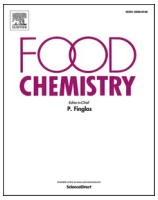
Food Chemistry 493 (2025) 145891

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Comparative efficiency of extraction techniques for bioactive compounds in *Cinnamomum zeylanicum*

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ARTICLE INFO

Keywords:
Ultrasonic-assisted extraction
Accelerated solvent extraction
Antioxidants
Phenols
Cinnamaldehyde

ABSTRACT

Cinnamomum zeylanicum is a plant well-known for its antioxidant-rich bioactives. This study assessed its total phenolic (TPC) and flavonoid contents (TFC), antioxidant activity, and key bioactives (cinnamaldehyde, eugenol, and cinnamic acid) using two extraction methods: accelerated solvent extraction (ASE) and ultrasonic-assisted extraction (UAE), with solvents including ethanol, methanol, acetone, and water. ASE with 50 % ethanol yielded the highest TPC (6.83 ± 0.31 mg GAE/g), TFC (0.50 ± 0.01 mg QE/g), cinnamaldehyde (19.33 ± 0.002 mg/g), eugenol (10.57 ± 0.03 mg/g), and cinnamic acid (0.18 ± 0.004 mg/g), making it superior to UAE. However, UAE with 50 % ethanol showed the strongest antioxidant activity via ABTS (IC50 = 3.26 μg/mL), while antioxidant activity showed no significant differences. A strong correlation ($R = 0.81$) between TPC and TFC in ASE extracts indicated that flavonoids are major contributors. This study addresses a research gap by systematically comparing UAE and ASE for extracting key bioactives from *Cinnamomum zeylanicum* in optimising its bioactive recovery for application in functional foods.

1. Introduction

Cinnamon is a widely used spice derived from the inner bark of trees belonging to the genus *Cinnamomum*. There are two major types of cinnamon: *Cinnamomum zeylanicum* (*C. zeylanicum*), and *Cinnamomum cassia* (*C. cassia*). *C. zeylanicum* Blume, which is endemic to Sri Lanka and one of the oldest and most commonly used spices in the world, is a tropical evergreen tree of the family Lauraceae. The bark is used as a flavouring agent in many foods and beverages. The use of cinnamon has even been documented in Chinese writings as early as 4000 BCE (Spence, 2024).

Although *C. zeylanicum* has long been valued for its distinctive flavour and aroma in culinary applications, recent years have seen a growing interest in this spice, driven by its potential health benefits and therapeutic properties (Błaszczyk et al., 2021). *C. zeylanicum* intake has been associated with antioxidant properties (Mathew & Abraham, 2006), antidiabetic activity (Ranasinghe & Galappaththy, 2016), blood pressure lowering (Ranasinghe et al., 2017), blood cholesterol lowering (Ranasinghe et al., 2017), anti-inflammatory, anticancer (Holkem et al., 2020), and antimicrobial properties (Gupta et al., 2008). These health benefits can be attributed to the bark of *C. zeylanicum*, which is a rich source of bioactive compounds such as cinnamaldehyde and eugenol (Weerasekera et al., 2021).

Extraction is considered an important step as it affects the composition of the active ingredient (Huie, 2002) and is an essential step in the separation, identification, and characterisation of biologically active compounds. The extraction of bioactive compounds is influenced by many factors such as the solubility of the compounds in the solvent, the extraction technique, temperature, time, and pressure that is employed during the extraction process (Jha & Sit, 2022). To further enhance the yields of extraction, modification to the procedure and conditions is also crucial (Bouloumpasi et al., 2024).

Conventional extraction methods, such as maceration and Soxhlet extraction, have been widely used for the extraction of plant bioactives; however, they suffer from several limitations, including the use of large amounts of organic solvents and prolonged extraction times and temperatures, which can lead to the degradation of bioactive compounds responsible for phenolic and antioxidant activity (Shrivastav et al., 2024). To overcome these limitations, innovative non-conventional techniques such as pressurised hot water extraction (PHWE), deep eutectic extraction (DEE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction

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https://doi.org/10.1016/j.foodchem.2025.145891
Received 19 April 2025; Received in revised form 4 August 2025; Accepted 8 August 2025
Available online 11 August 2025
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(SFE) have gained prominence. These green technologies, when combined with environmentally friendly solvents, provide a more sustainable and efficient approach to extracting natural compounds while minimising hazardous waste (Usman et al., 2022).

While the health benefits of *C. zeylanicum* have been widely studied, there are significant gaps in understanding the effectiveness of different extraction methods in isolating key bioactive compounds. This is particularly evident in the case of green extraction techniques such as UAE and accelerated solvent extraction (ASE). ASE is a modern technique that serves as an alternative to traditional soxhlet extraction and has enhanced benefits due to reduced extraction time and solvent consumption (Adam et al., 2023). The high pressure and temperature that can be used in the system can increase the extraction efficiency by increasing the diffusion rates for the bioactive (Ji et al., 2020). This emerging technique is often referred to as a green approach due to its simplicity, high efficiency, cost-effectiveness, lower organic solvent consumption, and shorter extraction time (Hong et al., 2023). UAE forms waves that produce cavitation bubbles that collapse and form cracks in the cell wall of the plant material. This phenomenon eases the solvent to diffuse into the cell wall, facilitating the transfer of bioactive of the plant material (Shirsath et al., 2012).

