Effect of Thermal Extraction Conditions on Antioxidant Capacity, Phenolic Composition and Pancreatic Lipase Inhibitory Activity of Hibiscus Sabdariffa Water Extract

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ABSTRACT

Thermal processing plays a critical role in modulating the phytochemical composition and biological activity of plant extracts. This study investigated the effects of extraction temperature (40–100 °C) and duration (15–180 min) on total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity, and pancreatic lipase inhibitory activity of Hibiscus sabdariffa water extract. TPC was maximized at 40 °C for 30 min (48 mg GAE/g), whereas TFC peaked at 60 °C for 30 min (650 mg CE/g). DPPH radical scavenging activity remained relatively stable across extraction conditions (75–90%), with optimal activity observed at 80 °C for 120 min. In contrast, ferric reducing antioxidant power (FRAP) increased with temperature, reaching a maximum at 100 °C for 120 min (2100 μM Fe^{2+}/g). Notably, pancreatic lipase inhibition was highest at 100 °C for 30 min (92%), approaching the activity of the reference inhibitor orlistat. Antioxidant activity did not directly correlate with total phenolic or flavonoid content, indicating that qualitative changes in phytochemical composition contribute significantly to bioactivity. These findings demonstrate that extraction parameters should be optimized based on the targeted functional endpoint, with high-temperature short-time conditions favoring enzyme inhibition, while moderate conditions preserve antioxidant-associated phytochemicals. This study provides a bioactivity-driven framework for optimizing aqueous extraction of H. sabdariffa for functional food and nutraceutical applications.

Keywords: Hibiscus sabdariffa; thermal extraction; polyphenols; antioxidant activity; DPPH; FRAP; pancreatic lipase inhibition; nutraceutical optimization

INTRODUCTION

Oxidative stress, resulting from excessive production of reactive oxygen species (ROS), is widely recognized as a major contributing factor to the development of chronic diseases such as cardiovascular disorders, diabetes mellitus, metabolic syndrome, and obesity (Reddy, 2023). Reactive oxygen species can initiate lipid peroxidation, impair cellular macromolecules, and disrupt metabolic homeostasis (Su *et al*., 2019). Dietary antioxidants derived from plant sources play an essential role in neutralizing free radicals and mitigating oxidative damage (Lobo *et al*., 2010). Among these, phenolic compounds and flavonoids have attracted considerable attention due to their strong radical-scavenging, reducing, and metal-chelating properties (Panche *et al*., 2016).

Hibiscus sabdariffa L., (*H. sabdariffa*) commonly known as roselle, is a tropical plant widely consumed as a herbal beverage and traditional remedy (Almajid *et al*., 2023). The calyces are particularly rich in anthocyanins, phenolic acids, and flavonoids, which contribute to its reported antioxidant, anti-inflammatory, antihypertensive, and hypolipidemic effects (Ghaly *et al*., 2025). Numerous studies have demonstrated that *H. sabdariffa* extracts exhibit substantial antioxidant activity through DPPH radical scavenging, FRAP, and other in vitro assays (Wu *et al*., 2018). In addition to antioxidant properties, *H. sabdariffa* has been associated with lipid-lowering and anti-obesity potential, possibly through modulation of lipid metabolism and digestive enzyme inhibition (Yang *et al*., 2025).

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Pancreatic lipase is a key enzyme responsible for the hydrolysis of dietary triglycerides into absorbable free fatty acids (Subramaniyan *et al*., 2025). Inhibition of pancreatic lipase reduces fat absorption and represents an established therapeutic strategy for obesity management (Tran *et al*., 2014). Synthetic inhibitors such as orlistat (Xenical) are clinically effective but may produce undesirable gastrointestinal side effects (Beyea *et al*., 2012). Therefore, identification of natural lipase inhibitors from plant sources has gained increasing research interest. Several phenolic-rich plant extracts have been shown to inhibit pancreatic lipase activity, suggesting that phenolic composition may contribute to anti-obesity potential (Yang *et al*., 2015).

Extraction methodology plays a critical role in determining the yield, composition, and bioactivity of phytochemicals (Fitri *et al*., 2025). Thermal processing, including variations in temperature and extraction duration, can significantly influence phenolic recovery and structural stability (Narra *et al*., 2024). While moderate heating may enhance extraction efficiency by disrupting plant cell matrices, excessive or prolonged heating may lead to degradation, oxidation, or polymerization of phenolic compounds (Sun *et al*., 2025). Consequently, thermal parameters may differentially affect antioxidant capacity and enzyme inhibitory activities (Frlin *et al*., 2025).

Although *H. sabdariffa* has been extensively investigated for its antioxidant properties, systematic evaluation of how thermal extraction conditions simultaneously influence phenolic profile, antioxidant capacity, and pancreatic lipase inhibitory activity remains limited. Understanding these interactions is essential for optimizing extraction strategies aimed at maximizing functional bioactivity.

Therefore, the present study aimed to investigate the influence of thermal processing (40–100°C; 15 min–3 h) on the phenolic composition, antioxidant potential (DPPH and FRAP), and pancreatic lipase inhibitory activity of *H. sabdariffa* water extract. By integrating antioxidant profiling with enzyme inhibition assessment, this study provides insight into the optimization of *H. sabdariffa* extraction for potential functional food and nutraceutical applications.

MATERIALS AND METHODS

Plant Material

Dried calyces of *H. sabdariffa* L. were obtained from a certified local supplier in Malaysia and authenticated by a botanist at the Forest Research Institute Malaysia (FRIM), Kepong, Selangor. The plant material was cleaned to remove foreign debris and ground into a fine powder using a laboratory grinder. The powdered sample was stored in airtight containers at room temperature (25 ± 2 °C) in a dry and dark environment until further analysis.

