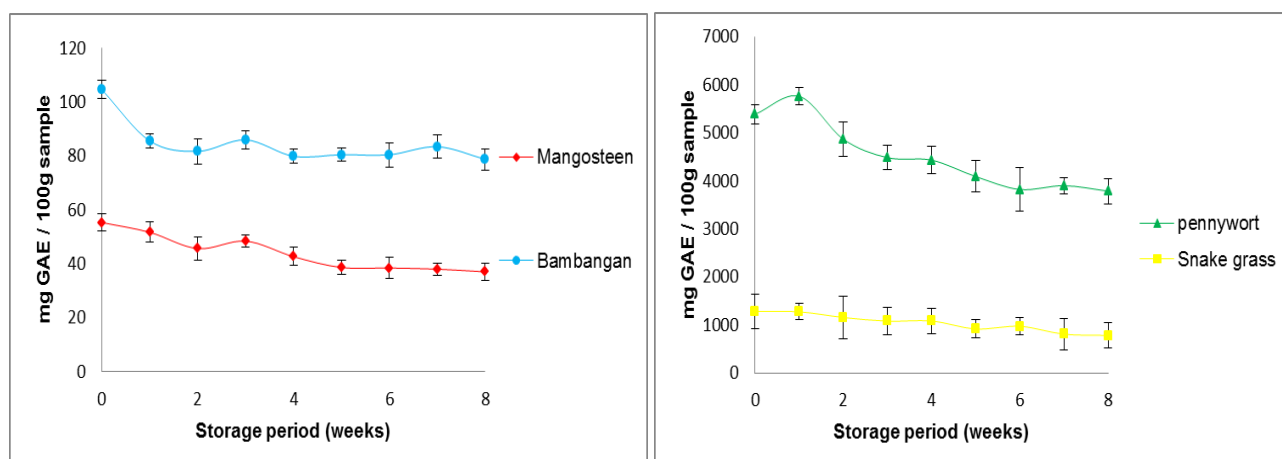


Figure 1. Total phenolic content of fruits and herbs during eight weeks of storage presented as mean \pm SD ($n = 3$).

Wet-weight reporting includes 70%–90% water, thus apparent TPC values are lower than dry-weight literature values despite similar actual phenolic yields. Comparative retention studies of fresh vs frozen produce emphasize this basis effect (Bouzari *et al.*, 2015). Differences from Naczka *et al.* (2011) and Ibrahim *et al.* (2010) likely reflect dry- vs wet-weight expression and preprocessing (e.g., drying, blanching) which alter extractability and apparent stability (Kaseke *et al.*, 2020; Zhang *et al.*, 2021).

Herbs

Pennywort and snake grass are known to contain high phenolic levels (Arullappan *et al.*, 2014; Rahman *et al.*, 2013). From Figure 1, pennywort shows a significantly higher TPC: 5381.84 ± 39.60 mg GAE/100 g (freeze dried) at week 0, a slight increase at week 2, then a gradual decline to 3783.67 ± 46.28 mg GAE/100 g at week 8 (–29.69%). Transient early rises followed by decline are reported in chilled or frozen storage as cell disruption increases extractability before oxidative or polymerization losses dominate (King *et al.*, 2022; Zhao *et al.*, 2025).

Snake grass began at 1281.98 ± 39.53 mg GAE/100 g (freeze dried) and decreased to 780.40 ± 66.34 mg GAE/100 g by week 8 (–39.13%), with a minor rebound at week 6. Losses of this magnitude over weeks are consistent with herb leaves when oxidative enzymes remain active unless blanched; enzyme-inactivation pretreatments moderate declines (Zhang *et al.*, 2021).

Fruits vs herbs (TPC)

Across all four samples, pennywort had the highest initial and final TPC (5381.84 ± 39.60 to 3783.67 ± 46.28 mg GAE/100 g). This was followed by snake grass, bambangan, and mangosteen. Although bambangan had the lowest relative loss (–24.69%), pennywort retained a much higher absolute TPC because of its very high baseline. Large composition datasets similarly rank herbs or spices among the top sources of phenolics and reducing capacity compared with most fruits (Carlsen *et al.*, 2010; Bouzari *et al.*, 2015). Retention behavior is commodity-specific, phenolic classes such as anthocyanins degrade faster than proanthocyanidins, so stability is driven more by phenolic profile than by fruit vs herb category (Zhang *et al.*, 2021; Zhao *et al.*, 2025). TPC varied week-to-week with overall reduction by week 8. One-sample t-tests indicated significant differences ($p < 0.05$) for

all samples; none retained $\geq 95\%$ phenolics. Comparable fluctuations and early-period losses are common in frozen plant tissues (King *et al.*, 2022; Zhang *et al.*, 2021).