Hot water bath (HWB) extraction is a conventional, simple, cost-effective technique commonly used for extracting bioactive compounds, particularly polar compounds like phenolics and flavonoids. However, it requires significantly longer extraction times and higher solvent volumes, with limited mass transfer, resulting in lower efficiency compared to advanced methods like ASE or UAE. Additionally, the extended heating required during the extraction process can cause thermal degradation of sensitive compounds, particularly those that are heat labile, thus diminishing the overall potency of the extracted bioactive compounds.

While previous studies have explored the extraction of bioactives from cinnamon using UAE or ASE individually, direct comparative analysis of these two techniques, specifically for *C. zeylanicum* and across various solvent systems, remains unexamined. Accordingly, this study aims to bridge this gap by providing a comprehensive assessment of UAE and ASE for optimising cinnamon bioactive extraction. Ahmad et al. (2023) extracted bioactive compounds from *C. zeylanicum* using UAE. However, their study did not evaluate the impact of different solvents and solvent concentrations on the bioactive yield, nor did it quantify key bioactives like cinnamaldehyde and eugenol, which are known for their significant health benefits. Cebi et al. (2019) performed UAE on *C. zeylanicum* with different ethanolic concentrations but did not provide a comparison of key bioactives such as cinnamaldehyde or the effect of different solvents in extraction. Thus, in evaluating UAE as a method of extraction, significant gaps exist in the comparative analysis of different solvents and the application of UAE for the extraction of key bioactives such as cinnamaldehyde, eugenol, and cinnamic acid.

Lim and Ko (2022) explored the extraction of flavouring compounds from *C. zeylanicum* using ASE, but their study did not evaluate the effect of solvent concentration. This study also did not include in-vitro assays such as total phenol content (TPC), total flavonoid content (TFC), and antioxidant activity. This highlights a significant gap in the use of ASE for the extraction of bioactive compounds from cinnamon, especially *C. zeylanicum*. Accordingly, the present research seeks to address these critical gaps by providing a systematic comparative analysis of UAE and ASE for the extraction of key bioactive compounds from *C. zeylanicum*. The findings of this study are expected to provide valuable insights into optimising extraction strategies for maximising the recovery of key bioactive compounds from *C. zeylanicum*, thereby contributing to its enhanced utilisation in functional foods and nutraceutical applications.

2. Materials and methods

2.1. Sample preparation and chemicals

Dried *C. zeylanicum* bark (100 %) originating from Sri Lanka, was bought from a local supplier in Palmerston North, New Zealand. The plant material was ground, sieved (250 μm), and stored in an air-tight container at room temperature in a dry place before extraction. Quercetin, gallic acid, and 2,2 diphenyl-1-picryhydrazylradical (DPPH) were obtained from Sigma-Aldrich, Inc. (Auckland, New Zealand). Folin-Ciocâlteu phenol reagent was purchased from Merck (Saint Louis, USA). Methanol, acetone, and ethanol (analytical grade) were from Thermo Fisher Scientific (Auckland, New Zealand). All other chemicals and solvents were of the highest analytical grade and used without further purification.

2.2. Accelerated solvent extraction (ASE)

Dry cinnamon powder (1.00 g) was loaded with diatomaceous earth into a 22 mL stainless steel extraction cell of the ASE system (Dionex ASE 350, Thermo Fischer Scientific, Massachusetts, USA). The extractions were performed using the method reported by Dahmoune et al. (2015) with some modifications (i.e., changes to the heating time and temperature of extraction using 25 %, 50 %, 75 %, 100 % ethanol, and 100 % water). The solvent used in the extraction was degassed using nitrogen and pumped under 1500 psi into the extraction cell, which was then heated for 3 min. Thereafter, the extraction was performed at 55 °C and three static cycles. Finally, the cells were pumped with fresh solvents and purged with nitrogen for 90 s at 1500 psi. Following extraction, the collected extracts (approximately 34 mL) were transferred into vials and adjusted to a final volume of 40 mL using the respective extraction solvent. The samples were then centrifuged at 3000 rpm for 10 min, filtered, and stored at 4 °C until further analysis.

The extraction yield was determined by concentrating the supernatants under vacuum (Caldwell, 2003) using a Savant™ SpeedVac Concentrator SC250 EXP (Thermo Scientific, Sunnyvale, CA, USA). The yield was then calculated using the following equation:

$$\text{Extraction yield}\ (\%) = (\text{Weight of dry extract} / \text{Weight of initial plant material}) \times 100 \quad (1)$$

2.3. Ultrasonic-assisted extraction (UAE)

UAE was conducted according to the method specified by Gilani and Najafpour (2022) using a laboratory ultrasonic device (QSonica, Q700, 20 kHz, 700 W, Newtown, USA) with modification to the solvent concentrations and sonication time. The samples of cinnamon powder (1.00 g) were extracted with 40 mL of water, 25 %, 50 %, 75 %, 100 % ethanol, 50 % acetone, 50 % methanol, and 100 % water, for 2 min at temperatures ranging from 50 to 55 °C in a glass beaker. The temperature was maintained using an ice bath. The extracts were then centrifuged for 10 min at 3000 rpm, filtered, and stored at 4 °C for further analysis. The extraction yield percentage was also calculated.

2.4. Hot water bath extraction (HWB)

In this method, cinnamon powder (1 g) was mixed with 40 mL of distilled water and extracted in a water bath at 55 °C, for 60 min. Following this, the mixture was cooled to room temperature and centrifuged for 10 min at 3000 rpm. Then the supernatant was collected into a different container for further analysis of TPC, TFC, DPPH, and ABTS scavenging activity. The supernatant was then stored at 4 °C for further analysis.

