

Optimisation of flavonoids extraction from *lucuma* fruits (*Pouteria campechiana*) in Tra Vinh province, Vietnam

*Le, Q. D.

School of Agriculture and Aquaculture, Tra Vinh University, Tra Vinh City 87000, Vietnam

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Abstract

Lucuma (*Pouteria campechiana*) fruits, cultivated in Tra Vinh province, Vietnam, currently hold limited economic value. The present work thus aimed to optimise the extraction of flavonoids and natural antioxidants with established health benefits from *lucuma* fruits. This optimisation not only enhances their economic potential but also promotes their conservation through sustainable utilisation. Flavonoid extraction was evaluated by high-performance liquid chromatography (HPLC) analysis. The investigation focused on the influence of various extraction parameters, including solvent type, extraction time, and temperature. The results identified ethanol (1:10, w/v) at 30°C for 8 h as the optimal extraction condition, yielding a noteworthy efficiency of 6.47%, based on dry weight. The present work highlighted the potential of *lucuma* fruits as a source of natural antioxidants, and contributed valuable knowledge for the development of value-added products from this underutilised fruit, particularly as a natural antioxidant additive in functional drinks, nutraceutical supplements, and fortified food products.

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Introduction

Lucuma (*Pouteria campechiana*), an Andean fruit gaining popularity for its potential health benefits, is native to South America, and rich in various bioactive compounds (Oliveira *et al.*, 2023). These compounds, including sugars, organic acids, phenolics, carotenoids, and sterols, contribute to the antioxidant, anti-inflammatory, antihyperglycemic, and antibacterial properties of *lucuma* fruits. Studies have shown high levels of genetic diversity among *lucuma* germplasms in Vietnam (Duy *et al.*, 2019a), suggesting their potential for future breeding programs. However, extracting high-quality flavonoids from plant materials remains challenging due to their sensitivity to extraction conditions, such as temperature and solvent choice. This highlights the need for optimised extraction methods tailored to specific plant sources to improve yield and quality (Jurinjak Tušek *et al.*, 2022).

In plants, flavonoids exist as free aglycones or glycosides bound to sugars. Acid or enzyme hydrolysis releases sugars, including D-glucose, D-galactose, L-rhamnose, L-arabinose, D-xylose, and D-apirose. Aglycones exhibit low solubility in non-

polar solvents (hexane, benzene, and petroleum ether), but high solubility in moderately and highly polar solvents (ethyl acetate, dimethyl ether, methanol, and ethanol). Additionally, flavonoid glycosides with hydroxyl groups (–OH) at the C7 position are acidic, rendering them soluble in solutions containing NaOH, Na₂CO₃, and NaHCO₃. Flavonoids readily form water-soluble salts with alkaline hydroxides, demonstrating their sensitivity to pH, temperature, and light. Furthermore, their ability to form complexes with metal ions results in characteristically coloured products. The conjugated double-bond system formed by the two benzene rings and the pyran ring allows flavonoids to absorb ultraviolet (UV) light, typically at two maximum wavelengths (λ_{max}): 320 - 380 nm and 240 - 280 nm (Jahan *et al.*, 2014). Additionally, temperature and solvent selection during extraction significantly impact flavonoid yield (Settharaksa *et al.*, 2012).

The present work thus focused on optimising the extraction of flavonoids from *lucuma* fruits. The present work aimed to develop a method for obtaining high-purity flavonoid extracts that could contribute to the development of value-added *lucuma* products, and promote wider utilisation of this promising fruit.

*Corresponding author.

Email: lequocduy@tvu.edu.vn

Materials and methods

Plant material

Lucuma fruits free from blemishes were harvested in March 2024 from Tra Vinh Province, Vietnam. After washing, peeling, and slicing, fruit samples were freeze-dried to preserve their bioactive compounds. The dried samples were ground into fine powder, and stored at -20°C in airtight containers until further analyses.

Standard calibration

A calibration curve for quercetin was constructed to quantify the flavonoids in the extracts. A series of standard solutions with varying concentrations (e.g., 10 - 200 ppm) was prepared, and their absorbance was measured at 370 nm using an HPLC system. The resulting data were used to generate a calibration curve, and the coefficient of determination (R^2) indicated a strong linear relationship. Standard deviations are represented as error bars where applicable, reflecting the variability in the measurements.