Fluctuations likely reflect polyphenol oxidase and peroxidase-mediated oxidation during thawing and handling, and slow non-enzymatic degradation (e.g., anthocyanin loss and polymerization). Blanching and oxygen-limiting packaging mitigate these effects (Kaseke *et al.*, 2020; Zhang *et al.*, 2021; Zhao *et al.*, 2025). Genotype, agronomy, maturity, and washing influence phenolic levels and stability, and water-soluble phenolics can leach during washing. Comparative studies across commodities show minimal average differences between properly handled fresh and frozen items, with species-specific deviations (Esseberri *et al.*, 2022). These findings support optimizing storage time and temperature, and pretreatments on a matrix-specific basis (Zhang *et al.*, 2021; Zhao *et al.*, 2025).

Ferric Reducing Antioxidant Power (FRAP)

Fruits

For mangosteen, FRAP was $448.12 \pm 19.51 \mu\text{mol Fe(II)}/100 \text{ g}$ at week 0, decreased slightly to week 2, rose at week 3, dropped sharply at week 4, and ended at $337.86 \pm 13.05 \mu\text{mol Fe(II)}/100 \text{ g}$ by week 8 (-24.61%) as shown in Figure 2. Bambang began at $1403.65 \pm 19.55 \mu\text{mol Fe(II)}/100 \text{ g}$, decreased to $900.09 \pm 14.85 \mu\text{mol Fe(II)}/100 \text{ g}$ at week 1, rebounded to $1106.22 \pm 123.52 \mu\text{mol Fe(II)}/100 \text{ g}$ at week 2, then declined to $945.17 \pm 28.04 \mu\text{mol Fe(II)}/100 \text{ g}$ by week 8 (-32.67%). The week 1 to 2 variation likely reflects analytical and biological variability. Similar rebound then decline behavior has been reported in frozen berries and tropical pulps due to early increases in extractability followed by oxidative/enzymatic losses (King *et al.*, 2022; Zhao *et al.*, 2025). Despite larger percentage loss, bambang maintained a far higher absolute FRAP than mangosteen throughout, consistent with its higher TPC and the typical TPC–FRAP linkage during storage (Zhang *et al.*, 2021).

Herbs

Pennywort's reducing ability was 30 to 40 times higher than snake grass on a freeze dried-weight basis, $70863.21 \pm 1090.83 \mu\text{mol Fe(II)}/100 \text{ g}$ at week 0, declining to $53153.33 \pm 4126 \mu\text{mol Fe(II)}/100 \text{ g}$ at week 8 (-24.99%) as per Figure 2. Snake grass started at $1927.84 \pm 4.10 \mu\text{mol Fe(II)}/100 \text{ g}$, fell to $1602.49 \pm 63.23 \mu\text{mol Fe(II)}/100 \text{ g}$ by week 8 (-20.30%), with a slight uptick at week 6. The markedly lower absolute FRAP of snake grass relative to pennywort is consistent with *C. asiatica*'s high phenolic content and reducing capacity compared with many leafy herbs, while *C. nutans* shows more modest absolute values but similar directional change (King *et al.*, 2022; Zhang *et al.*, 2021).

Interestingly, despite snake grass recording a moderate total phenolic content (around 1,000–1,300 mg GAE/100 g), its FRAP values remained relatively low at approximately $1,000 \mu\text{mol Fe(II)}/100 \text{ g}$. This pattern has been previously observed in *Clinacanthus nutans* and is consistent with published data (Kong *et al.*, 2019). The discrepancy likely reflects the specific phytochemical composition of the plant: C-glycosyl flavones such as schaftoside and orientin dominate its phenolic profile, and these compounds typically exhibit lower ferric-reducing activity compared to aglycone polyphenols. The FRAP assay is sensitive to the redox potential of antioxidants, and not all phenolics contribute equally. Thus, while snake grass contains a substantial amount of phenolics, their structural characteristics result in reduced efficacy in the FRAP test. This highlights the need to interpret antioxidant activity not solely by quantity of phenolics, but also by compound type and assay suitability.