2.5. Determination of total phenol content (TPC)

The Folin-Ciocâlteu method was used to determine the TPC in the obtained cinnamon extract. The extracted sample (20 μL) was mixed with 10 % Folin-Ciocâlteu reagent (100 μL). Following this, 7.5 % sodium carbonate solution (80 μL) was added and the mix was allowed to stand for 30 min before the absorbance was measured at 765 nm using a microplate reader (BioTek, Synergy 2 Multimode, Santa Clara, CA, USA). A standard calibration curve was constructed using gallic acid solutions of varying concentrations. From this, only the linear portion of the curve (0–50 μg/mL) was used for the quantification of total phenolic content (TPC). The TPC was calculated using the following equation:

$$\text{TPC (mg GAE/g)} = [(A - b)/m] \times [(V \times D)/(W \times 1000)] \quad (2)$$

where, A = absorbance of the sample at 765 nm; b = y-intercept of the calibration curve; m = slope of the calibration curve; V = volume of the sample solution (mL); D = dilution factor; W = weight of the test material (g), and 1000 = conversion factor from μg to mg.

2.6. Determination of total flavonoid content (TFC)

The flavonoid content of the cinnamon extracts was determined using a method specified by Pękal and Pyrzynska (2014) with modifications to adapt to the microplate setup. Standard solutions of quercetin were prepared at various concentrations, and the linear portion of the calibration curve (0–125 μg/mL) was used for quantification. Results were expressed as milligrams of quercetin equivalent (QE) per gram of dry sample. Briefly, 100 μL of the test sample was added to the microplate, following which another 100 μL of 2 % (w/v) $AlCl_3$ in methanol was added and incubated for 15 min at room temperature. The absorbance was then measured at 435 nm using a microplate reader (BioTek, Synergy 2 Multimode, Santa Clara, CA, USA). The TFC was calculated using the following equation:

$$\text{TFC (mg QE/g)} = [(A - b)/m] \times [(V \times D)/(W \times 1000)] \quad (3)$$

where, A = absorbance of the sample at 435 nm; b = y-intercept of the calibration curve; m = slope of the calibration curve; V = volume of the sample solution (mL); D = dilution factor; W = weight of the test material (g), and 1000 = conversion factor from μg to mg.

2.7. Determination of antioxidant activity

2.7.1. DPPH radical scavenging activity

The method described by Gulcin et al. (2019) was employed in the determination of DPPH radical scavenging activity with modification to adapt to a 96-well microplate reader. Briefly, 5 μL of the test sample was added to each well of the plate, following which 195 μL of 0.1 mM DPPH solution was added. Then, the prepared test samples were kept in the dark for 30 min. The DPPH inhibition was determined by measuring the absorbance of the samples by a microplate reader (BioTek, Synergy 2 Multimode, Santa Clara, CA, USA) set at 518 nm using the following equation:

$$\text{Inhibition (\%)} = [(A_c - A_s)/(A_b)]^* 100 \quad (4)$$

where, A_c = absorbance of the control at 518 nm; A_s = absorbance of the sample at 518 nm.

2.7.2. ABTS radical scavenging activity

The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid; ABTS) radical scavenging activity was assessed using the method described by Song et al. (2010), with modifications to adapt to a 96-well microplate reader. A 7 mmol/L ABTS stock solution and a 2.45 mmol/L potassium persulfate solution were prepared and stored in the dark at 0–4 °C. Equal volumes of these solutions were mixed and incubated in the dark for 12–16 h to generate the $ABTS^{\bullet+}$ working solution. Before the assay, 2 mL of this working solution was diluted with 40 mL of ultrapure water. For the assay, 5 μL of each sample or standard was added to a well, followed by 195 μL of the prepared ABTS working solution. The mixture was incubated under continuous shaking in the dark for 5 min, and the absorbance was measured at 734 nm at approximately 27 °C, using a reagent blank as the reference. The results were expressed as IC_{50} values, representing the concentration of the sample required to reduce ABTS radical absorbance by 50 % compared to the blank. IC_{50} was determined from individual curves constructed for each extract at varying concentrations against inhibition percentage.

2.8. Quantification of cinnamaldehyde, eugenol, and cinnamic acid by HPLC

The chromatographic experiments were conducted using high-performance liquid chromatography (HPLC) using a Dionex Ultimate 3000 instrument equipped with a UV–Vis detector and a 5 μm, C18 reverse-phase column (25 cm × 4.6 mm, Supelco Analytical, Bellefonte, USA). The separation method was based on the approach proposed by Ding et al. (2011) with modifications to solvent A composition and the gradient program to optimise analyte separation and detection.

The mobile phase consisted of water/formic acid (0.1 %) (solvent A) and acetonitrile (solvent B), with a constant flow rate of 1 mL per minute. The gradient elution profile started with 5 % solvent B, reaching 100 % B in 20 min, and returning to 5 % B in 24 min. The total run time was 29 min. The column temperature was 25 °C. Compound identification relied on comparing retention times and UV/Vis spectra of standards with those found in the samples. Analyte quantification was achieved using a standard calibration curve generated at different concentrations (0 to 1 mg/mL), utilising analyte areas in the chromatogram. Standards (Sigma Aldrich) of cinnamaldehyde, eugenol, and cinnamic acid diluted in HPLC grade ethanol at concentrations ranging from 0 to 1 mg/mL, were employed for identifying and quantifying analytes in the samples. Cinnamaldehyde, eugenol, and cinnamic acid were detected at a wavelength of 287 nm.