High-performance liquid chromatography (HPLC) analysis

The HPLC analysis employed a reversed-phase ODS-C18 column to separate flavonoid compounds based on their varying polarities. The flavonoids were detected using a photodiode array (PDA) detector at 370 nm, which corresponds to the maximum absorbance of several flavonoid compounds, such as quercetin. The mobile phase consisted of a gradient system of acetonitrile (solvent A) and 3% formic acid in deionised water (solvent B) at a flow rate of 1 mL/min, and a constant temperature of 30°C. Quantification of flavonoids in *lucuma* fruit extracts was performed using a quercetin standard curve at 370 nm.

Optimisation of flavonoid extraction

Solvent selection

Five test tubes, each containing 100 mg of *lucuma* powder, were used to evaluate the influence of various solvents (ethanol, isopropanol, ethyl acetate, *n*-hexane, and acetone) on extraction efficiency. A constant solid-to-liquid ratio of 1:10 (mg/mL) was maintained by adding 10 mL of the solvent to each tube. All the tubes were stirred at 30°C and 500 rpm for 6 h. Flavonoid content was quantified using the established quercetin calibration

curve. All extraction experiments were conducted in triplicates ($n = 3$). Results are expressed as mean $\pm$ standard deviation. Statistical analysis was performed using one-way ANOVA, followed by Tukey's *post hoc* test, with significance defined at $p < 0.05$. The solvent that yielded the highest amount of extracted flavonoids was selected for further optimisation.

Solid-to-liquid ratio

Five new test tubes were prepared, each containing 100 mg *lucuma* powder. Different volumes of the selected solvents were added to achieve solid-to-liquid ratios of 1:5, 1:10, 1:20, 1:40, and 1:80 (w/v). All tubes were stirred under identical conditions (30°C, 500 rpm) for 6 h. All extraction experiments were conducted in triplicates ($n = 3$). Results are expressed as mean $\pm$ standard deviation. Statistical analysis was performed using a one-way ANOVA, followed by Tukey's *post hoc* test, with significance defined at $p < 0.05$. The extracted flavonoids were then quantified using a calibration curve. The ratio that yielded the highest amount of extracted flavonoids was selected for further optimisation.

Extraction time

Using the optimal solid-to-liquid ratio, five new test tubes were prepared with 100 mg *lucuma* powder. The tubes, each containing 100 mg of *lucuma* powder and solvent, were stirred at a constant speed (500 rpm) and temperature (30°C) for varying durations (2, 4, 6, 8, and 10 h). All experiments were performed in triplicates ($n = 3$). Results are expressed as mean $\pm$ standard deviation. Statistical analysis was performed using a one-way ANOVA, followed by Tukey's *post hoc* test, with significance defined at $p < 0.05$. The extracted flavonoids were then quantified using a calibration curve. The extraction time that yielded the highest amount of extracted flavonoids was selected for further optimisation.

Extraction temperature

Using the optimal solvent, solid-to-liquid ratio, and extraction time, five new test tubes were prepared with 100 mg *lucuma* powder. Flavonoid extraction was conducted at different temperatures (30, 35, 40, 45, and 50°C) with constant stirring (500 rpm) for the chosen optimal extraction time. All experiments were performed in triplicates ($n = 3$). Results are expressed as mean $\pm$ standard deviation. Statistical analysis was performed using a one-way ANOVA, followed by

Tukey's *post hoc* test, with significance defined at $p < 0.05$. The extracted flavonoids were then quantified using a calibration curve. The extraction temperature that yielded the highest amount of extracted flavonoids was selected for further optimisation.

Results and discussion

The constructed quercetin standard curve and absorbance measurements at 370 nm using HPLC is shown in Figure 1.

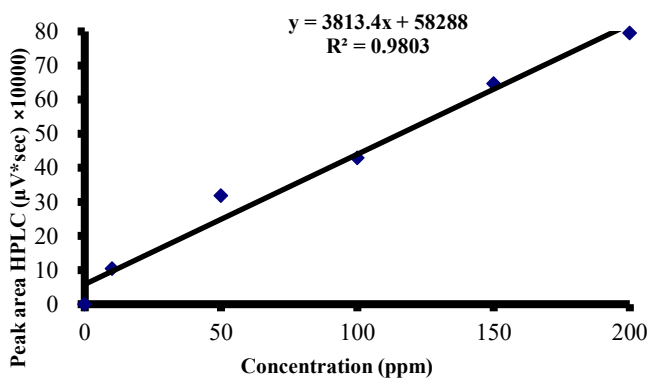


Figure 1. Quercetin standard calibration curve for total flavonoid concentration determination.