Broad horticultural screenings reveal genotype-driven ranges for phenolics and antioxidant capacity, reinforcing likely cultivar effects (Cantín *et al.*, 2009). Environmental conditions such as

temperature, water, light, minerals, and soil, and cultivation system of organic vs conventional method also affect phenolics (Esseberri *et al.*, 2022). Metabolic changes begin immediately after harvest where storage temperature, atmosphere, and chemical treatments influence phenolic quantity and quality. Enzyme-inactivation and oxygen-limiting approaches before or during freezing mitigate phenolic losses and can improve retention (Kaseke *et al.*, 2020; Zhang *et al.*, 2021).

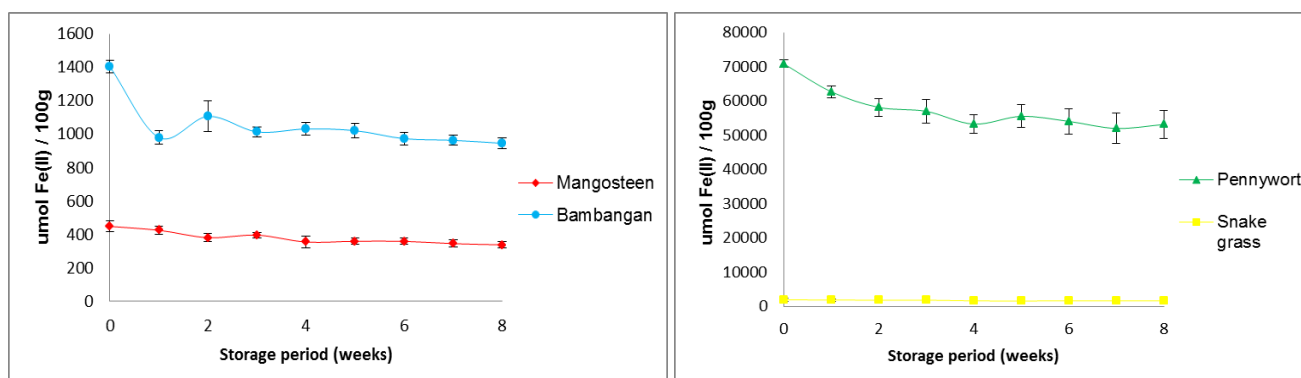


Figure 2. Antioxidant activities of fruits and herbs measured using FRAP for a duration of eight weeks; presented as mean \pm SD ($n = 3$).

Fruits vs herbs (FRAP)

On their respective reporting bases (freeze-dried herbs vs fresh fruit pulp), herbs show higher antioxidant activities. However, because these values are expressed on different mass bases, they should not be interpreted as a direct absolute comparison. By week 8, herbs retained about 75%–80% of initial FRAP, while fruits retained about 67%–75%. These findings are consistent with Carlsen *et al.* (2010), where herbs and spices ranked highest in antioxidant content among >3100 foods. Our pattern also agrees with current storage studies showing that matrices with higher initial phenolic content (often herbs and spices) maintain higher absolute reducing capacity throughout storage despite proportional declines (King *et al.*, 2022; Zhao *et al.*, 2025).

As shown in Table 1, total phenolic content was strongly and positively correlated with ferric reducing antioxidant power in all four samples, with correlation coefficients ranging from 0.836 to 0.971 ($p < 0.01$). The highest correlation was observed in mangosteen ($r = 0.971$), followed by bambangan ($r = 0.895$), snake grass ($r = 0.847$), and pennywort ($r = 0.836$). These strong correlations indicate that, within each plant matrix, higher phenolic content is associated with greater ferric reducing capacity, and support the view that phenolic compounds are important contributors to the antioxidant activity of these fruits and herbs. The slightly lower correlation coefficients in pennywort and snake grass may reflect the contribution of other non-phenolic antioxidants or differences in phenolic composition that influence the FRAP response. Overall, the correlation analysis confirms that changes in total phenolic content during frozen storage are closely mirrored by changes in antioxidant activity across all samples.

Table 1. Pearson's correlation of total phenolic content and ferric reducing antioxidant power of fruits and herbs.

TPC and FRAP pairs	Correlation Value
Mangosteen	.971**
Bambangan	.895**
Pennywort	.836**
Snake grass	.847**

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

CONCLUSION

TPC and FRAP of mangosteen, bambangan, pennywort and snake grass fluctuated and declined during eight weeks of frozen storage at -20°C . Herbs exhibited higher absolute antioxidant activities than fruits and retained a larger fraction of initial capacity by week 8. Pennywort showed the highest values, followed by snake grass, bambangan and mangosteen. These findings reinforce that freezing is a viable preservation method that retains a substantial portion of phenolic-linked antioxidant capacity, though stability is matrix and compound dependent.

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