Preparation of Water Extract under Different Thermal Conditions

The aqueous extracts were prepared by varying extraction temperature and duration to evaluate the influence of thermal processing on bioactivity. Briefly, 5 g of dried *H. sabdariffa* powder was mixed with 100 mL of distilled water (solid-to-solvent ratio 1:20, w/v) in a conical flask. The mixture was subjected to extraction at controlled temperatures of 40, 60, 80, and 100 °C using a thermostatically regulated water bath.

For each temperature, extraction was performed at different time intervals: 15 min, 30 min, 1 h, 2 h, and 3 h. The flasks were covered with aluminum foil to minimize evaporation and oxidation during heating. After extraction, the mixtures were cooled to room temperature and filtered through Whatman No. 1 filter paper. The filtrates were centrifuged at 4000 rpm for 10 min to remove residual particulates.

The supernatants were collected and stored at −20 °C until analysis. All extractions were performed in triplicate.

Determination of TPC

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method with slight modifications. Briefly, 200 µL of appropriately diluted extract was mixed with 1.0 mL of 10% (v/v) Folin–Ciocalteu reagent. After 5 min of incubation at room temperature, 800 µL of 7.5% (w/v) sodium carbonate solution was added. The reaction mixture was vortexed and incubated in the dark for 30 min at room temperature.

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Absorbance was measured at 765 nm using a UV–Vis spectrophotometer. Gallic acid was used to construct a calibration curve (0–200 µg/mL), and results were expressed as milligrams of gallic acid equivalents per gram of dry sample (mg GAE/g). All measurements were performed in triplicate.

Determination of TFC

Total flavonoid content was determined using the aluminum chloride colorimetric method. Briefly, 500 µL of extract was mixed with 1.5 mL of methanol, followed by 100 µL of 10% aluminum chloride solution, 100 µL of 1 M potassium acetate, and 2.8 mL of distilled water.

The mixture was incubated at room temperature for 30 min. Absorbance was measured at 415 nm against a reagent blank. Catechin was used as the standard reference compound, and results were expressed as catechin equivalents (CE). All analyses were conducted in triplicate.

DPPH Radical Scavenging Assay

The antioxidant capacity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. A 0.1 mM DPPH solution was prepared in methanol.

An aliquot of 1.0 mL of extract was mixed with 2.0 mL of DPPH solution and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer.

The percentage of radical scavenging activity was calculated using the following equation:

$$\%\text{DPPH inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ represents the absorbance of the control (DPPH solution without extract) and A_{sample} represents the absorbance in the presence of extract.

Ascorbic acid was used as a positive control. All assays were performed in triplicate.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was conducted according to the method described by Benzie and Strain with minor modifications. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1 (v/v/v).

Briefly, 100 µL of extract was added to 3.0 mL of FRAP reagent and incubated at 37 °C for 4 min. Absorbance was measured at 593 nm.

A standard curve was constructed using ferrous sulfate ($FeSO_4 \cdot 7H_2O$), and results were expressed as µM Fe^{2+} equivalents per gram of sample. All measurements were carried out in triplicate.

Pancreatic Lipase Inhibitory Assay

Pancreatic lipase inhibitory activity was determined spectrophotometrically using porcine pancreatic lipase and p-nitrophenyl palmitate (pNPP) as the substrate.

Briefly, 100 µL of extract was pre-incubated with 100 µL of pancreatic lipase solution (1 mg/mL in phosphate buffer, pH 7.4) at 37 °C for 15 min. Subsequently, 100 µL of pNPP substrate solution was added to initiate the reaction. The reaction mixture was incubated at 37 °C for 30 min.

The release of p-nitrophenol was measured at 405 nm using a microplate reader. Xenical (orlistat) was used as a positive control.

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The percentage of lipase inhibition was calculated as:

$$\%\text{Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ represents enzyme activity without inhibitor and A_{sample} represents enzyme activity in the presence of extract.

All experiments were performed in triplicate.

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine significant differences among extraction conditions. Differences were considered statistically significant at $p < 0.05$.