2.9. Statistical analyses

All tests were conducted in triplicate, and the results are presented as mean ± SD. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for comparisons. A significance level of $P < 0.05$ was considered acceptable. The correlation coefficients (R) between TPC and TFC were also analysed. Data analysis was performed using Minitab 21 (Minitab Inc., State College, PA, USA).

3. Results and discussion

3.1. Effect of different solvents on the extraction of phenols and flavonoids of C. zeylanicum

The TPC and TFC values of *C. zeylanicum* extracts obtained using different solvents are presented in Fig. 1 and Table 1, respectively. The extraction was conducted using UAE with aqueous solutions of ethanol, methanol, acetone, and water. The highest TPC and TFC values were observed in extracts that were obtained using aqueous ethanol. Although the TPC of aqueous ethanolic extracts was higher than that of other solvent extracts, statistical analysis showed that there was no significant difference ($P > 0.05$) between the TPC of ethanol and acetone extracts. However, the TFC of the aqueous ethanolic extract was significantly higher than that of all other extraction solvents at the 0.05 significance level, suggesting that aqueous ethanol is particularly effective in extracting flavonoid-rich fractions from *C. zeylanicum* bark extracts.

Solvent selection plays a crucial role in optimising the extraction of phenolic and flavonoid compounds from plant materials. Previous

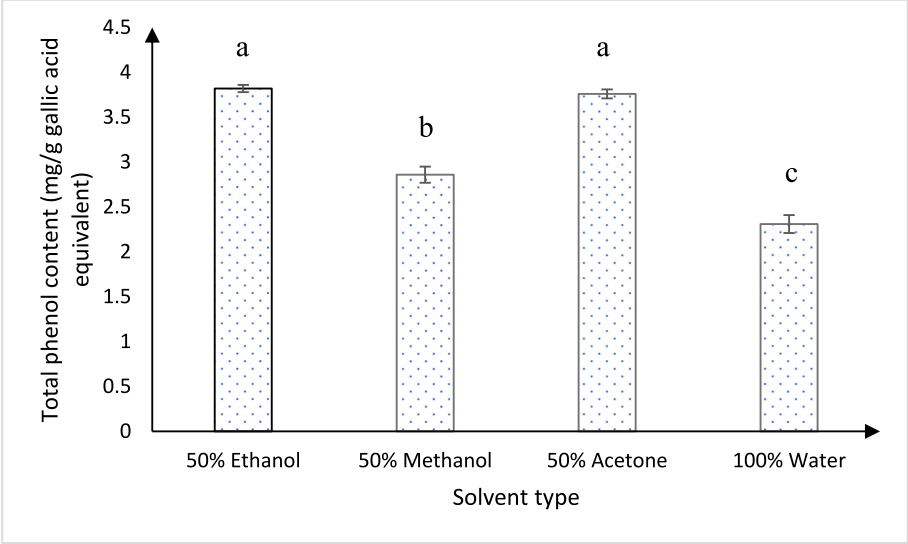

Fig. 1. The mean total phenol content (TPC) of *C. zeylanicum* extracts extracted with different solvents. Means within a column followed by different letters were significantly different at $P < 0.05$.

Table 1
Total flavonoid content of *C. zeylanicum* extracted by ultrasonic extraction with different solvents.

Solvent type	TFC mg/g QE
50 % Ethanol	0.35 ± 0.005^{a}
50 % Methanol	0.31 ± 0.003^{b}
50 % Acetone	0.31 ± 0.002^{b}
100 % Water	0.29 ± 0.001^{b}

TFC: Total flavonoid content (mg/g), Quercetin equivalents; Means within a column followed by different letters were significantly different at $P < 0.05$.

studies have demonstrated that the efficiency of phenolic recovery varies depending on the solvent that is being used (Boeing et al., 2014). While research has explored the impact of solvents such as ethanol, methanol, acetone, and water on *C. cassia* and Indian cinnamon, studies focusing on *C. zeylanicum* remain limited.

Dvorackova et al. (2015) evaluated five different solvents such as methanol, ethanol, acetone, ethyl acetate, and water for the extraction of phenolic compounds from *C. cassia*, reporting maximum recovery with aqueous ethanol. Similarly, Gilani and Najafpour (2022) used ethanol, methanol, chloroform, and water to extract cinnamaldehyde and cinnamic acid from Indian cinnamon, also identifying aqueous ethanol as the most effective solvent. In the study of Sree Satya Nandam and Vangalapati (2012), cinnamon from Visakhapatnam, India was extracted with water, ethanol, methanol, and ethyl acetate, and the highest phenol recovery was observed with the extract extracted with 50 % methanol. Additionally, Koffi et al. (2010) found that ethanol exhibited superior efficiency in extracting phenolic compounds from 26 Ivorian plant species compared to acetone, methanol, and water.

The present study corroborates the findings of the previous studies presented above, with aqueous ethanol demonstrating the highest efficiency in extracting both phenols and flavonoids from *C. zeylanicum*. There was no significant difference in the TPC of the extracts extracted by acetone and ethanol ($P > 0.05$), suggesting that ethanol and acetone may extract phenolic compounds similarly. However, the significantly higher TFC in the ethanolic extract compared to the acetone extract ($P < 0.05$) indicates that ethanol may be more effective for flavonoid recovery. Given these results, aqueous ethanol was selected as the optimal solvent for subsequent extraction and optimisation experiments, as it effectively extracts both phenolic and flavonoid compounds while being relatively safer than other organic solvents.