Effect of solvent on flavonoid extraction

The efficacy of various solvents (ethanol, isopropanol, ethyl acetate, *n*-hexane, and acetone) for extracting flavonoids from *lucuma* fruit was evaluated. As illustrated in Figure 2 and Table 1, ethanol demonstrated the highest extraction efficiency, achieving a peak area of 11.50×10^4 $\mu\text{V}\cdot\text{sec}$, and a recovery efficiency of 17.42%. This difference was statistically significant compared with the efficiency of the other solvents ($p < 0.05$). Conversely, *n*-hexane exhibited the lowest efficiency (peak area: 1.86×10^4 $\mu\text{V}\cdot\text{sec}$, recovery rate: 2.9%). The extraction efficiencies of isopropanol, ethyl acetate, and acetone were lower than that of ethanol, ranging from 5.07 to 12.37%. These observations can be attributed to the interplay between solvent polarity and flavonoid structure. Solvents with higher dielectric constants, indicative of greater polarity, were more effective at extracting flavonoids from *lucuma* fruits, which increased their solubility in less polar solvents such as *n*-hexane and ethyl acetate (Khoddami *et al.*, 2013). Conversely, flavonoids with multiple hydroxyl groups are more polar, and have a stronger affinity for polar solvents, such as ethanol.

Based on these findings and the anticipated composition of *lucuma* fruit flavonoids (predominantly flavanols and flavanones, which are typically more polar), ethanol was chosen as the optimal solvent for further optimisation. This selection aligned with previous research on tea leaf flavonoid extraction, where ethanol exhibited superior efficiency compared to the other tested solvents (Wang and Helliwell, 2001). Ethanol is the preferred solvent for extracting flavonoids from diverse plant materials. Studies have consistently reported its advantages over water in this regard, as exemplified by propolis (Mokhtar, 2019), orange peels (Feng *et al.*, 2020), and *Moringa oleifera* leaves (Oktaviana *et al.*, 2022). However, the optimal ethanol concentration for extraction is plant specific. For instance, bitter oranges yielded the highest flavonoid content in 100% ethanol (Feng *et al.*, 2020), whereas 70% ethanol was most effective for papaya leaves (Nugraha *et al.*, 2023), and 96% for *Moringa oleifera* (Oktaviana *et al.*, 2022). In addition to solvent selection, other parameters significantly influence extraction efficiency. Temperature and

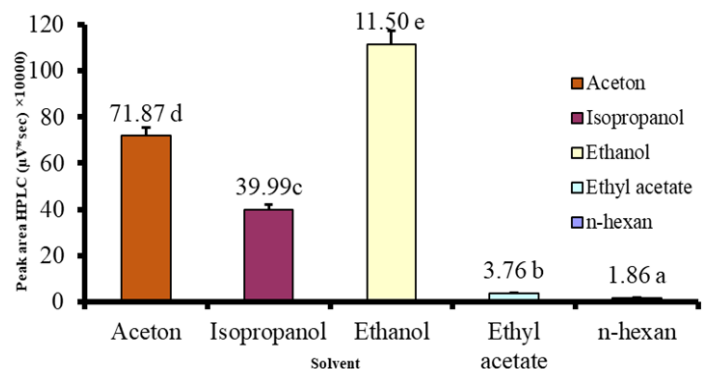


Figure 2. Effect of solvent on flavonoid extraction.

Table 1. Flavonoid yields of *Pouteria campechiana* extracts in different solvents.

Solvent	Mean $\pm$ SD
Acetone	71.87 $\pm$ 3.59 ^d
Isopropanol	39.99 $\pm$ 2.00 ^c
Ethanol	11.50 $\pm$ 4.46 ^e
Ethyl acetate	3.76 $\pm$ 0.15 ^b
<i>n</i> -hexane	1.86 $\pm$ 0.07 ^a

Data are mean $\pm$ standard deviation of triplicates ($n = 3$). Different lowercase superscripts indicate significant differences ($p < 0.05$, one-way ANOVA followed by Tukey's test). Means are expressed in mg quercetin equivalents per gram dry weight (mg QE/g DW).

extraction time are crucial factors, with papaya leaves achieving optimal results at 30 - 50°C and between 10 - 48 h (Nugraha *et al.*, 2023). The sample-to-solvent ratio is another essential consideration, as evidenced by propolis extracts exhibiting the highest total phenolic and flavonoid contents when a 1:5 ratio was employed (Mokhtar, 2019). These findings underscore the critical role of optimising the extraction parameters to maximise flavonoid yield from various plant sources.