Results

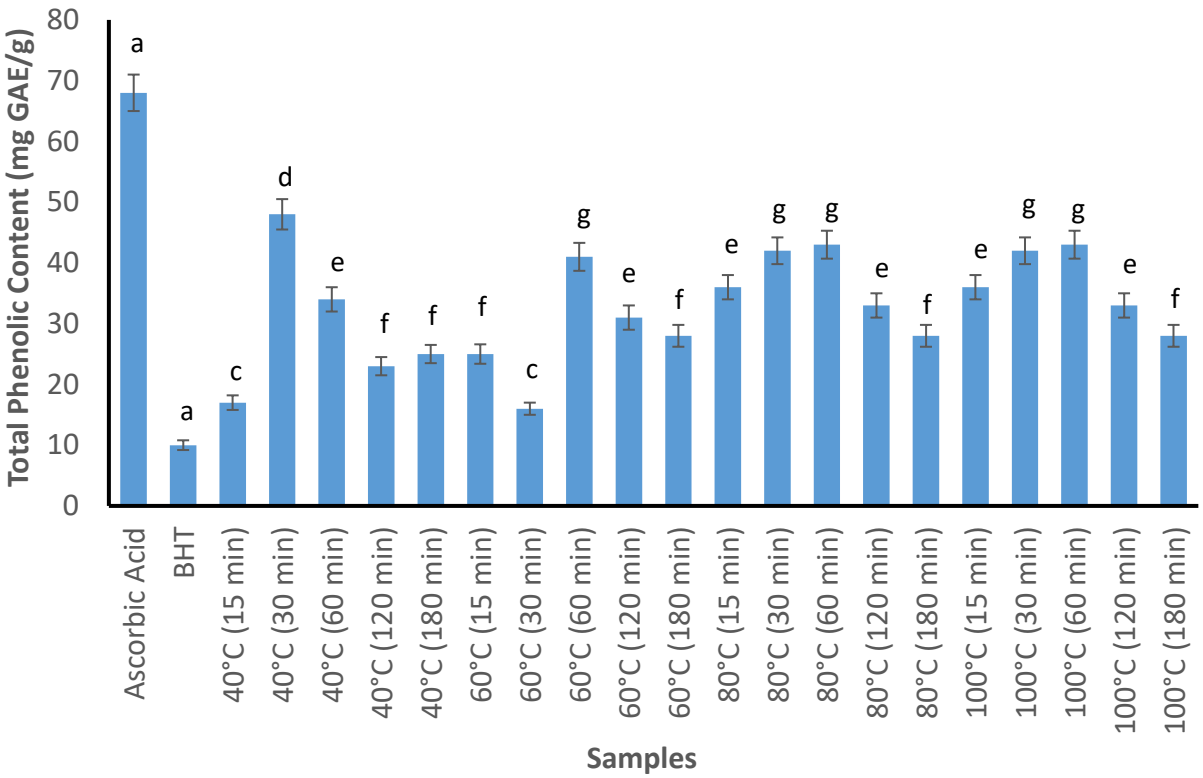

Figure 1. Total phenolic content (TPC) of *H. sabdariffa* water extracts obtained under different thermal extraction conditions (40–100 °C; 15 min–3 h). Results are expressed as mg gallic acid equivalents (GAE)/g sample. Ascorbic acid and BHT were used as reference standards. Data are presented as mean ± SD. Different superscript letters indicate statistically significant differences between samples at $P < 0.05$.

The TPC of *H. sabdariffa* water extracts was significantly influenced by extraction temperature and duration ($P < 0.05$). Ascorbic acid exhibited the highest Folin-reactive reducing capacity, although it is not a phenolic compound, while BHT showed the lowest, reflecting its non-phenolic nature. At lower temperature (40 °C), TPC increased with extraction time, reaching a maximum at 30 min (48 mg GAE/g), followed by a gradual decline with prolonged extraction (60–180 min). This suggests that moderate extraction time enhances phenolic release, whereas extended exposure may lead to degradation or oxidation of thermolabile compounds.

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A similar trend was observed at 60 °C, where TPC remained low at shorter durations (15–30 min) but increased significantly at 60 min (40 mg GAE/g), before decreasing at longer extraction times. This indicates that higher temperature requires an optimal duration to maximize phenolic recovery.

At elevated temperatures (80–100 °C), TPC values were generally higher and reached their peak within shorter extraction durations (30–60 min; 41–43 mg GAE/g). This suggests that increased temperature enhances mass transfer and facilitates the release of bound phenolics from plant matrices. However, prolonged exposure (120–180 min) resulted in reduced TPC, likely due to thermal degradation, polymerization, or oxidation of phenolic compounds, particularly heat-sensitive anthocyanins.

Broadly, these results demonstrate that phenolic extraction is governed by a balance between enhanced release at higher temperatures and degradation under prolonged thermal conditions. The findings highlight that optimal extraction parameters are both temperature- and time-dependent, with high-temperature short-time conditions providing efficient phenolic recovery without significant degradation.