Although previous studies have shown that aqueous ethanol is effective for extracting phenolic compounds from cinnamon species, it is important to consider the differences in species and growing conditions. The present study focuses on *C. zeylanicum* from Sri Lanka, which may have a unique phenolic composition due to variations in climate and soil. These environmental factors can influence the efficiency of different solvents in extracting bioactive compounds. Furthermore, differences in extraction methods, such as temperature and solvent concentration, can also impact phenolic recovery. Future research comparing cinnamon from various regions under the same extraction conditions would provide a clearer understanding of these variations.

3.2. *Effect of solvent concentration and method of extraction on the extraction of phenols and flavonoids*

The TPC, TFC, and extraction yield percentage of *C. zeylanicum* extracts obtained using UAE and ASE at different ethanol concentrations (25 %, 50 %, 75 %, and 100 %) are presented in Tables 2 and 3, respectively. The highest TPC and TFC were observed in extracts obtained with 50 % ethanol for both UAE and ASE. The TPC for ASE was 6.83 ± 0.31 mg/g GAE, while in the case of UAE, it was significantly lower at 3.82 ± 0.04 mg/g GAE ($P < 0.05$). Similarly, the TFC in the case of ASE was 0.50 ± 0.003 mg/g QE, whereas UAE yielded 0.35 ± 0.006 mg/g QE, showing that ASE was more efficient in extracting phenols and flavonoids compared to UAE.

For both extraction methods, the TFC values followed the trend: 50 % ethanol >100 % ethanol >75 % ethanol >25 % ethanol. In contrast, the TPC values for ASE followed the same trend, while for UAE, the TPC values followed the order: 50 % ethanol >75 % ethanol >100 % ethanol >25 % ethanol.

The extraction yield of *C. zeylanicum* varied significantly depending on the extraction method and the ethanol concentration used. In both UAE and ASE, the highest yields were observed at 50 % ethanol extraction method, with yields of 18.3 % and 18.8 %, respectively. This suggests that a moderate polar solvent mixture is more effective in solubilising a broader range of bioactive compounds, including both hydrophilic and hydrophobic compounds. The research conducted by Yang et al. (2012), which extracted *Cinnamomum cassia* using 95 % ethanol, reported an extraction yield of 12.73 %. This is comparable to our findings, where 100 % ethanolic extraction using UAE and ASE resulted in yields of 13.41 % and 12.61 %, respectively. Nawaz et al. (2020)

Table 2
Total phenol content (TPC), total flavonoid content (TFC), DPPH scavenging activity, and extraction yield percentage of *C. zeylanicum* extracted by ultrasonic extraction with different ethanolic concentrations.

Solvent type	TPC mg/g GAE	TFC mg/g QE	DPPH free radical scavenging activity	ABTS Ic50 (μg/mL)	Extraction yield (%)
25 % Ethanol	2.12 ± 0.07^{c}	0.29 ± 0.007^{d}	90.49 ± 0.11^{a}	5.46 ± 0.01^{a}	8.24 ± 0.81^{d}
50 % Ethanol	3.82 ± 0.04^{a}	0.35 ± 0.005^{a}	91.07 ± 0.15^{a}	3.26 ± 0.05^{b}	18.27 ± 0.65^{a}
75 % Ethanol	2.70 ± 0.04^{b}	0.32 ± 0.004^{c}	90.60 ± 0.10^{a}	3.46 ± 0.33^{b}	16.27 ± 0.73^{b}
100 % Ethanol	2.53 ± 0.02^{b}	0.33 ± 0.003^{b}	91.20 ± 0.24^{a}	3.36 ± 0.005^{b}	13.41 ± 0.44^{c}

TPC: Total phenolic content (mg/g), gallic acid equivalents; TFC: Total flavonoid content (mg/g), Quercetin equivalents; Means within a column followed by different letters were significantly different at $P < 0.05$.

Table 3
Total phenol content (TPC), total flavonoid content (TFC), DPPH scavenging activity, and extraction yield percentage of *C. zeylanicum* extracted by accelerated solvent extraction.

Solvent type	TPC mg/g GAE	TFC mg/g QE	DPPH free radical scavenging activity	ABTS Ic50 (μg/mL)	Extraction yield (%)
25 % Ethanol	3.99 ± 0.45^{b}	0.44 ± 0.009^{c}	89.11 ± 1.31^{ab}	6.48 ± 0.42^{b}	15.65 ± 0.64^{b}
50 % Ethanol	6.83 ± 0.31^{a}	0.50 ± 0.003^{a}	86.9 ± 2.58^{ab}	4.89 ± 0.15^{c}	18.83 ± 0.86^{a}
75 % Ethanol	4.83 ± 0.60^{ab}	0.45 ± 0.006^{bc}	91.07 ± 2.00^{a}	5.50 ± 0.21^{c}	16.99 ± 0.33^{b}
100 % Ethanol	6.82 ± 0.41^{a}	0.47 ± 0.004^{b}	89.02 ± 4.2^{ab}	17.23 ± 0.04^{a}	12.61 ± 0.73^{c}

TPC: Total phenolic content (mg/g), gallic acid equivalents; TFC: Total flavonoid content (mg/g), Quercetin equivalents; Means within a column followed by different letters were significantly different at $P < 0.05$.