Effect of solid-to-liquid ratio on flavonoid extraction

Following the selection of ethanol as the optimal solvent for flavonoid extraction from *lucuma* fruit, a series of experiments were conducted to determine the ideal solid-to-liquid ratio. Different ratios (1:5, 1:10, 1:20, 1:40, and 1:80 w/v) were evaluated at a constant extraction time (8 h). As depicted in Figure 3, and summarised in Table 2, a 1:10 (w/v) ratio resulted in the highest extraction efficiency, indicating that it was the optimal solvent-to-solid ratio to use for further experiments. These results indicated that increasing the solvent-to-sample ratio to 1:20, 1:40, and 1:80 (w/v) led to a reduction in flavonoid yield. This may be attributed to the dilution effect and disruption of solvent-solute interactions at high solvent volumes, which reduced the extraction efficiency. Similar findings have been reported in previous studies (Dai and Mumper, 2010; Azwanida, 2015). Higher ratios provide a greater volume of solvent per unit mass of plant material, facilitating mass transfer and extraction. Conversely, lower plant material concentrations might lead to increased intermolecular interactions, and the potential degradation of target flavonoids. These findings partially aligned with those of Handayani *et al.* (2016), who reported that a 1:10 solid-to-solvent ratio, along with a proportional increase in solvent volume, improved extraction efficiency in *Limnophila aromatica*. Similarly, our study found that increasing the solvent volume relative to the sample mass enhanced flavonoid yield in *lucuma* fruit extracts, suggesting a comparable influence of solvent proportion on extraction performance across different plant materials. It is important to acknowledge that specific structural features of flavonoids, such as the presence of *ortho*-dihydroxy groups, can influence their susceptibility to oxidation during extraction. Although ethanol is generally considered as a satisfactory solvent for flavonoid extraction, high ethanol concentrations can promote

oxidation and alter the final extract profile (Naróg and Sobkowiak, 2023). This is further supported by Soimaloon *et al.* (2018), who demonstrated that solvent choice significantly affected the colour, pH, and flavour profile of *Limnophila aromatica* extracts. They observed that methanol facilitated the detection of specific ribonucleotides, highlighting the potential influence of solvent polarity on the extraction of diverse phytochemicals. Notably, *Limnophila aromatica* is recognised for its antimicrobial and antioxidant properties, which are attributed to its flavonoid and terpenoid contents (Roy *et al.*, 2014). This underscores the importance of optimising the extraction conditions for specific target compounds based on their chemical properties and potential interactions with the solvent. Similarly, Oktaviana *et al.* (2022) identified 96% ethanol as the most effective solvent for flavonoid extraction from *Moringa oleifera*. Sari *et al.* (2022) compared the effects of maceration and sonication on mango leaf flavonoid extraction. Acetone demonstrated higher solvent efficiency than ethanol in both methods, while a 1:10 solvent-to-plant material ratio yielded

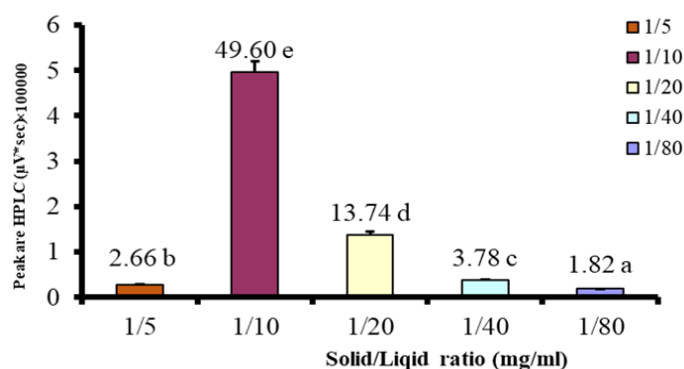


Figure 3. Effect of solid-to-liquid ratio on flavonoid extraction.

Table 2. Flavonoid yields of *Pouteria campechiana* extracts in different sample-to-solvent ratios.