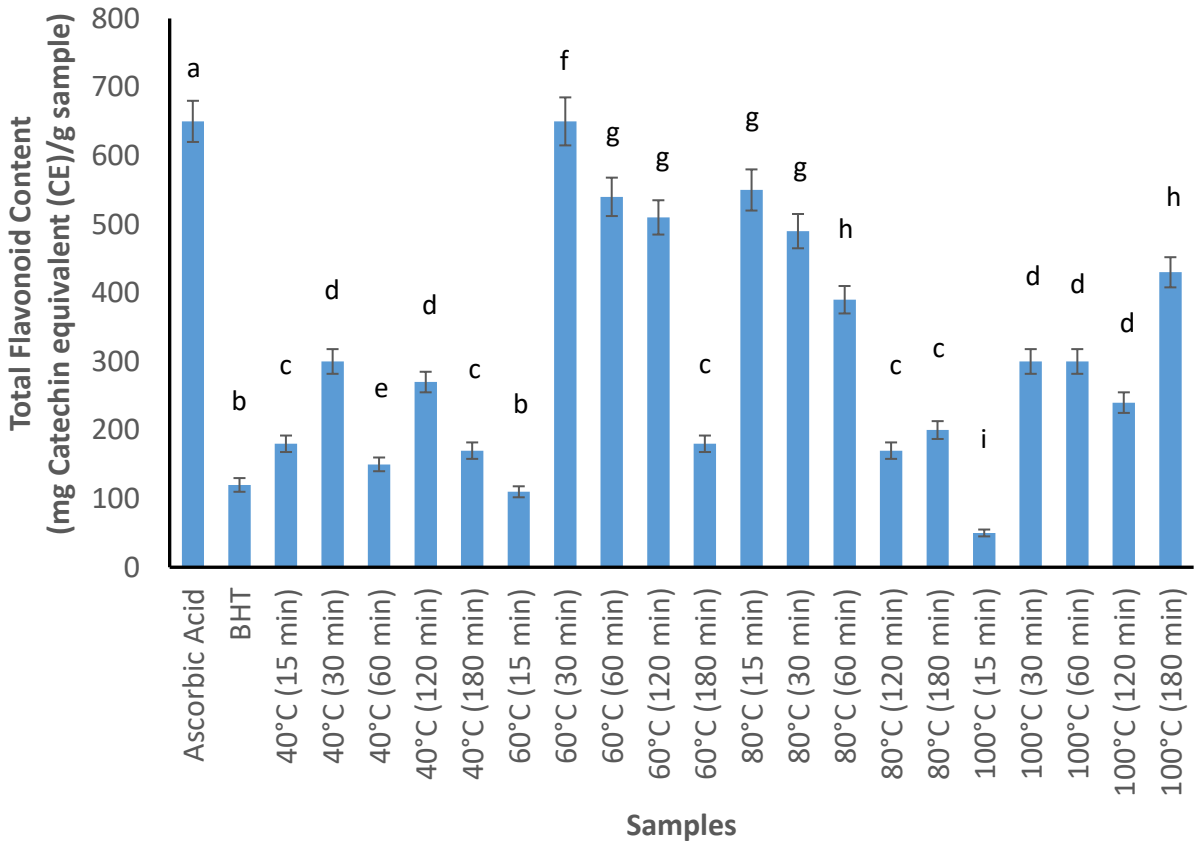

Figure 2. Total flavonoid content (TFC) of *H. sabdariffa* water extracts obtained under different thermal extraction conditions (40–100 °C; 15 min–3 h). Results are expressed as mg catechin equivalents (CE)/g sample. Ascorbic acid and BHT were used as reference standards. Data are presented as mean ± SD. Different superscript letters indicate statistically significant differences between samples at $P < 0.05$.

The TFC of *H. sabdariffa* water extracts was significantly affected by extraction temperature and duration ($P < 0.05$). Ascorbic acid exhibited the highest flavonoid equivalent value, while BHT showed the lowest, confirming the validity of the assay.

At 40 °C, TFC increased with extraction time, reaching a maximum at 30 min (300 mg CE/g), followed by a decline at longer durations (60–180 min). This suggests that moderate extraction time enhances flavonoid release, whereas prolonged exposure may lead to degradation of heat-sensitive flavonoids. At 60 °C, TFC values were markedly higher compared to 40 °C, with a peak observed at 30 min (650 mg CE/g), representing the highest flavonoid content among all conditions. However, further prolongation of extraction time resulted in a

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gradual decrease in TFC, indicating possible thermal degradation or structural modification of flavonoid compounds.

At 80 °C, relatively high TFC values were also observed, particularly at shorter durations (15–30 min; 500–550 mg CE/g). However, a consistent decline was noted with increasing extraction time, suggesting that elevated temperature accelerates both extraction and degradation processes. In contrast, extraction at 100 °C showed a pronounced reduction in TFC at shorter duration (15 min; 50 mg CE/g), indicating severe thermal degradation of flavonoids under harsh conditions. Although TFC partially recovered at longer durations (30–180 min), the values remained lower than those observed at 60–80 °C, suggesting that excessive heat compromises flavonoid stability.

Generally, these results demonstrate that flavonoid extraction is highly sensitive to thermal conditions, with optimal recovery achieved at moderate temperature and controlled extraction time. Excessive temperature or prolonged exposure leads to significant degradation, highlighting the importance of balancing extraction efficiency with compound stability.