found that extraction with more polar solvents, such as water, resulted in higher overall yields but lower phenolic and flavonoid contents compared to less polar solvents. Similarly, in our study, while 25 % ethanol (a more polar solvent) produced a moderate to high extraction yield in the ASE, it yielded significantly lower levels of total phenolics and flavonoids. In the same study, it was reported that extraction yield increased with increasing solvent polarity. However, our findings showed that the highest extraction yields were achieved with moderately polar solvents, such as 50 % ethanol, rather than with highly polar solvents like absolute ethanol. In the study of Al-Garadi et al. (2023) an extraction yield of 12.48 and 13.50 % were observed with 100 % ethanol and 50 % ethanol when refluxed in a water bath. These yields are slightly lower than those observed in our study, highlighting the significant influence of both the extraction method and solvent concentration on the overall extraction yield when needing to optimise bioactive recovery.

The antioxidant activity of the extracts was evaluated using DPPH and ABTS assays. At 25 mg/mL, all UAE and ASE extracts exhibited DPPH scavenging activity exceeding 85 %, confirming strong antioxidant potential. The highest DPPH scavenging activity was observed with 50 % ethanol for UAE and 75 % ethanol for ASE, although no significant differences were noted among different ethanol concentrations ($P > 0.05$). The IC50 values for ABTS scavenging activity were lowest for 50 % ethanolic extracts, indicating the highest antioxidant potential. However, this value was not significantly different ($P > 0.05$) from that of 75 % ethanol extracts. To compare ASE and UAE with a conventional extraction method, HWB extraction was performed. Fig. 2 illustrates the TPC of cinnamon extracts obtained through HWB, UAE, and ASE using water as a solvent. Although ASE was the most effective method when using ethanol, UAE yielded significantly higher phenolic content when water was used. In contrast, HWB extraction resulted in the lowest TPC compared to UAE and ASE.

Solvent concentration plays a crucial role in the extraction efficiency of bioactive compounds from *C. zeylanicum* (Gilani & Najafpour, 2022). Previous studies have demonstrated that binary solvent systems are more effective in extracting phenolic compounds than mono-solvent systems (Dvorackova et al., 2015). This is because aqueous solvent has better extraction power than pure solvents due to their ability to extract

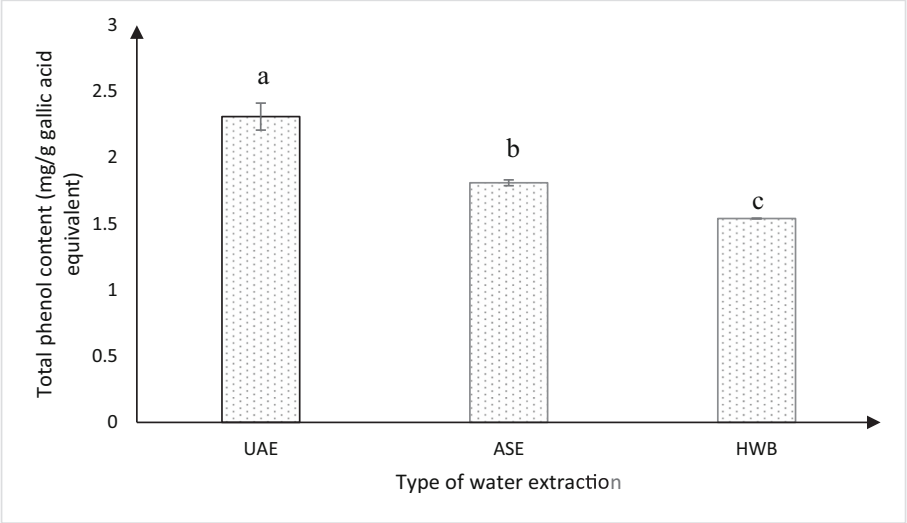

Fig. 2. The mean plot of the TPC value of the water extracts of cinnamon obtained via hot water bath extraction, ultrasonic extraction, and accelerated solvent extraction. Different letters (a–c) above the bars represent significant differences among the water extracts ($P < 0.05$). Each data point represents the mean ± SD of the results. UAE: ultrasonic-assisted extraction, ASE: accelerated solvent extraction, HWB: hot water bath extraction.

both hydrophilic and lipophilic compounds. The polarity of absolute ethanol is 5.2 and this value increases as the concentration of water increases, making it easier to extract compounds across a wider polarity (Hikmawanti et al., 2021).

Previous studies on *C. cassia* using varying ethanol concentrations have revealed a nonlinear relationship between solvent concentration and extraction efficiency, with the highest bioactive extraction observed at intermediate ethanol levels (Dvorackova et al., 2015; Gilani & Najafpour, 2022; Kim & Kim, 2000). The results of this study align with the previous findings on *C. cassia*. However, the study results of Yang et al. (2012) showed that phenols extraction of *C. cassia* was better with 95 % ethanol than 50 % ethanol. There have no studies been done so far to understand the effect of varying ethanolic concentration on the bioactive profile of *C. zeylanicum* extracts to the best of our knowledge.