Sample: Ethanol (mg/mL)	Mean ± SD
1:5	2.66 ± 0.99 ^b
1:10	49.60 ± 4.37 ^e
1:20	13.74 ± 0.43 ^d
1:40	3.78 ± 0.13 ^c
1:80	1.82 ± 0.03 ^a

Data are mean ± standard deviation of triplicates ($n = 3$). Different lowercase superscripts indicate significant differences ($p < 0.05$, one-way ANOVA followed by Tukey's test).

the highest flavonoid content with maceration. In summary, the optimal extraction method for *lucuma* flavonoids was identified as maceration using ethanol at a 1:10 (w/v) solid-to-liquid ratio for 8 h at 30°C. This condition outperformed the other tested solvents and ratios, which displayed reduced flavonoid yields, likely due to dilution effects or weaker solvent-solute interactions. These findings emphasised the importance of selecting appropriate solvent polarity and extraction parameters to maximise efficiency and preserve compound stability.

Effect of extraction time on flavonoid yield

Beyond solvent selection, several factors significantly influence flavonoid yield during ethanol extraction. These include concentration, temperature, and extraction time. Although studies suggested a general effectiveness range of 50 - 80% ethanol (Hakim and Saputri, 2020), the optimal concentration can vary depending on the plant material (Nugraha *et al.*, 2023). Similarly, extraction temperatures between 30 - 70°C are often suitable. The extraction time can vary considerably, ranging from 30 min to 48 h for different plant sources (Hakim and Saputri, 2020; Nugraha *et al.*, 2023). Specific methods, such as maceration with heating and stirring, can expedite extraction, as demonstrated for *melinjo* peels (Husna and Kumalaningsih, 2021). Notably, ultrasound-assisted extraction (UAE) is particularly effective when combined with ethanol, further enhancing its efficiency (Hakim and Saputri, 2020). Importantly, exceeding the optimal extraction time can lead to decreased yield, owing to potential degradation, as observed for *Averrhoa bilimbi* leaf extracts (Niawanti *et al.*, 2019).

Building on the established optimal solid-to-liquid ratio of 1:10 (mg/mL), we investigated the influence of extraction time on flavonoid yield from *lucuma* fruit, using ethanol as the solvent (Figure 4 and Table 3). As extraction time increased, efficiency initially increased, reaching a maximum at 8 h (recovery rate: 7.37%, peak area: $55.74 \times 10^4 \mu\text{V}\cdot\text{sec}$). Lower efficiencies were observed at shorter extraction times (2 h), indicating an insufficient extraction time. Conversely, extending the extraction time beyond 8 h resulted in decreased flavonoid content, potentially due to thermal degradation, as inferred from the decrease in peak intensity, and the appearance of minor peaks in the HPLC chromatograms associated with prolonged heating. These findings aligned with those of Niawanti *et al.*

(2019), who reported a decrease in flavonoid yield from *Averrhoa bilimbi* leaves after 4 h of extraction using the Soxhlet method with ethanol.

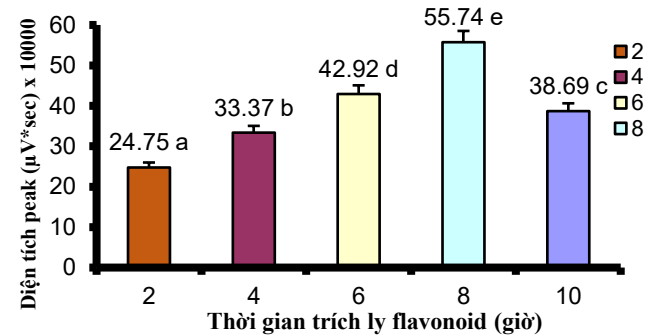


Figure 4. Effect of extraction time on flavonoid extraction.

Table 3. Flavonoid yields of *Pouteria campechiana* extracts at different extraction times.

Time (hour)	Mean ± SD
2	24.75 ± 1.24 ^a
4	33.37 ± 1.67 ^b
6	42.92 ± 2015 ^d
8	55.74 ± 2.79 ^e
10	38.69 ± 1.93 ^c

Data are mean ± standard deviation of triplicates ($n = 3$). Different lowercase superscripts indicate significant differences ($p < 0.05$, one-way ANOVA followed by Tukey's test).