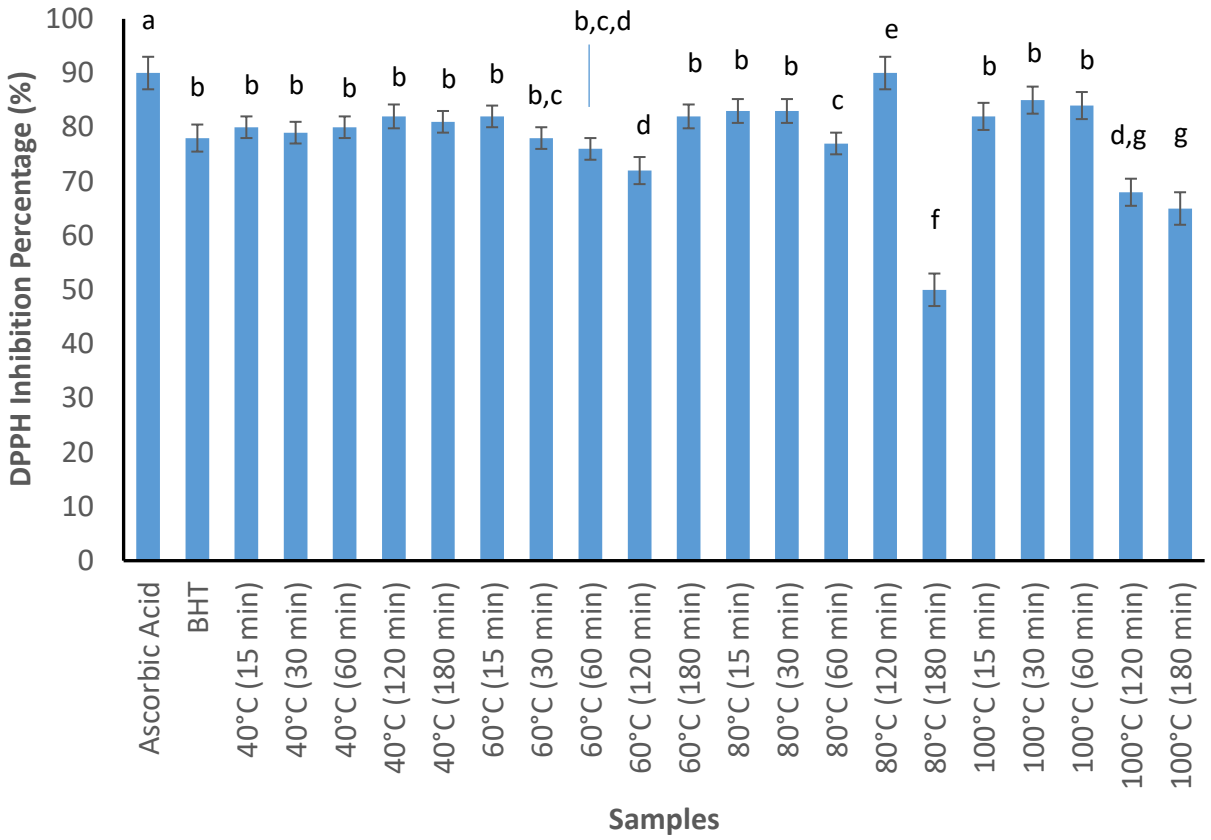

Figure 3. DPPH radical scavenging activity (%) of *H. sabdariffa* water extracts obtained under different thermal extraction conditions (40–100 °C; 15 min–3 h). BHT and ascorbic acid were used as reference standards. Data are presented as mean ± SD. Different superscript letters indicate statistically significant differences between samples at $P < 0.05$.

The DPPH radical scavenging activity of *H. sabdariffa* water extracts was significantly influenced by extraction temperature and duration ($P < 0.05$). Ascorbic acid exhibited the highest antioxidant activity (90%), while BHT and most extracts showed slightly lower but comparable values, confirming the reliability of the assay. As a whole, the majority of extracts demonstrated relatively high DPPH scavenging activity, ranging between approximately 75–85%, indicating that antioxidant capacity was largely preserved across a wide range of extraction conditions. At 40 °C, DPPH activity remained relatively stable regardless of extraction time, suggesting that mild thermal conditions do not significantly affect the radical scavenging ability of the extract.

At 60 °C, a slight variation in activity was observed, with a minor decrease at intermediate durations (30–60 min), followed by recovery at longer extraction time. This may reflect partial degradation of certain antioxidant

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compounds followed by the release of other active constituents. At 80 °C, a notable increase in DPPH activity was observed at 120 min (90%), representing one of the highest antioxidant activities among all conditions. This suggests that elevated temperature may enhance the formation or release of compounds with strong hydrogen-donating capacity. However, a sharp decline was observed at 80 °C for 180 min (50%), indicating significant degradation of antioxidant compounds under prolonged thermal exposure.

At 100 °C, DPPH activity remained relatively high at shorter durations (15–60 min; 80–85%), but decreased at longer durations (120–180 min), further supporting the idea that excessive heat and prolonged extraction lead to the breakdown of bioactive compounds. Overall, these findings indicate that DPPH radical scavenging activity is relatively robust across different thermal conditions but is sensitive to prolonged exposure at high temperatures. Optimal antioxidant activity appears to be achieved at moderate-to-high temperatures with controlled extraction duration.