The highest TPC and TFC values in this study were obtained using 50 % ethanol. This observation aligns with the principle that a mixture of water and an organic solvent like ethanol can better solubilise a wider range of compounds with varying polarities, leading to higher yields of phenolics and flavonoids, confirming that optimal extraction occurs at a balanced polarity. The significantly higher TPC and TFC in ASE extracts compared to UAE extracts suggest that high pressure and temperature in ASE facilitate greater mass transfer and solubilization of bioactives (Zhang, Hawboldt, & MacQuarrie, 2024). The elevated temperature and pressure in ASE may also enhance the solubility of target compounds in a slightly less polar solvent mixture (50 % ethanol), whereas in UAE, the cavitation forces might be more effective with a slightly higher ethanol concentration (75 %) for disrupting the plant matrix and releasing these specific bioactives. The strong correlation between TPC and TFC for UAE ($R = 0.79$) and ASE ($R = 0.81$) further suggests that flavonoids are a major phenolic group in *C. zeylanicum*. This strong positive correlation also indicates that extraction conditions favoring the recovery of total phenolics are likely to enhance flavonoid extraction as well and vice versa. Furthermore, this relationship implies that the observed antioxidant activity is largely attributed to the phenolic, particularly flavonoid, components of the cinnamon extracts, reinforcing their significance.

In terms of antioxidant activity, all extracts exhibited high DPPH radical scavenging activity (>85 %), indicating their strong potential in neutralizing free radicals. The IC50 values confirmed that 50 % ethanol extracts had the highest antioxidant potential, likely due to enhanced extraction of hydrophilic and lipophilic antioxidants. The absence of significant differences between 50 % and 75 % ethanol extracts suggests that both solvent concentrations are optimal for extracting antioxidant compounds from cinnamon. While ASE yielded higher phenolic and flavonoid content when ethanol was used as the solvent, UAE was more effective for water-based extraction. This suggests that UAE is better suited for extracting water-soluble bioactives, whereas ASE is more efficient when using ethanol-based solvents. The lower efficiency of HWB extraction can be attributed to longer extraction times, lower mass transfer rates, and potential thermal degradation of heat-sensitive compounds.

Overall, these findings provide critical insights into optimising solvent concentration and extraction methods for maximising bioactive yields in *C. zeylanicum* extracts. Lastly, while the results in this study provide valuable insights into optimising extraction from this specific source, future studies could investigate the variability in bioactive extraction efficiency across different geographical origins and varieties of *C. zeylanicum*.

3.3. Effect of solvent concentration and extraction method on the extraction yields of cinnamaldehyde, cinnamic acid, and eugenol

The amounts of cinnamaldehyde, eugenol, and cinnamic acid extracted from *C. zeylanicum* bark powder using UAE and ASE with different ethanol concentrations are presented in Tables 4 and 5, respectively. The highest yields of these bioactive compounds were obtained with 75 % ethanol using UAE and 50 % ethanol using ASE.

Table 4
Amounts of cinnamaldehyde, eugenol, and cinnamic acid present in stem bark powder of *C. zeylanicum* extracted by ultrasonic extraction.

Type of extract	Cinnamaldehyde mean amounts (mg/g)	Eugenol mean amounts (mg/g)	Cinnamic acid mean amounts (mg/g)
25 % ethanol	13.57 ± 0.004^{c}	7.06 ± 0.028^{c}	0.0822 ± 0.001^{c}
50 % ethanol	16.02 ± 0.006^{b}	8.68 ± 0.044^{b}	0.0922 ± 0.001^{b}
75 % ethanol	18.03 ± 0.025^{a}	10.22 ± 0.003^{a}	0.1026 ± 0.002^{a}
100 % ethanol	11.68 ± 0.001^{d}	6.13 ± 0.013^{d}	n/d

Means within a column followed by different letters were significantly different at $P < 0.05$; n/d: not detected.

Table 5
The concentration of cinnamaldehyde, eugenol, and cinnamic acid present in stem bark powder of *C. zeylanicum* extracted by accelerated solvent extraction.

Type of extract	Cinnamaldehyde mean amounts (mg/g)	Eugenol mean amounts (mg/g)	Cinnamic acid mean amounts (mg/g)
25 % ethanol	12.45 ± 0.002^{c}	5.85 ± 0.001^{c}	0.13 ± 0.001^{c}
50 % ethanol	19.33 ± 0.002^{a}	10.57 ± 0.026^{a}	0.18 ± 0.004^{a}
75 % ethanol	17.52 ± 0.006^{b}	9.40 ± 0.038^{b}	0.15 ± 0.001^{b}
100 % ethanol	7.45 ± 0.001^{d}	3.70 ± 0.011^{d}	n/d

Means within a column followed by different letters were significantly different at $P < 0.05$; n/d: not detected.

These results highlight the influence of both solvent concentration and extraction method on bioactive compound recovery.

The standard chromatographic peaks for cinnamaldehyde, cinnamic acid, and eugenol were identified using HPLC and are presented in Fig. 3, which shows a representative chromatogram of these marker compounds under the initial gradient elution profile. Although several studies have investigated the presence of cinnamaldehyde, cinnamic acid, and eugenol in *C. zeylanicum* bark oil, there is limited research quantifying these bioactive compounds in bark extracts. The available literature primarily explores extraction using different solvents and methodologies, but a comprehensive analysis of how these bioactive compounds vary with ethanol concentration and extraction methods, particularly high-pressure techniques like ASE remains largely unexplored.