Effect of temperature on flavonoid extraction and importance of plant-specific optimisation

Building on previously established optimal extraction conditions (ethanol solvent, 1:10 mg/mL solid-to-liquid ratio, and 8 h extraction time), the present work investigated the influence of temperature on flavonoid yield from *lucuma* fruits (Figure 5 and Table 4). Interestingly, increasing the temperature resulted in a decrease in flavonoid extraction efficiency, likely due to the thermal degradation of flavonoids. At elevated temperatures, flavonoids are prone to structural breakdown through oxidation, glycosidic bond hydrolysis, and ring-opening reactions, leading to reduced stability and diminished extraction yields. The highest extraction efficiency (6.47% yield, $97.90 \times 10^4 \mu\text{V}\cdot\text{sec}$ peak area) was achieved at 30°C. Conversely, the lowest efficiency (2.01% yield, $33.99 \times 10^4 \mu\text{V}\cdot\text{sec}$ peak area) was observed at 50°C, indicating a statistically

significant decrease ($p < 0.05$) compared to the other temperatures tested.

Optimising the extraction parameters is crucial for maximising flavonoid yield across various plant materials, with studies on melon species exemplifying this point. Muhamad *et al.* (2018) found that drying melon at 40°C with methanol extraction yielded the highest total phenolics and flavonoids. In pepino melon, 70% ethanol was optimal for extracting flavonoids, phenolics, and beta-carotene (Firsty *et al.*, 2023). In contrast, acetone was the most effective for bitter melon flavonoid extraction (Tan *et al.*, 2014), whereas water extracted only 5.4%. Dimtsas *et al.* (2024) maximised the polyphenol yield and antioxidant capacity of Galia melon peels using 50% ethanol at 80°C for 150 min. Similar variations have also been observed in other plants. According to Adamtsevich *et al.* (2020), maximum flavonoid yield ($> 2\%$) was achieved for littlewale (*Lithospermum officinale*) using 50% ethanol at 65 - 70°C for 35 - 45 min. Conversely, yellow velvet leaves (*Limncharis flava*) and water lettuce (*Pistia stratiotes*) yielded the highest flavonoid content at lower temperatures (Sudirman *et al.*, 2024). Roselle (*Hibiscus sabdariffa*) extracts yielded optimal flavonoid content within 60 - 80°C, with extraction times between 90 - 150 min, and a 1:20 solid-to-solvent ratio (Duy *et al.*, 2019b). These studies highlighted the significant influence of plant origin on optimal flavonoid extraction conditions. Temperature, extraction time, and solid-to-solvent ratio are only a few of the factors that significantly impact the yields of various plant materials. Optimising extraction parameters is important not only for enhancing flavonoid yield, but also for preserving compounds typically linked to antioxidant potential, although antioxidant activity was not evaluated in the present work.

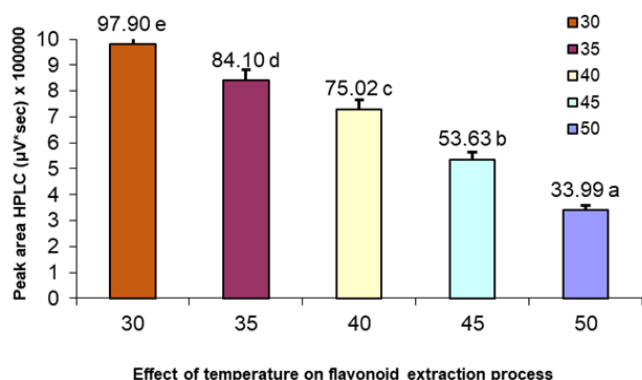


Figure 5. Effect of temperature on flavonoid extraction.

Table 4. Flavonoid yields of *Pouteria campechiana* extracts at different extraction temperatures.

Temperature (°C)	Mean $\pm$ SD
30	97.90 $\pm$ 0.35 ^e
35	84.10 $\pm$ 0.30 ^d
40	75.02 $\pm$ 0.25 ^c
45	53.63 $\pm$ 0.25 ^b
50	33.99 $\pm$ 0.30 ^a

Data are mean $\pm$ standard deviation of triplicates ($n = 3$). Different lowercase superscripts indicate significant differences ($p < 0.05$, one-way ANOVA followed by Tukey's test).

Conclusion

In the present work, HPLC was employed to optimise the extraction of flavonoids from *Pouteria campechiana* powder. The optimal extraction conditions were identified as ethanol for solvent, 1:10 mg/mL for solid-to-liquid ratio, 8 h for extraction time, and 30°C for extraction temperature. These findings provided valuable insights into the efficient extraction of flavonoids from this underutilised fruit. This optimised method would pave the way for further research on the bioactivity and potential applications of *P. campechiana* flavonoids. Future studies should focus on evaluating their antioxidant, anti-inflammatory, and anticancer activities using *in vitro* and *in vivo* assays to validate their health-promoting properties.

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