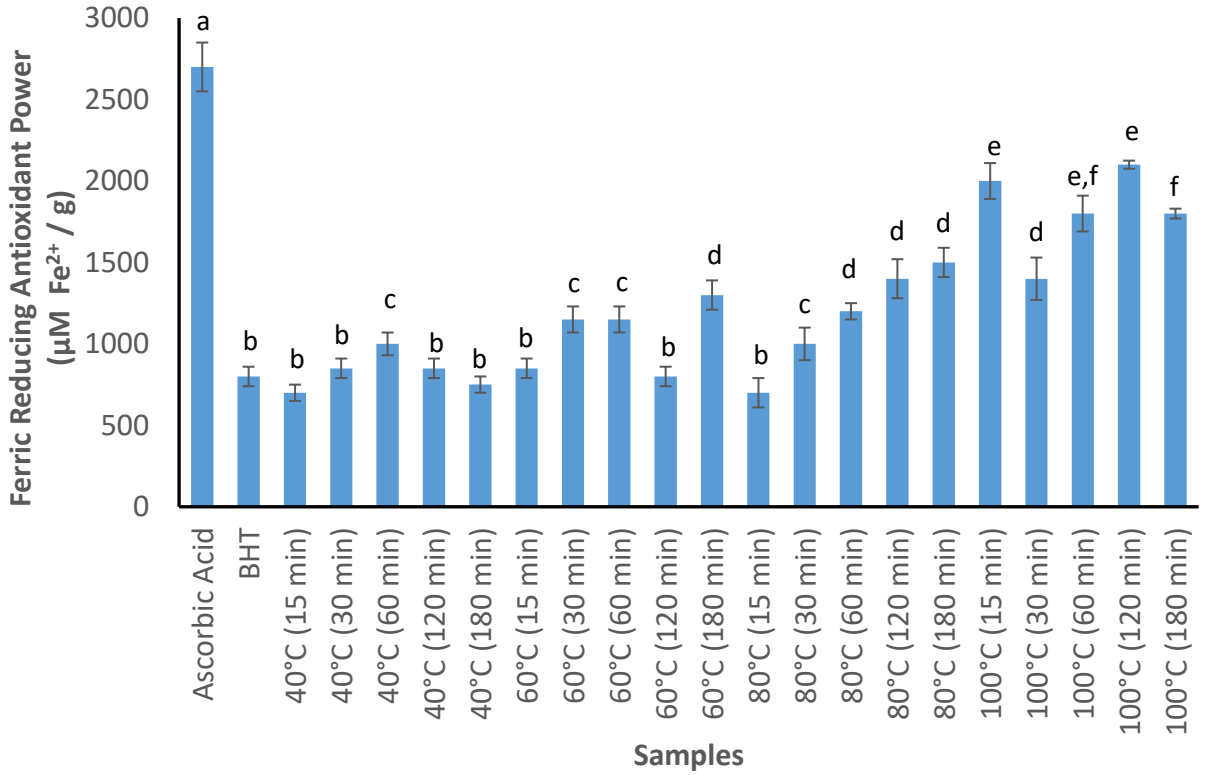

Figure 4. Ferric reducing antioxidant power (FRAP) of *H. sabdariffa* water extracts obtained under different thermal extraction conditions (40–100 °C; 15 min–3 h). Results are expressed as µM Fe^{2+} /g. Ascorbic acid was used as a reference standard. Data are presented as mean ± SD. Different superscript letters indicate statistically significant differences between samples at $P < 0.05$.

The FRAP of *H. sabdariffa* water extracts was significantly influenced by extraction temperature and duration ($P < 0.05$). Ascorbic acid exhibited the highest reducing power (2700 µM Fe^{2+}/g), confirming the sensitivity and validity of the assay.

At lower temperature (40 °C), FRAP values were relatively low, ranging from approximately 700 to 1000 µM Fe^{2+}/g. A slight increase was observed at 30–60 min, followed by a decline at longer extraction durations, suggesting limited extraction efficiency under mild conditions and possible degradation with prolonged exposure. At 60 °C, FRAP values increased compared to 40 °C, with peak values observed at 30–60 min (1100–1200 µM Fe^{2+}/g). This indicates improved extraction of reducing compounds at moderate temperatures. However, a decrease was observed at extended duration (120 min), suggesting that prolonged heating may reduce the availability of redox-active compounds.

At 80 °C, a further increase in FRAP values was observed, particularly at longer extraction durations (120–180 min), reaching approximately 1300–1500 µM Fe^{2+}/g. This suggests that elevated temperature enhances the release of compounds with strong electron-donating capacity.

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At 100 °C, the highest FRAP values were recorded, especially at 30–120 min (1800–2100 μM Fe^{2+}/g). This indicates that high temperature significantly enhances reducing power, likely due to increased extraction efficiency and/or the formation of thermally stable reducing compounds. However, a slight decrease at prolonged duration (180 min) suggests that excessive thermal exposure may lead to partial degradation.

Generally, these findings demonstrate that FRAP activity increases with temperature, indicating enhanced reducing capacity at elevated thermal conditions. However, prolonged extraction may reduce activity due to degradation of redox-active compounds, highlighting the importance of optimizing both temperature and extraction time.

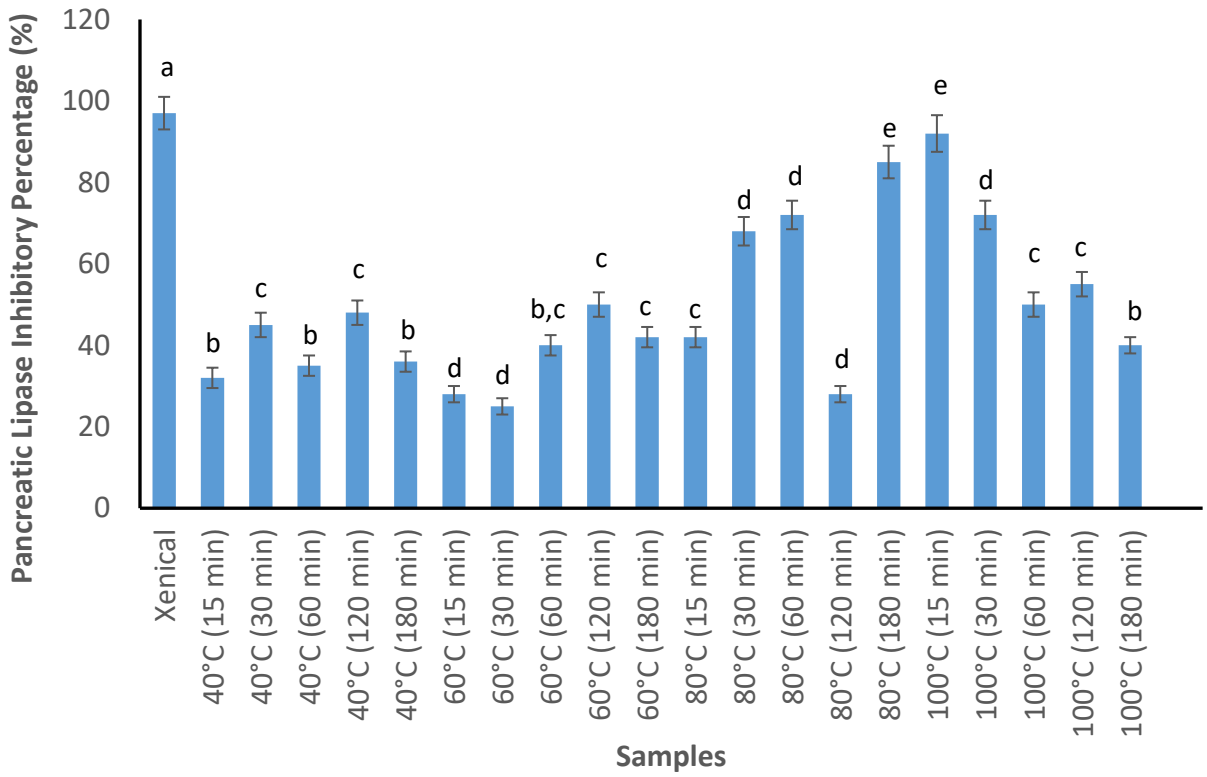

Figure 5. Pancreatic lipase inhibitory activity (%) of *H. sabdariffa* water extracts obtained under different thermal extraction conditions (40–100 °C; 15 min–3 h). Xenical® (orlistat) was used as the positive control. Data are presented as mean ± SD. Different superscript letters indicate statistically significant differences between samples at $P < 0.05$.