In a study by Gursale et al. (2010), *C. zeylanicum* bark was extracted using methanol, yielding a cinnamaldehyde content of 8.76 mg/g. Dhillon and Amarjeet (2013) used ethanol as the extraction solvent and reported a much lower cinnamaldehyde yield of 1.42 mg/g of cinnamon dry weight, indicating that solvent choice significantly influences bioactive recovery. Foudah et al. (2021) applied UAE with methanol and used high-performance thin-layer chromatography (HPTLC) for quantification, obtaining a remarkably high cinnamaldehyde content of 111.57 mg/g, emphasising the impact of extraction techniques on yield.

Zhou et al. (2024) studied the yields of cinnamaldehyde and cinnamic acid from Guangxi cinnamon under varying ethanol concentrations using ultrasonic-microwave extraction. Their results showed that the yields of both compounds increased up to 75 % ethanol, followed by a decline. The findings of UAE in the current study align with these results, as the highest cinnamaldehyde, eugenol, and cinnamic acid yields were also observed with 75 % ethanol. However, the highest yields for ASE were obtained with 50 % ethanol, differing from UAE and the ultrasonic-microwave extraction results reported by Zhou et al. (2024). This suggests that both the extraction method and compound

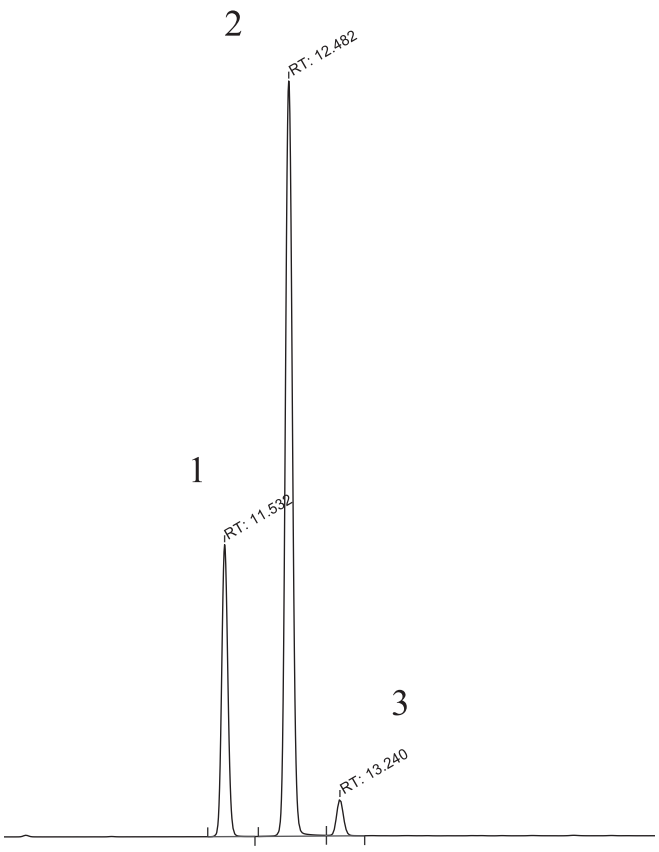

Fig. 3. Representative chromatogram of marker compounds on a C18 column with the initial gradient elution profile: 0 min, 5 % B; 5 min, 100 % B; 20 min, and held at 100 % B for 3 min, flow rate 1 mL/min, detection wavelength UV 287 nm. Peaks: (1) cinnamic acid (125 μg/mL), (2) cinnamaldehyde (250 μg/mL), and (3) eugenol (125 μg/mL).

solubility in the solvent play a critical role in the extraction efficiency of cinnamon bioactives.

Despite previous studies, to the best of our knowledge, no research has systematically investigated the variation of cinnamaldehyde, cinnamic acid, and eugenol across different ethanol concentrations using HPLC in *C. zeylanicum* bark extracts. This study aims to address this gap by evaluating ethanol concentration effects on extraction efficiency, providing valuable insights for optimising extraction methodologies in food, nutraceutical, and pharmaceutical applications.

4. Conclusions

Among the three methods tested in this study (ASE, UAE, and conventional HWB), ASE demonstrated the highest extraction efficiency of bioactive compounds from *C. zeylanicum* across most parameters, confirming its superiority over UAE and HWB. Using 50 % ethanol as the solvent resulted in the highest bioactive recovery, outperforming 25 %, 75 %, and 100 % ethanol, as well as methanol, acetone, and water. However, scalability, commercialisation costs, solvent recovery, and bioavailability of the extracted bioactives could present key challenges for industrial applications. These findings provide valuable insights into optimising cinnamon extraction for functional food and nutraceutical applications, potentially leading to the development of bioactive-rich products with enhanced health benefits. Taken together, this study highlights the potential of ASE as a superior method for extracting bioactive compounds from *C. zeylanicum*. By addressing the challenges of scalability and bioavailability, future advancements in extraction technology could significantly enhance the commercial viability of bioactive-rich cinnamon products. Future research should focus on optimising processes for large-scale extraction, developing encapsulation strategies to improve bioactive stability and bioavailability, and creating sustainable solvent recovery systems to enhance industrial feasibility.

CRediT authorship contribution statement

M.S. Culas: Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **L. Kaur:** Writing – review & editing, Validation, Supervision, Methodology. **D.G. Popovich:** Writing – review & editing, Supervision, Methodology, Conceptualization. **A. Rashidinejad:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors wish to thank Maggie Zou from the Riddet Institute for the assistance in the laboratory and David Lun from the School of Food Technology and Natural Sciences, Massey University, for the help provided with the HPLC work. The first author would also like to acknowledge the Doctoral scholarship granted by Massey University (Palmerston North, New Zealand).

Data availability

Data will be made available on request.

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