The pancreatic lipase inhibitory activity of *H. sabdariffa* water extracts was significantly influenced by extraction temperature and duration ($P < 0.05$). Xenical® (orlistat) exhibited the highest inhibition (~95–100%), confirming the validity of the assay.

At 40 °C, lipase inhibition ranged between approximately 30–50%, with a moderate increase observed at 30–120 min, followed by a slight decline at prolonged duration. This suggests that mild extraction conditions are insufficient to fully release potent lipase-inhibiting compounds. At 60 °C, inhibition activity remained relatively low (25–50%), with slight improvement at longer durations. This indicates partial extraction of bioactive compounds, but not at optimal efficiency.

At 80 °C, a marked increase in lipase inhibition was observed, particularly at 30–60 min (~65–75%), suggesting enhanced extraction or formation of active compounds at elevated temperature. However, a notable decrease at 80 °C for 120 min (~30%) indicates degradation or loss of active constituents under prolonged thermal exposure. At 100 °C, the highest lipase inhibitory activity was recorded, particularly at 30 min (~90%), followed by slightly lower values at 15 and 60 min. This demonstrates that high-temperature short-time extraction is most effective

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for maximizing lipase inhibition. However, prolonged extraction (120–180 min) resulted in a decline in activity, indicating thermal degradation of enzyme-inhibiting compounds.

Therefore, these findings demonstrate that pancreatic lipase inhibition is strongly temperature-dependent and is maximized under high-temperature short-duration conditions. Prolonged exposure to elevated temperatures reduces bioactivity, highlighting the importance of optimizing extraction parameters to preserve active compounds.

Table 1. Optimal extraction conditions for different bioactivity endpoints of *H. sabdariffa* water extract

Bioactivity Endpoint	Optimal Temperature	Optimal Duration	Maximum Value Achieved	Comparison to Positive Control
Total Phenolic Content (TPC)	40 °C	30 min	48 mg GAE/g	Lower than ascorbic acid
Total Flavonoid Content (TFC)	60 °C	30 min	650 mg CE/g	Lower than ascorbic acid
DPPH Radical Scavenging	80 °C	120 min	90%	Comparable to ascorbic acid (90%)
Ferric Reducing Power (FRAP)	100 °C	120 min	2100 μM Fe^{2+}/g	Lower than ascorbic acid (2700 μM Fe^{2+}/g)
Pancreatic Lipase Inhibition	100 °C	30 min	92%	Approaching Xenical (97.33%)

Table 1 presents a comparative overview of the optimal extraction parameters required to achieve maximum bioactivity across different functional endpoints of *H. sabdariffa* water extract. The data clearly indicate that extraction conditions must be tailored according to the specific bioactivity target, as no single parameter set simultaneously optimizes all responses.

With respect to TPC, the highest value (48 mg GAE/g) was achieved under mild extraction conditions (40 °C, 30 min). This suggests that phenolic constituents, particularly heat-sensitive compounds such as anthocyanins, are more effectively preserved at lower temperatures and shorter exposure times. Increasing temperature or extending extraction duration appears to promote degradation processes, including oxidation and structural breakdown.

A different pattern was observed for TFC, which reached its maximum (650 mg CE/g) at 60 °C for 30 min. This indicates that moderate thermal input facilitates the release of flavonoids, potentially through the disruption of plant cell matrices and the conversion of bound forms into more extractable species. However, further increases in temperature may lead to instability and loss of these compounds.

In terms of antioxidant performance, distinct trends were evident between the two assays employed. The highest DPPH radical scavenging activity (90%) was recorded at 80 °C for 120 min, reflecting enhanced availability of hydrogen-donating antioxidants under these conditions. In contrast, ferric reducing antioxidant power (FRAP) peaked at 100 °C for 120 min (2100 μM Fe^{2+}/g), indicating that elevated temperatures favor compounds with strong electron-donating capacity, which may include both native phenolics and thermally generated products.

A different extraction behavior was evident for pancreatic lipase inhibitory activity. Maximum inhibition (92%) was obtained at 100 °C for 30 min, approaching the efficacy of the reference drug, Xenical. This suggests that high-temperature short-time extraction enhances the release or transformation of specific bioactive molecules with strong affinity toward the enzyme active site.

Taken together, these findings demonstrate that thermal processing exerts both beneficial and detrimental effects, depending on the conditions applied. While increased temperature can improve extraction efficiency and bioactivity in certain cases, excessive heat or prolonged exposure may compromise compound stability. Consequently, the selection of extraction parameters should be guided by the intended functional outcome, particularly in the context of nutraceutical and functional food development.

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DISCUSSION

The present study demonstrates that thermal extraction parameters exert a profound influence on the phenolic composition, antioxidant capacity, and pancreatic lipase inhibitory activity of *H. sabdariffa* water extract. Importantly, the results reveal that optimal extraction conditions are highly dependent on the targeted biofunctional endpoint, reflecting the complex interplay between compound stability, extraction efficiency, and thermal transformation processes (Sun *et al*., 2025).

Total phenolic content (TPC) was maximized under mild extraction conditions (40 °C), indicating that lower temperatures favor the preservation of thermolabile phenolic compounds. Moderate heating enhances cell wall permeability and facilitates solvent penetration, thereby improving the diffusion of soluble phenolics without inducing substantial degradation (Qin *et al*., 2025). However, prolonged exposure or elevated temperatures resulted in reduced TPC, which can be attributed to oxidative degradation, structural instability, and polymerization of phenolic compounds, particularly anthocyanins known for their heat sensitivity (Enaru *et al*., 2021; Wu *et al*., 2018; Narra *et al*., 2024). These findings highlight that phenolic yield is governed by a balance between enhanced extraction and thermal degradation.

In contrast, TFC was highest at moderate temperature (60 °C), suggesting that flavonoids require greater thermal energy for efficient release from the plant matrix. Thermal treatment may disrupt flavonoid–polysaccharide interactions and promote hydrolysis of glycosylated forms, thereby increasing solubility and extractability (Panche *et al*., 2016). However, extraction at 100 °C resulted in a marked reduction in TFC, particularly at shorter durations, indicating rapid degradation of thermolabile flavonoids under harsh thermal conditions (Nastasi *et al*., 2025). This divergence between TPC and TFC responses underscores the structural heterogeneity of phenolic compounds and their differential sensitivity to thermal processing.

Antioxidant activity exhibited assay-dependent behavior. DPPH radical scavenging activity remained relatively stable across most extraction conditions, indicating that hydrogen-donating antioxidant compounds in *H. sabdariffa* are moderately resistant to thermal degradation. This stability suggests that multiple antioxidant constituents contribute synergistically to radical scavenging activity, thereby buffering the effects of thermal variation (Lobo *et al*., 2010). However, a pronounced decline observed under prolonged high-temperature conditions indicates degradation or structural modification of key antioxidant molecules (Wu *et al*., 2018).

Conversely, FRAP values showed a strong temperature-dependent increase, with the highest reducing power observed at 100 °C. Unlike DPPH, which reflects hydrogen atom transfer mechanisms, FRAP measures electron-donating capacity. The enhanced FRAP activity at elevated temperatures may be attributed to the release of thermally stable phenolic acids or the formation of new reducing compounds, including Maillard reaction products generated during thermal processing (Chaipoot *et al*., 2026). This indicates that different antioxidant mechanisms respond distinctly to thermal conditions, emphasizing the importance of employing multiple assays for comprehensive antioxidant evaluation.

Notably, antioxidant capacity did not directly correlate with TPC or TFC values. For example, although TPC was highest at lower temperature, maximal DPPH and FRAP activities were observed at higher temperatures. This suggests that antioxidant activity is governed not only by total phenolic concentration but also by qualitative changes in compound composition, structural modifications, and synergistic interactions among phytochemicals (Lobo *et al*., 2010; Narra *et al*., 2024). Therefore, thermal processing influences both the quantity and functional properties of bioactive compounds.

The most pronounced thermal effect was observed in pancreatic lipase inhibitory activity, which was strongly enhanced at high temperature with short extraction duration. Maximum inhibition was achieved at 100 °C for 30 min, approaching the efficacy of Xenical. This suggests that high-temperature short-time extraction promotes the release or transformation of specific bioactive compounds with strong enzyme-binding affinity. Phenolic acids and flavonoids, including anthocyanins and chlorogenic acid derivatives, have been reported to inhibit pancreatic lipase through hydrogen bonding and hydrophobic interactions at the catalytic site (Tran *et al*., 2024). Furthermore, thermal processing may facilitate the hydrolysis of glycosylated flavonoids into aglycone forms, which exhibit greater lipophilicity and enhanced enzyme interaction.

However, prolonged heating resulted in a decline in lipase inhibitory activity, indicating degradation or structural modification of active compounds. This pattern is consistent with the behavior observed for TPC and TFC, further supporting the concept that thermal processing exerts both beneficial and detrimental effects depending on the extraction conditions. Interestingly, maximal lipase inhibition did not coincide with peak TPC or TFC values, indicating that enzyme inhibition is mediated by specific phenolic subclasses or thermally transformed metabolites rather than TPC alone. This finding reinforces the concept that bioactivity is determined by compound quality and structural characteristics rather than quantity.

Collectively, the results demonstrate that thermal extraction exerts a dual effect: moderate heating enhances phytochemical liberation, whereas excessive temperature and prolonged exposure promote degradation. Moreover, antioxidant activity and enzyme inhibition respond differently to thermal conditions, highlighting that extraction optimization must be tailored to specific functional targets (Wu *et al*., 2018; Narra *et al*., 2024; Subramaniyan *et al*., 2025; Tran *et al*., 2024). From an application perspective, these findings suggest that no single extraction condition is universally optimal. Instead, extraction parameters should be selected based on the desired bioactivity. Mild conditions are preferable for maximizing phenolic preservation, moderate conditions for flavonoid enrichment, and high-temperature short-time conditions for enhancing lipase inhibitory activity. This endpoint-specific optimization strategy is particularly relevant for the development of functional foods and nutraceutical products derived from *H. sabdariffa*.

CONCLUSION

Thermal extraction conditions significantly influence the phytochemical composition and bioactivity of *H. sabdariffa* water extract. Optimal conditions were endpoint-dependent, with mild temperature favoring phenolic preservation, moderate conditions enhancing flavonoid extraction, and high-temperature short-time extraction maximizing lipase inhibitory activity. Antioxidant responses varied by assay, and bioactivity did not directly correlate with TPC or TFC, indicating the importance of qualitative compositional changes. These findings highlight the need for bioactivity-driven extraction strategies in functional food and nutraceutical development.

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